Chapter 12
Applications of Carbon-Based Nanomaterials for Antimicrobial Photodynamic Therapy

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12.1 Introduction

The discovery of antibacterial agents is found to be the most significant development of the twentieth century in healthcare sector and has become a pioneer in current medicine in reducing the percentage of folds mortality due to bacterial diseases. In earlier periods, specifically at the time of the pre-antibiotic era, bacterial infections were found to be a major threat to humans, where the case of mortality rate for the infections caused by *Streptococcus pneumoniae* and *Staphylococcus aureus* claimed as high as 40% and 80%, respectively. Furthermore, amputation was the only option to treat the wound infection before the emergence of antibiotics; the report said that nearly 70% of amputations were exhibited during World War I as an impact of wound infections (Friedman et al. 2016). Antibiotics have facilitated the relief to the patients with bacterial infections, which drastically changed the way to treat and cure such deadly bacterial diseases. Nevertheless, antibiotics stood as supporting pillars in the advancement of modern medicine like complex surgery, transplantation, and chemotherapy. Unfortunately, the spread of bacterial pathogens with multidrug resistance to antibacterial agents found to be a major threat and significant challenge for the current treatment modalities (Sharland et al. 2015).

Development of the resistance by bacteria could be achieved either by mutation or accepting mobile genetic elements that hold resistant genes. It could occur due to the consequences of indiscriminate use of drugs which created selective pressure to the bacteria. There was a driving force behind the gradual increase in the rate of drug resistance due to the misuse of antimicrobial agents, either prescribed to the

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patients or deposited into the environment (Roca et al. 2015). Furthermore, food chain is found to be another important cause for the development of multidrug-resistant bacteria, which subsequently alters both the commensal and pathogenic bacteria in humans through dietary contact (Fig. 12.1) (Liebana et al. 2013; Tetro 2018). In a generalized view, the bacterial infections that are caused by multidrug-resistant strains contribute comparatively greater adverse effects than similar infections caused by susceptible bacterial strains. The adverse effects cover both clinical such as death or failure of treatment and economic aspects, including cost of treatment and duration of the hospital stay during the bacterial infection treatment process. The hardships of these adverse effects have been pronounced in several ways including disease severity and strain virulence (Chaudhary 2016). There was a continuous encountering of challenges in the prevention of bacterial diseases with the current situation where there is a demand for effective drug to combat the antibiotic-resistant bacterial infections. Though few new antibiotics are in the clinical pipeline, the situation seeks for the development of different novel therapeutic alternatives (Frieri et al. 2017).

In the recent years, understanding the virulence strategies and molecular identification of the disease increased significantly, which provided a novel path where virulence-associated traits of the pathogens can be targeted. This approach has been greatly appreciated due to their mechanism of targeting pathogenic factors or virulence traits rather than providing selective pressure on the pathogen to develop resistance (Hancock et al. 2012). As a result considerable efforts have been rendered to formulate drug molecules that invade the activity of the pathogenic factors which arrest pathogens until the activation of host immune system to kill the pathogens. The frequent administration of antipathogenic drugs could not show any sign of the resistance, unlike conventional antimicrobial agents, because they do not directly show adverse effects on the growth of pathogens and therefore prevent the development of resistant bacteria (Hauser et al. 2016). The bacterial toxins play a major role in the pathogenicity of the bacteria. The bacterial toxins were synthesized inside the

