

# Chapter 14

## Carbon Nanoparticles as the Next-Generation Antimicrobial Agents



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**Abstract** In the wake of increasing antimicrobial resistance due to the indiscriminate use of antibiotics, the need of the hour has been to look for novel antimicrobial agents that have least potential for eliciting resistance from microbes. Current antibiotics that target bacterial cell wall or biochemical processes in the cell have thus largely failed as microbes evolve faster to counteract these mechanisms. Carbon nanomaterials (CNMTs) have been studied as a novel class of antibiotics in the last decade, and functionalized carbon nanoparticles and nanocomposites have shown to possess antimicrobial activity. Carbon nanocomposites like nanotubes, fullerenes, and graphene and their derivatives have also shown great potential to act as antimicrobial compounds. One of the main contributors to antimicrobial activity is the large surface to volume ratio of CNMTs that allows easy and efficient binding to microbes to elicit the antimicrobial activity by various mechanisms including physical cell membrane damage, oxidative stress, affecting bacterial enzymes, and respiration. This chapter focusses on the antimicrobial properties of various types of CNMTs, their mechanism of action, their advantages for combating antimicrobial resistance, and challenges in use as next-generation antimicrobial agents.

**Keywords** Antimicrobial resistance · Antimicrobial agents · Bacterial cell wall · Antibiotics · Carbon nanocomposites · Carbon nanoparticles · Carbon nanotubes · Membrane disruption · Nanoliposomes · Oxidative stress · Antioxidant · Reactive oxygen species · Photosensitizers · Multidrug resistance · Fullerenes · Photodynamic therapy · Gram-positive bacteria · Gram-negative bacteria · Liposomes · Graphene

### Abbreviations

CNTs	Carbon nanotubes
DWCNTs	Double-walled carbon nanotubes
GO	Graphene oxide

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Gt	Graphite
GtO	Graphite oxide
MDR	Multidrug resistance
MWNTs	Multiwalled nanotubes
PDT	Photodynamic therapy
rGO	Reduced graphene oxide
ROS	Reactive oxygen species
SWNTs	Single-walled carbon nanotubes

## 14.1 Introduction

Antimicrobial resistance is a global crisis, affecting lives of millions of people suffering from various infections. Developing countries are the worst affected. Indiscriminate use of antibiotics and the lack of new effective drugs have made multidrug-resistant infections common, impacting the clinical outcomes of many especially in hospitals and intensive care units, surgery patients, and immunocompromised patients including cancer patients and transplant patients (Ventola 2015). Typically treating drug-resistant bacteria is not only cost-intensive but extremely long, impacting not only the affected individual but also hospitals and governments. This is especially causing epidemics in the case of communicable infectious diseases, especially in developing countries like Africa, India, South America, and Southeast Asia, making it a worldwide public threat (Mogasale et al. 2021).

With the increasing global population, and pollution in the environment and lack of sanitation, there is a wide spread of infectious diseases, and therefore, antibiotics are being indiscriminately used. However, over the years, bacteria both individual and those in biofilms have developed resistance through adaptations, selection of mutants. There are several mechanisms by which bacteria protect themselves—some secrete enzymes that destroy antibiotics, and others produce efflux pumps that pump out antibiotics from cellular interior. Others excrete extracellular polymeric substances that preserve them from host responses. Due to existence of bacteria in biofilms in respiratory infections, chronic wounds, infective endocarditis, and osteomyelitis, bacteria are protected due to advantages of spatial and chemical heterogeneities allowing for persistence of infections for reasons other than resistance mutations.

Currently treatment of MDR requires multiple or high dosages of antibiotic agents that result in long and expensive treatments, with the possibility of adverse effects and uncertain outcomes. Nanomaterials are not naturally present in the environment and hence are new materials for bacteria, and any mechanisms against them have not yet been developed. This presents an opportunity for carbon nanomaterials to be used as nanocarriers to address multidrug-resistant microbes—both planktonic and in biofilms in therapeutics (Mulani et al. 2019).

Carbon allotropy is one of the most interesting phenomena in material science giving rise to a variety of nanostructures that differ in their properties while all being composed of the same carbon atoms. In the last 20 years, apart from the two well-known natural allotypes, graphite and diamond, there have been other allotypes synthesized in labs, exploiting the versatile sp<sup>2</sup> and sp<sup>3</sup> bonding ability of carbon nanotubes (CNTs), graphite, graphene/graphene oxide (G/GO), and fullerenes (Rajeswari et al. 2021; Wang et al. 2014a). This chapter focuses on each of the different types of carbon nanotubes, their antimicrobial properties, and their mechanism of action allowing them to be effective as next generation of antibiotics.

## 14.2 Carbon Nanotubes and Their Antimicrobial Properties

Carbon nanotubes are hollow carbon-walled structures that are broadly classified into single-walled carbon nanotubes (SWNTs), double-walled carbon nanotubes (DWCNTs), and multiwalled nanotubes (MWNTs) (Iijima 1991). The differences are in the wall diameter and thickness and properties that are conferred due to addition of cylinders. Their methods of synthesis have been detailed in Azizi-Lalabadi et al. (2020). They are known for their high electrical conductivity (~3000 W/m/K) high adsorption (17.44 mg g<sup>-1</sup>), and surface/volume ratios. These structures not only possess high electrical and thermal conductivity but also mechanical resistance and photoluminescence, making them useful for many biosensing applications at the interface of electronics and have thus been used in a variety of biosensing applications in agriculture and medicine (Rdest and Janas 2021; Chik et al. 2019; Wang et al. 2017; Cataldo and Da Ros 2008).

The antimicrobial properties of carbon nanotubes have come to light while investigating the possible interactions of carbon nanotubes with human cells for assessing toxicity. These studies were important as carbon nanotubes have wide applications in electronics and could be used as biosensors, hence could contaminate our environment. Their constant presence and interactions would help us evaluate their applications which are in vivo sensing capabilities. Carbon nanotubes are hydrophobic in nature. Therefore, in order to use carbon nanotubes for biological applications, they need to be functionalized. Functionalization is carried out using solubilizing agents like surfactants, harsh chemical treatments that could be toxic to human cells and elicit immune responses if used in medical applications (Vardharajula et al. 2012; Sun et al. 2002). Additionally, physicochemical properties of SWCNTs, such as properties such as structure, diameter, and cleanliness (e.g., % metal), also contribute to toxicity (Mohanta et al. 2019; Allegri et al. 2016; Jafar et al. 2016). These studies propelled groups to look into carbon nanotubes' effect on microbes (Kang et al. 2007).

### ***14.2.1 Carbon Nanotubes Damage Bacterial Cell Membranes by Direct Contact***

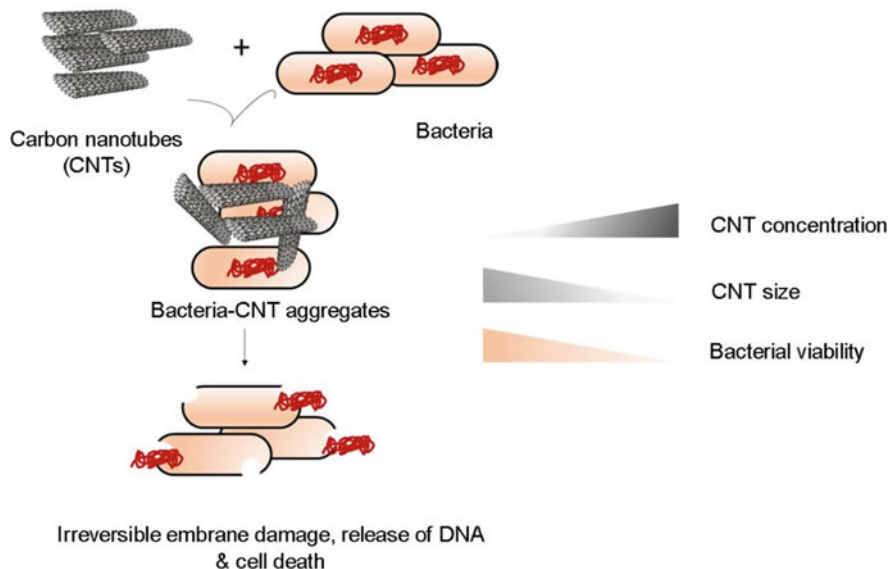
In 2007, for the first time, the antimicrobial properties of commercial SWCNTs (diameter: 0.75 nm to 1.2 nm; length: ~13  $\mu\text{m}$ ) were described on *E. coli* using fluorescence-based cytotoxicity assays utilizing dyes like propidium iodide (PI) and 4',6-diamidino-2-phenylindole (DAPI) whose emission spectra did not overlap with SWCNTs. *E. coli* treated with SWCNTs were found to exhibit severe cell membrane damage (Kang et al. 2007). They had concluded that direct contact of SWCNTs with cells was essential and that the membrane damage increased with time. Damage observed in the first 30 min was associated with loss of viability at 73.1% that increased to 87.6% viability loss in 120 min. Direct contact is dependent on many factors: size and concentration of nanotubes and time of treatment (Kang et al. 2007). This was confirmed from many subsequent studies showing direct contact by microscopical observations under different treatment conditions and from different types of nanotubes and bacteria studied. The main factors contributing to direct contact required to elicit antimicrobial activity against bacteria are discussed below.

### ***14.2.2 Antimicrobial Activity Is Dependent on CNT Concentration, Buffer, and Treatment Time***

Antimicrobial activity of SWCNTs and MWCNTs was studied against various groups of bacteria—gram-positive and gram-negative cocci/rods (Arias and Yang 2009). There was no significant difference in activities of nanotubes against various groups of bacteria studied. All of them showed approximately a 7-log reduction in cell count in viability testing.

This study also shed a light on the following properties of carbon nanotubes and their effect on antimicrobial activity: surface charge due to functionalization (presence of  $-\text{OH}$  and  $-\text{COOH}$  groups), concentration of nanotubes, and the buffer composition. They concluded that surface charge due to  $-\text{OH}$  and  $-\text{COOH}$  groups does not seem to be a factor for determining toxicity as SWNTs and MWNTs with the same surface charges behaved differently. While charged SWCNTs exhibited strong antimicrobial activity, MWCNTs did not exhibit significant antimicrobial property. They found that increasing concentration of nanotubes increased antimicrobial activities on both types of charged SWCNTs (with surface groups of  $-\text{OH}$  and  $-\text{COOH}$ ), starting at 50–250  $\mu\text{g/ml}$  (at the highest concentration, these could inactivate  $10^7$  cfu/ml *Salmonella* cells in 15 min). Interestingly, buffer composition was also found to be a critical factor in eliciting antimicrobial properties. SWCNTs in deionized water or 0.9% NaCl solution exhibited extremely strong antimicrobial activity but lost activity in PBS buffer and brain heart infusion broth.

Another study investigating antimicrobial property of SWCNTs against *Salmonella enteric*, *E. coli*, and *Enterococcus faecium* at concentrations 0.3–1 mg/ml showed that antibacterial activity was positively dependent on nanotube



**Fig. 14.1** Antimicrobial mechanisms of CNTs. When introduced to suspensions containing bacteria, CNTs aggregate with them and cause membrane damage, resulting in the release of DNA (red) and microbial cell death (left). Bacterial viability decreases with increasing CNT concentration and decreasing size (right)

concentration (Dong et al. 2012). SWCNTs are generally treated with surfactants for dispersing bundles into individual tubes for applications. Many of these surfactants themselves show antimicrobial activities, and hence the use of the right one is essential for applicability in biomedical applications. A study of various surfactants—sodium dodecyl benzenesulfonate (SDB), sodium cholate, and sodium dodecyl sulfate (SDS) in combination with SWCNTs—showed that sodium cholate exhibited least inhibitory effect on bacterial growth at concentrations of 1% while allowing for SWCNT dispersion effectively; SDS and SDB showed total effectiveness against antimicrobial growth at 1% (Dong et al. 2009). Moreover, sodium cholate shows lower toxicity to 1321Ni human astrocytoma cells and hence is optimal for use in biomedical applications. This allows for use of sodium cholate SWCNTs in drug delivery applications especially those that require long treatment times like those against multidrug-resistant microbes. In contrast to previous study, the time of treatment from 5 min to 2 h did not seem to affect antimicrobial activity (Dong et al. 2012). The antimicrobial mechanisms of CNTs are depicted in Fig. 14.1.