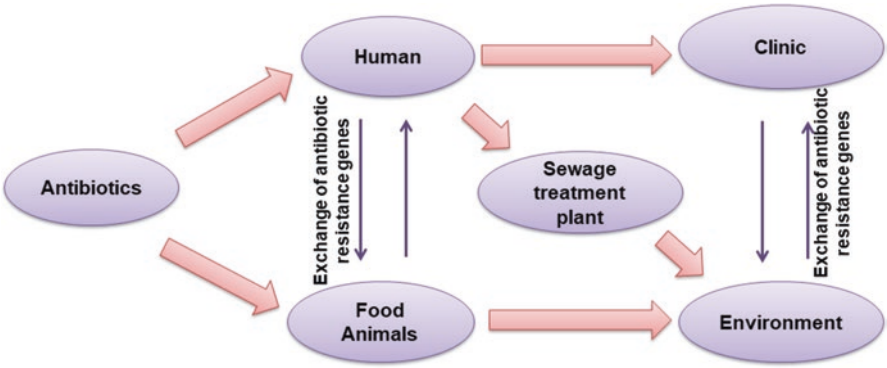

Fig. 12.1 Different modes of antibiotic resistance spread between people, animals, and the environment

cytosol, which need to cross the bacterial cell wall before they invade into the host cell. To accomplish this, the bacteria evolve with several numbers of secretion systems that facilitate the transport of the bacterial toxins to the surrounding environment. Recently, a drug has been developed to inhibit the type 3 secretion system as it is widely accepted as a potential drug target to develop anti-pathogenic drugs (Marshall and Finlay 2014). Furthermore, several leading approaches were formulated often to expand the efficacy of the antipathogenic agents because bacteria continuously alter their pathogenic mechanism; hence, the development of drug-targeting virulent traits may enhance the probability of success in the antimicrobial drug development process.

Besides the bacterial toxins, bacterial biofilm formation is a major pathogenic factor that often occurs on the inert surfaces like catheters or prosthetic joints and even in human body parts including heart valves and teeth (Khameneh et al. 2016). The biofilms are the physiological states of the bacteria found difficult to eradicate since the extracellular matrix prevents the penetration of antimicrobial agents into the biofilms (Smith 2005). The steps to eradicate this organization of cells demand significantly greater concentration of drugs for persistent periods, and these efforts have often failed due to the prolonged period of the infections. Apart from the clinical complications and treatment limitations, biofilms can also be considered as a major source of infection, which are observed in the medical apparatus (Frieri et al. 2017). The constant research in science provides a better understanding about the biofilm formation and the factors required which provide insights for the development of novel therapeutic approaches to prevent biofilm formation and also for the destruction of mature biofilms (Brooks and Brooks 2014). As the results are not promising, new strategies were reported with significant activity; however, very few of them are in the pipeline for the clinical testing (Teh et al. 2018).

Currently, an impressive approach, the inhibition of bacterial quorum sensing, has been found to be a promising alternative strategy to combat the biofilm-forming bacteria, since bacterial quorum sensing (QS) plays a significant role in the formation of biofilms (Li and Tian 2012). Moreover, the small diffusible signaling molecules in the bacterial QS participate as switching regulators that promote the planktonic bacterial cells to form biofilms. Hence, targeting these signaling molecules could be considered as a promising method to prevent infection from the biofilm-forming bacteria. Similarly, the target could be focused on the factors that facilitate bacteria in biofilms to develop resistance to antibacterial drugs, which might render a situation where biofilms can be sensitive to antibacterial drugs. In addition, other novel methods like phage therapy (Fu et al. 2010; Harper et al. 2014) and modulation of the microbiome (Biliński et al. 2016; Crow et al. 2015) have been reported as alternative approaches for the conventional antibiotics to prevent the bacterial infections and minimize the development of resistance to antibacterial drugs.

Resistant strains are developing rapidly with certain pathogenic isolates such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and members of the family *Enterobacteriaceae*, for example, *Klebsiella pneumoniae*, *E. coli*, and *Proteus* sp. (Basak et al. 2016). Among various novel antimicrobial

approaches, as discussed above, antimicrobial photodynamic therapy (aPDT) has become a potential alternative against various microorganisms. Antimicrobial efficacy of photodynamic therapy was described by Oskar Raab in 1900, who observed reduction in the viability of *Paramecium caudatum* upon light exposure in the presence of acridine dye. Though Oskar Raab introduced this technique 30 years before penicillin discovery, research on antimicrobial photodynamic therapy (aPDT) was limited after the discovery of antibiotics. However, currently, due to the rapid increase in the multidrug-resistant strains, aPDT is extensively studied and used in the treatment of localized infections (Oruba et al. 2015).