### 14.2.3 The Size of Nanotubes Determines Antimicrobial Activity

Nanotube size depends upon its diameter, length, and volume/surface area. A detailed study on the types of NTs and their effect showed that SWCNTs displayed

better antibacterial activity compared to MWCNTs owing to their smaller nanotube diameter and large surface area allowing for better interaction with bacterial membranes (Maleki Dizaj et al. 2015; Yang et al. 2010).

SWCNTs of similar weights but varying lengths were studied, and 5  $\mu\text{m}$  long were found to exhibit higher antibacterial activity compared to smaller-length tubes of  $<1 \mu\text{m}$  and  $<5 \mu\text{m}$ . The longer nanotubes aggregated better with bacterial cells, while shorter ones were more prone to self-aggregation excluding the bacterial cells. Interestingly, longer nanotubes also showed better antibacterial activity that increased with nanotube concentration and treatment time (Vecitis et al. 2010; Kang et al. 2008).

#### ***14.2.4 Carbon Nanotube Composites and Their Antimicrobial Properties***

Carbon nanotube composites with biopolymers and nanoparticles like CuO, Ag, TiO<sub>2</sub>, ZnO, etc. are known for their high antimicrobial activity (Kim et al. 2008; Cha et al. 2005). However, the utilization of these NPs requires much care considering their toxicity. To resolve this problem, carriers or supporters such as polymers, magnetic NPs, or CNMs like CNTs, GO, and fullerenes can be used, which can potentially boost the antimicrobial activity of the NPs too (Ballatore et al. 2015; Duri et al. 2017). CNMs have good functionalization potentials considering their chemical groups and excellent dispersion capability. The synergistic effect in antimicrobial properties is very important. CNT chitosan, CNT Ag, GO–Ag, and C60 ZnO and C60 CuO show increased antibacterial properties against microbes, including *E. coli* (Hussain et al. 2019; Sakib et al. 2019; Dinh et al. 2015; Das et al. 2011). This synergist effect is related to their mechanisms in disrupting the cell wall or membrane which permits easier availability for other small antimicrobial materials to penetrate into cells. AgNP deposition on MWCNTs with polyamidoamine has been shown to induce bacteriostatic effects against *S. aureus*, *E. coli*, and *P. aeruginosa* (Yun 2013; Yuan et al. 2008).

#### ***14.2.5 Carbon Nanotubes for Delivery of Antibiotics***

Due to the high adsorption properties of carbon nanotubes, imparted on it by its high surface area, electronic properties, and stability, CNTs have been used as drug delivery systems in therapeutic use.

CNTs have been used for delivery of vectors and biomolecules (like DNA, RNA, and proteins) to cells, tissues, and organs (He et al. 2013). And they have been used as biosensors for diagnostic applications as well (Zhang et al. 2011).

Due to the hollow nature of carbon nanotubes, drugs can be loaded into two ways (Martincic and Tobias 2015). It can either be packed into the hollow CNT structure or covalently conjugated to the CNT surface (Heister et al. 2012). CNT has been shown to act as an antimicrobial both as a drug deliverer without internalization into cells and being internalized into the cells along with the drugs. This was found to occur via either by penetration followed by diffusion mechanism or the endocytosis pathway. The internalization of drug into CNT hollow tube has been shown to be more effective than surface attachment. CNT works by releasing conjugated drug inside the cells, once internalized. If the drug were to be attached to the surface, they are in danger of degradation by the physiological fluids prior to be internalized by the cells (Debnath and Srivastava 2021; Roldo 2016; Bianco et al. 2005). CNTs have therefore promising agents for antibiotic drug delivery.

### 14.3 Fullerenes and Their Antimicrobial Properties

Fullerenes are a group of cage-like carbon nanostructures of various shapes from hollow spheres to ellipsoids. C60 or buckminsterfullerene is a well-known and the most abundant form of fullerene, resembling a soccer ball. Due to their unique structure and electronic properties, fullerenes like C60 have been shown to possess high antioxidant activity among other functions—organic photovoltaics and biopharmaceuticals and treatment of water.

They show bacteriostatic properties against gram-positive (*B. subtilis*) and gram-negative (*E. coli*) bacteria in saline or buffer systems; the antimicrobial properties of fullerene decrease with certain types of buffers that result in aggregation of fullerenes in solution (Fortner et al. 2005).

#### 14.3.1 *Functionalized Fullerenes Affect Viral Proteases and Possess Bacteriostatic Properties*

Functionalization is essential for fullerenes to be used in biomedical applications due to their insolubility (Li et al. 2012a). Thus, functionalization with different agents imparts different properties leading to different mechanisms of action against microbes.

Functionalization of C60 has shown to impart antimicrobial activity due to better interaction with microbes. Studies of C60 with viral proteases showed that similar to carbon nanotubes, direct contact was shown to be essential for antimicrobial activity of fullerenes (Zhu et al. 2003; Shoji et al. 2013).

Heightened antimicrobial and antiviral activity was seen due to incorporation of hydroxyl, carboxylic acid, and glycolic oxide groups in C60, due to enhanced interaction of C60 with virus protease active site cavity due to the formation of

various stable hydrogen bonds with supporting interactions between C60 and aromatic Phe53/Arg8 in protease active site (Barzegar et al. 2017; Thota et al. 2012). Amino derivatives of fullerenes have demonstrated potent antimicrobial activity in the water treatment. Fulleropyrrolidine-containing amino groups have been shown to deactivate HIV-1 and HIV-2 proteases (Szunerits et al. 2015).

Hexakis carboxylic acid fullerene derivatives have shown to protect mice from *Streptococcus* infection (Tsao et al. 2001). Fulleropeptides, prepared by solid-phase peptide synthesis, have been shown to possess bacteriostatic activities and have been effective against *Streptococcus* and *E. coli*. Interestingly, fulleropeptides have shown better antimicrobial activity to gram-positive bacteria than parent peptide. But these showed decreased potency against gram-negative bacteria and yeasts (D'Souza and Kadish 2012). In silico studies have shown that fullerenes can be effective against SARS-CoV-2 virus, causative agent of COVID-19 pandemic (Hurmach et al. 2021; Serrano-Aroca et al. 2021).

### 14.3.2 Fullerenes Affect Microbial Energy Metabolism

Antimicrobial properties of fullerenes are due to multiple properties relating to structure, solubility, and electronic chemistry. Like carbon nanotubes, membrane contact is important for the mechanism of action of fullerenes, but they do not necessarily cause membrane damage. Below are detailed accounts of mechanism of action of fullerenes in context of antimicrobial activity.

#### 14.3.2.1 Lipophilic Nature of Fullerenes Allow for Membrane Permeability

Due to their lipophilic nature, amino and other biofunctionalized fullerenes show membranotropic properties, i.e., easy absorption by cellular membranes (Kotelnikova et al. 1996). Studies show that even though direct contact is important, unlike carbon nanotubes, physical disruption of cell wall/membrane was not responsible for antimicrobial activity of fullerenes. Rather, fullerenes were shown to pass through cell membranes and interact with components of the cellular metabolism to affect their growth and survival (Zhang et al. 2021).

Fullerenes being capable of generating reactive oxygen species are thought to harm cell membrane metabolism. Studies in gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria on the effect of C60 on cell membrane lipid composition showed that the presence of fullerenes changed the proportion of unsaturated fatty acids and saturated fatty acids and impacted membrane fluidity. *P. putida* responded by decreasing its unsaturated fatty acids and increasing cyclopropane fatty acids, while *B. subtilis* levels of iso- and anteiso-branched fatty acids or monosaturated fatty acids are dependent of the concentration of C60 (Fang et al. 2007).



### 14.3.2.2 Cationic Fullerenes Use ROS-Mediated Antimicrobial Mechanism

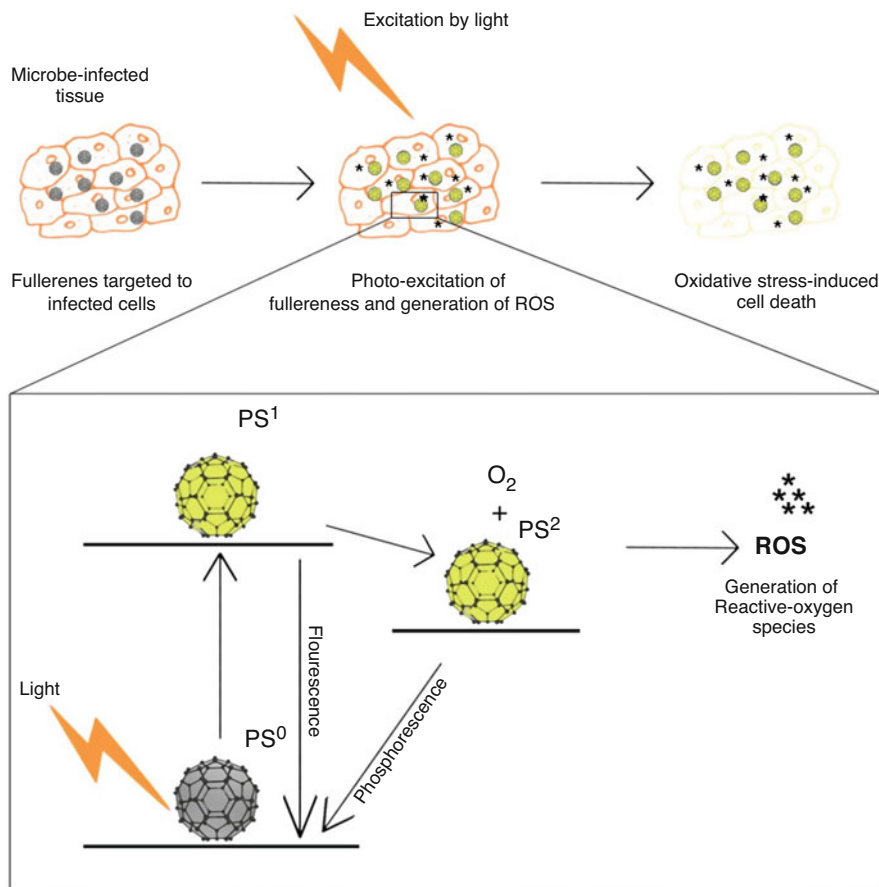
Functionalization of fullerene compounds has been carried out to generate positively charged, neutral, and negatively charged derivatives. Positively charged or cationic derivatives show increased antimicrobial activity on *Shewanella oneidensis* and *E. coli*, while the anionic derivatives are not that effective (Nakamura and Mashino 2009). Cationic fullerenes are thought to inhibit the bacterial respiratory chain by ROS production or direct reduction (Cataldo and Da Ros 2008).

Another study comparing a protonated amine and deprotonated carboxylic conjugated to fullerene cage via organic linkers showed that positively charged fullerenes bound effectively to *E. coli* and showed antibacterial activity, whereas those negatively charged did not bind nor show antimicrobial activity (Deryabin et al. 2014).

### 14.3.3 Fullerenes Used for Photodynamic Therapy Against Infections

Oxidative damage by ROS has been known to be toxic to human cells and microbial cells. Molecules that make ROS in response to light, called photosensitizers, have been used for generation of ROS for targeting cancers and microbes in vivo (Abrahamse and Hamblin 2016). This is called photodynamic therapy (PDT). Using localized light delivery, PDT can be performed to target specifically diseased tissue, cancer cells, or microbial cells while keeping the normal cells unharmed. Since functionalized fullerenes possess the ability to form excited singlet state, followed by transition to the long-lived excited triplet state, and can react with oxygen to make ROS, they are excellent candidates for photodynamic therapy (Li et al. 2012a). Figure 14.2 represented the PDT of microbe-infected tissue using fullerenes.

Functionalization of fullerenes with the addition of hydrophilic groups enhances production of superoxides which selectively increases cytotoxicity toward microbial cells compared to mammalian cells in PDT. Photoradiation of fulleropyrrolidinium salts resulted in toxicity of 99.99% of bacterial and fungal cells (Tegos et al. 2005). Sulfobutyl fullerenes were found to be effective against environmental bacteria (Lu et al. 2010). Cationic fullerenes are found to be effective against a broad range of microbes in PDT (Sharma et al. 2011). Cationic fullerenes with quaternary amino groups are found to be most effective against *S. aureus* and *E. coli* with most resistance from *C. albicans* (Cataldo and Da Ros 2008).



**Fig. 14.2** Photodynamic therapy (PDT) of microbe-infected tissue using fullerenes. Microbe-infected tissues can be targeted using fullerenes that can specifically enter infected cells. In the presence of light, fullerenes, effective photosensitizers by nature, are excited to higher electronic states that react with oxygen to form reactive oxygen species that induce oxidative stress-induced bacterial cell death (**top**). The excitation states are shown in the boxed figure (**bottom**). Gray fullerenes represent unexcited or ground state fullerenes, and yellow-green fullerenes represent excited states.  $PS^1$  and  $PS^2$  are the two excited states that give rise to fluorescence and phosphorescence as they give off energy to return to the ground state ( $PS^0$ ). \* indicates ROS

#### 14.3.4 Fullerene Nanocomposites as Antimicrobial Agents

Alekseeva et al. found that the fullerene/polystyrene film had bacteriostatic properties versus *S. aureus*, *E. coli*, and *C. albicans* (Alekseeva et al. 2013). Duri et al. (2017) investigated the effect of combining fullerenes (C60 or hydroxyC60) with polysaccharides such as cellulose, chitosan, and  $\gamma$ -cyclodextrin. The results indicated that the  $\gamma$ -cyclodextrin/chitosan/fullerene film had considerable antimicrobial activity against vancomycin-resistant enterococci. Thus,  $\gamma$ -cyclodextrin and chitosan

with fullerenes in composite films can be used in food packaging. Ballatore et al. (2015) assessed the inactivation of microorganisms in electrogenerated porphyrin-fullerene C60 polymeric films. These films exhibited photocytotoxic features versus *S. aureus* and *E. coli*; in the irradiated films, the microbial population decreased by as much as 4 logs after 30 and 60 min for *S. aureus* and *E. coli*, respectively. Therefore, porphyrin-fullerene films feature a fascinating, flexible, photodynamic, active surface that can annihilate microorganisms.

## 14.4 Antimicrobial Properties Graphene, Graphene Oxide (GO), and Their Derivatives

Graphene is the simplest form of carbon, made of carbon atoms arranged tightly in a monolayer, essentially thin sheets of graphite. It has unique electrochemical properties like high thermal conductivity, inertness to chemicals, very hydrophobic nature, and optical transmittance. The other graphite family members include graphene oxide, reduced graphene oxide, and graphite oxide.

A comparative study of antibacterial activity among graphite (Gt), graphite oxide (GtO), graphene oxide (GO), and reduced graphene oxide (rGO) toward *E. coli* showed that GO showed maximum antibacterial activity. After GO, rGO, Gt, and GtO showed decreasing antibacterial activity (Liu et al. 2011). Graphene oxide nanostructures are formed by functionalization of graphene with hydroxyl, epoxy, or carboxyl groups (Sanchez et al. 2012). These hydrophilic functional groups along with hydrophobic graphenic regions allow for hydrophobic interactions in polar and hydrophilic solvents. Its large surface area also enables its use as an adsorbent or catalyst. The amphiphilic nature of GO makes them suitable for in vivo drug delivery of water-insoluble drugs and other applications in bioimaging and biosensing applications. Both graphene and graphene oxide are known for their antibacterial properties owing to two main properties: membrane damage and oxidative damage to bacterial cells. But recent studies have shown controversial results, indicating that other mechanisms may be responsible. In the coming sections, studies indicating the different mechanisms of action will be touched upon.

### 14.4.1 Graphene and Derivatives Cause Physical Damage to Microbial Membrane

Similar to carbon nanotubes, graphene and its derivatives show antimicrobial activity through direct contact and damage of cell membranes due to the sheets being sharp to penetrate membranes. This is why they are called nanoknives. Studies on both gram-positive and gram-negative bacteria showed that sheets caused membrane damage and consequent RNA efflux (Akhavan and Ghaderi 2010). Graphene

nanowalls, vertically standing graphene nanostructures, have also been found effective against *S. aureus* (Gurunathan et al. 2012). Damage of cell membranes by GO and rGO has been reported against *E. coli* (Hu et al. 2010). Another report suggests that apart from physical piercing of cell membrane, hydrophobic regions of GOs have been found to strongly interact with lipids on the cell membrane resulting in their extraction and further destruction of cell membrane. In *E. coli*, large amounts of phospholipids were found to be extracted by graphene nanosheets (Tu et al. 2013).

Interestingly a study in understanding GO uptake by *E. coli* through atomic force microscopy studies found that hydrophilic GO sheets face high-energy barrier before entry into the cell due to repulsive interactions with the outer cell membrane. This was thought to result in lower incidence of adhesion events possibly bringing into question the physical piercing of cell membrane as the mechanism of action of GO sheets (Romero-Vargas Castrillón et al. 2015). Nano-wrapping of bacteria by carbon nanowalls has been another proposed antimicrobial mechanism (Akhavan et al. 2011).

#### **14.4.2 GOs Cause Oxidative Damage to Microbial Cells**

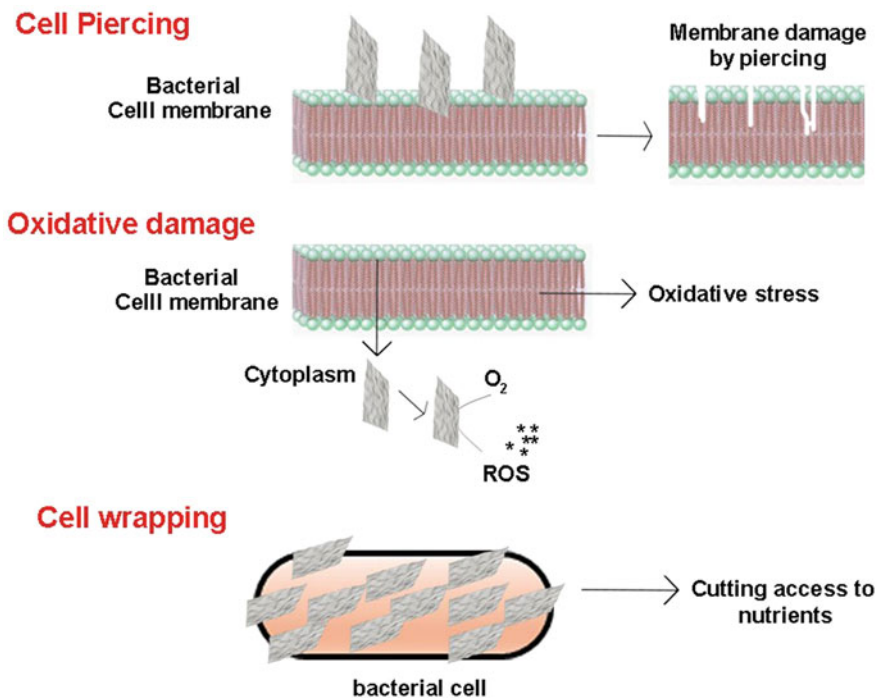
GO being an oxidated state of graphene has also been shown to induce cellular damage by oxidation of bacterial cell membrane components. But the type of oxidation event has not yet been clear due to contrasting reports. On the one hand, oxidative stress has been thought to be caused by generation of ROS and on the other hand, through nonsuperoxide-mediated oxidation of cellular components directly by GO (Fig. 14.3).

Study on *P. aeruginosa* evaluating the antibacterial activities of GO and rGO showed that the presence of these nanostructures induces significant production of ROS, leading to cell death, in a time- and dose-dependent manner of GOs (Gurunathan et al. 2012).

Another report showed that ROS formation did not take place in the presence of GO and rGO, but oxidation of glutathione added for checking oxidation events in vitro indicated that GOs may be oxidating cellular substrates in microbial cells to induce oxidative stress (Gurunathan et al. 2012; Vecitis et al. 2010; Kang et al. 2008).

#### **14.4.3 Size and Solubility of GO Determines Antimicrobial Activity**

Solubility plays an important role in determining the antimicrobial activity in GO sheets. Depending on solubility antimicrobial activity of GO changes. In the case of GO suspensions, antimicrobial activity is through cell entrapment mechanism (Das



**Fig. 14.3** Antimicrobial properties of GO sheets. Sheets of graphene oxide and derivatives use three mechanisms against microbes: (1) cell piercing due to the sharp edges; (2) oxidative damage to cells by production of ROS or direct reduction of cellular respiration contents; and (3) cell wrapping preventing access of microbes to nutrients

et al. 2011). This antimicrobial effect of GO increases with increasing sheet area and size. In other cases, the antimicrobial activity of GO is attributed to oxidative mechanisms, where decreasing sheet area or smaller size was found to increase antimicrobial activity by fourfold. The higher antimicrobial effect of smaller GO sheets is attributed to higher defect density of smaller sheets. The oxidative damage is also more permanent in nature, while cell entrapment is reversible (Perreault et al. 2015).

#### 14.4.4 GO Nanocomposites in Antimicrobial Therapy

Nanocomposites of GO with metal ions and other molecules have been shown to be effective antimicrobials. GO nanostructures, graphene oxide chlorophyllin and graphene oxide chlorophyllin Zn, were found to act against *E. coli*. Membrane damage was their proposed mechanism of action. Surface chemistry by way of hydrogen bonding of tetrapyrroles with cellular surface and metal toxicity due to

Zn<sup>2+</sup> leaching was thought to be additional antimicrobial mechanisms (Azimi et al. 2014).

#### 14.4.4.1 Metal Nanocomposites Most Effective Antimicrobial Agents

GO-Ag nanocomposites have shown effective antibacterial activity against bacteria, gram-positive and gram-negative (Perreault et al. 2015; Yun 2013). GO-Ag composites significantly reduced *E. coli* and *S. aureus* populations by up to 99.99% and 99.96%, respectively. Membrane penetration, oxidative stress, ROS production, and disruption of bacterial DNA replication were found to be responsible. These were thought to be dependent on temperature, time, pH, and concentration.

A bacteriostatic activity was observed against *E. coli* by GO-ZnO nanocomposites (Wang et al. 2014b). Although gold NPs themselves have antimicrobial properties against *E. coli*, addition of GO or rGO resulted in enhanced action against *S. aureus* and *B. subtilis* as well. The antibacterial activity of GO-Ag and GO-ZnO against *E. coli*, *Enterococcus faecium*, *S. aureus*, and *Klebsiella pneumonia* was assessed. GO-Ag was found to be more effective than GO-ZnO in terms of antibacterial activity (Whitehead et al. 2017).

Nanocomposite of rGO, CuO, and poly-L-lysine (PLL) showed high antimicrobial activity against *E. coli* and *S. aureus* with a lethality rate of 99.9% (Ouyang et al. 2013). GO-Au or rGO-Au composites show enhanced activity against microorganisms such as *E. coli*, *S. aureus*, and *B. subtilis* (Hussain et al. 2014). Irradiated (758 nm) nanocomposites have also been found to be effective antimicrobial properties. GO-Au, GO-Fe, and rGO-TiO<sub>2</sub>Au were found to work against gram-positive, gram-negative, and fungal microorganisms. GO-TiO<sub>2</sub> composites have been found to be cytotoxic to mammalian cells as well. They announced that this composite could damage the mitochondria, increase the number of lysosomes, and consequently disrupt and destroy the cell (Díez-Pascual 2020). rGO nanocomposites containing polyvinyl alcohol and Cu<sub>2</sub>O or TiO<sub>2</sub> have shown bacteriostatic activity against *Streptococcus oralis*, *S. aureus*, *E. coli*, and *P. aeruginosa* (Dhanasekar et al. 2018). TiO<sub>2</sub> particles and their nanocomposites have excellent potential to be used in food packaging applications. rGO-FeNP composites have shown bacteriostatic activity against *S. aureus*. These produce hydroxyl radicals inactivate vital cells and kill bacteria both in vivo and in vitro.

#### 14.4.4.2 Other GO Nanocomposites and Their Antimicrobial Properties

Studies on polymer-GO composites like polyurethane-rGO-polyethyleneimine and GO-polyurethane composites showed that polyurethane-rGO-PEI composite had higher antibacterial property and hence has practical application in biomolecule encapsulation or immobilization (Tang et al. 2016). Agarose has also been used as an antibacterial hydrogel for its bacteriostatic properties. GO along with cationic surfactants such as benzalkonium bromide can be used to produce novel GO

hydrogels for their antibacterial properties (Wang et al. 2015; Guo et al. 2020). The combination of polyvinyl-N-carbazole (PVC) and GO shows enhanced bacteriostatic activity against *E. coli*, *Cupriavidus metallidurans*, *B. subtilis*, and *Rhodococcus opacus* and few gram-positive bacteria (Carpio et al. 2012). For this reason, GO-PVC composites could be used to prevent biofilm formation.

GOs with surfactants like Tween are shown to be effective in inhibiting the bacterial growth (Szunerits and Boukherroub 2016). A composite with GO, LF, and chitosan enhanced antimicrobial activity of GO against bacteria (Nanda et al. 2015). GO-lysozyme composite showed excellent activity against *E. coli*. Nisin-G composites produced an active matrix that was used to identify, separate, and disinfect water contaminated with methicillin-resistant *S. aureus*. Due to the numerous properties of GO, they are under consideration for medical applications. One of the major challenges is its low biocompatibility. Functionalized GO are hence the solution for applicability.

## 14.5 Carbon Dots as Emerging Class of Photosensitizers for Antimicrobial Therapy

Carbon dots, also known as quantum carbon dots, are extremely tiny, quasi-spherical nanoparticles with diameter below 10 nm. They are a new class of carbon nanomaterials with enhanced photosensitizing properties. They have the ability of specific detection and inactivation of different bacterial species. Like other carbon nanoparticles, they possess chemical stability and outstanding photoelectric properties. Interestingly, they show high water solubility, low toxicity, and hence are known to be extremely biocompatible. They are easy to prepare and are affordable and hence ideal candidates for antimicrobial therapy. Discovered in 2004, CDs have been found to have applications ranging from use in semiconductors to biomedicine and agricultural applications (Li et al. 2012b).

Their photosensitizing properties have been exploited for testing their use in photodynamic therapy, like GOs. Bright carbon dots (Cipro@Cdots) synthesized using microwave assistance and gum arabic have been shown to be used to deliver broad-spectrum antibiotic nad ciprofloxacin hydrochloride into mammalian cells where release of antibiotic was regulated under physiological conditions. Release of ciprofloxacin was found to be extremely regulated under physiological conditions. Cipro@Cdots conjugate also exhibited antimicrobial activity against gram-positive and gram-negative microbes. CDs hence could be used as nanocarriers with abilities for controlled drug release, contributing to high antimicrobial activity. Table 14.1 summarizes the different types of nanoparticles described in this chapter and their mechanisms of action.

**Table 14.1** Summary of types of carbon nanoparticles and their antimicrobial mechanisms

Type of carbon nanoparticle (CNP)	Mechanism of action
<i>Carbon nanotubes</i> SWCNTs, MWCNTs, DWCNTs, and metal carbon nanotube composites	Physical damage of the cell membrane Oxidative stress by ROS production
<i>Fullerenes</i> Fullerene family of materials, C60 (common), and metal fullerene composites	Oxidative stress by ROS production Damaging cellular respiration directly
<i>Carbon nanosheets</i> Graphene, GO, rGO, GO polymer composites, and GO metal composites	Membrane piercing (physical damage) Oxidative stress by ROS production

## 14.6 Future Perspectives for the Use of Carbon Nanomaterials as Antibiotics: Advantages and Challenges

Nanoparticles by themselves or in conjugation with other molecules are the next-generation antibiotics due to their varied mechanisms of action against bacterial cells. Current antibiotics fail due to the fast rate of evolution of microbes against the mechanisms of action of antibiotics. This is especially true for gram-negative ESKAPE pathogens that are more resistant than others (Santajit and Indrawattana 2016). This resistance is therefore due to the biological warfare and continuous arms race between biological molecules. Hence the only way to put an end to this never-ending fight is the use of physical forces that bacteria cannot evolve against. This is largely absent in the current *in vivo* antimicrobial therapies. Carbon nanomaterials hence have an advantage due to their properties of piercing cell membranes and carrying out physical damage to microbial cells. Carbon nanotubes and graphene and its derivatives are very well known for this property and hence can be used as carriers with antibiotics or standalone for antimicrobial *in vivo* therapy.

One of the main issues in the fight against antimicrobial resistance is the lack of biotherapeutic molecules for use as antibiotics in the case of multidrug-resistant infections. Thus, combinatorial therapy with existing antibiotics can be carried out to generate new drug combinations. Combination of nanoparticles along with existing antibiotics show a better therapeutic strategy to combat multidrug-resistant infections, targeting different mechanisms of action against bacteria. This would also mean more combinations can be created in therapy to prevent the rise of resistant strains.

Non-toxic inert carbon nanoparticles are used as carriers of antibiotics to allow for more permeability into bacterial cells due to the lipophilic nature of carbon nanoparticles like fullerenes. This makes better drug permeability and availability and also allows for targeted drug delivery mechanisms.



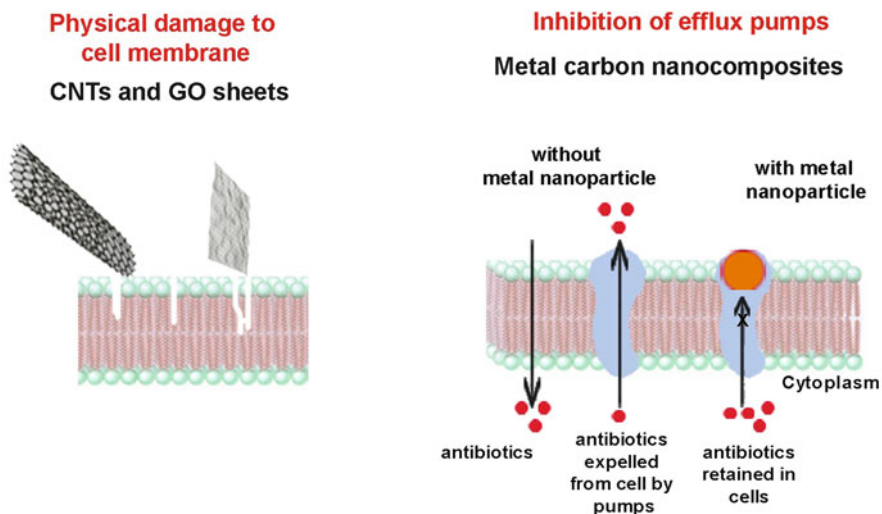
Graphene and metal nanocomposites of carbon nanoparticles have been shown to be very effective against multidrug-resistant bacteria due to their thermo-plasmonic properties. Therefore, antibiotic functionalized carbon nanoparticles could be used for photothermal lysis to increase the efficacy of antimicrobial therapy. Some composites of carbon nanodots have been shown to regulate the release of antibiotics as well depending on physiological conditions, allowing for controlled release of antibiotics *in vivo*.

One of the major factors in antibiotic resistance is efflux of antibiotics by the increased number of efflux pumps on membranes of resistant bacteria, affecting drug availability in the cellular interior. Carbon nanotubes and fullerenes have been shown to inhibit the activity of efflux pumps and components in bacterial membrane and metabolism, neutralizing the resistance mechanisms. This is thus a huge advantage of using nanoparticles in conjugation with antibiotics that will allow for more drug availability.

Although antibacterial effects of carbon nanoparticles have been studied for two decades, only few have come into mainstream treatment. The three main reasons for it are the lack of comprehensive understanding of cytotoxicity of nanoparticles on biological systems, selection of narrow size range of nanoparticles, and emerging news of resistance against nanoparticles that have mechanism of action other than physical damage. Cytotoxicity of carbon nanoparticles essentially is imparted by chemical treatments carried out during their preparation to arrive at the right size range as well as to functionalize them for use in biological applications. Although toxicity of various carbon nanoparticles and their functionalized derivatives have been studied in mammalian and microbial cells, and even mice, there needs to be studies carried out in higher mammalian models to understand accumulation of carbon nanoparticles (CNP) in tissues, organs, their half-life and clearance, and potential long-term effects. The antimicrobial mechanisms of CNPs effective against the evolving microbes are summarized in Fig. 14.4.

Synthesis of carbon nanoparticles is fairly a complex process and extremely sensitive to various factors, including choice of precursors and method of synthesis to temperature and chemical environment. This makes their synthesis unpredictable as getting a narrow range of size of CNPs in nanoscale is a challenge, consistently, even with the same production method. Even if synthesis is achieved, slight variations may cause changes in their antimicrobial properties which need to be tested every time, unlike drugs that will behave similarly if a particular SOP is followed for its synthesis. Therefore, carbon nanoparticles used in therapy would require exhaustive structural and functional characterization studies.

Non-toxic carbon nanoparticles have been in use for nano-delivery and sustained release of antibiotics, to counter-attack toxicity from antibiotics themselves. Polymer-based and nonpolymeric nanoparticles as liposomes are used for nano-delivery for the treatment of infectious diseases (Zazo et al. 2016). There are now commercially available carbon nanoparticles used for treatment. Respiratory infections are generally difficult to treat and have longer treatment time that leads to systemic side effects. Broad-spectrum antibiotics like ciprofloxacin used for lung infections are now commercially available as a liposome formulation such as



**Fig. 14.4** CNP mechanisms effective in combating the evolving microbial defense. Microbes develop antimicrobial resistance to antibiotics by either evolving enzymes that breakdown membrane-targeting antibiotics or evolving transmembrane efflux pumps that pump out antibiotics from cell interior reducing drug availability. Carbon nanoparticles in combination with traditional antibiotics are advantageous as CNPs by themselves can carry out physical damage and prevent efflux of antibiotics by blocking bacterial membrane pumps

Lipoquin™, designed as an inhaled formula that releases drug in 24 h, eliminating the systemic effects of the high-dose antibiotic (Cipolla et al. 2016). Antifungal liposomal carrier AmBisome® (amphotericin B) reduces toxicity of amphotericin B and hence is used for highly immunocompromised patients with HIV infection and disseminated histoplasmosis (Meletiadiis et al. 2008). Nano-drugs containing silver nanoparticles are also used in biosensors and medical devices like venous catheters to prevent biofilm formation (Wu et al. 2015; Wang et al. 2017). Other nanoparticles used in diagnosis or as medical devices include Endorem™ SPIONs, Verigene®, Silverline®, and Acticoat™ (Beal et al. 2013).

Recent reports indicating resistance against nanomaterials show an emerging threat against the use of nanomaterials as antibiotics. These concerns have arisen due to the widespread use of silver nanoparticles in consumer products as well as healthcare settings. Studies have shown the possibility of *Acinetobacter baumannii*, an opportunistic pathogen, causing nosocomial infections, to become resistant to silver nanoparticles. This raises concern over the use of other nanoparticles, especially those with heavy metal composites in antimicrobial therapy. Further studies are required to understand and combat these challenges before carbon nanoparticles could be deployed as antibiotics for the next generation therapeutics.

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**Conflict of Interest** None to declare.

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