12.2 Antimicrobial Photodynamic Therapy

Photodynamic therapy involves the use of mainly three components to kill undesired microorganisms: a nontoxic light-sensitive compound (photosensitizer), a visible light source, and oxygen (Wong et al. 2005). aPDT is an oxygen-dependent photochemical reaction that occurs upon light-mediated activation of a photosensitizer compound which leads to the generation of cytotoxic reactive oxygen species or singlet oxygen (Garcez et al. 2011). On exposure to light of a specific wavelength, the photosensitizer absorbs energy and undergoes transition from a low-energy ground state to an excited singlet state. In this excited singlet state, the boosted electron in the higher energy orbital still maintains its opposite spin. But this state is short-lived (nanoseconds) and subsequently loses its energy by fluorescence or internal heat conversion. The excited singlet-state PS can also invert its electron to parallel spins by a process called intersystem crossing. The change of electron to parallel spins results in the formation of an excited triplet-state PS which is a long-lived state (microseconds). The triplet-state PS can transfer its energy in two different pathways: type I and type II photoprocesses, both of which are oxygen dependent (Robertson et al. 2009). Type I reaction involves electron transfer from the excited triplet-state PS to organic substrates of the cell resulting in the production of free radicals which are highly reactive and react with molecular oxygen to generate reactive oxygen species (ROS) like superoxide, hydroxyl radicals, and hydrogen peroxide. These reactive oxygen species are cytotoxic in nature which cause damage to cell membrane integrity and permeability. In type II pathway, the excited triplet-state PS transfers its electron to a ground-state molecular oxygen (triplet state) that turns into a highly reactive singlet oxygen. This species oxidizes many compounds like nucleic acid, proteins, and lipids leading to oxidative damage of the cell wall (Hamblin and Hasan 2004). Both the mechanisms target mainly biomolecules like amino acids, unsaturated lipids, and purine and pyrimidine bases of nucleic acids (DNA/RNA). Since aPDT targets a wide range of biomolecules at a time, it is considered as a broad-spectrum antimicrobial therapy. This broad-spectrum activity gives advantage to aPDT over antibiotics as it is difficult for microbes to gain resistance against wide targets of aPDT at a time (Sperandio et al. 2013).

12.2.1 Mechanisms of aPDT

Photodynamic therapy is a nonthermal photochemical reaction based on the excitation of a photosensitizer upon exposure to visible light of a particular wavelength. Delivery of light with appropriate wavelength to a PS leads to absorption of photon resulting in the transformation of PS to an excited singlet state (^{1}PS) from ground state (^{0}PS). The reason behind this excitation is electron transfer from the highest occupied molecular orbital to one of the unoccupied molecular orbital. This state is called the excited singlet state where an opposite electronic spin is maintained as in the ground state. The excited singlet state is a short-lived state (nanoseconds), and the molecule can revert back by releasing the absorbed energy via fluorescence or by interconversion where energy lost is in the form of heat (Oruba et al. 2015). From the excited singlet state, it can change to the excited triplet state by spin conversion where it forms a parallel spin (intersystem crossing). The excited triplet state has lower energy than the singlet state but is stable and long-lived (micro to milliseconds). This triplet-state molecule can also release energy and revert back to the ground state through phosphorescence or through a nonradiative pathway. There is also another mechanism by which it can transfer its energy to the surrounding molecules (Fig. 12.2). Based on the molecules to which they transfer energy, there are two different types of PDT—type 1 and type 2 (Sudhakara et al. 2012).

Type 1 reaction involves generation of superoxide and hydroxyl radicals. The excited triplet-state molecule transfers its electron to the surrounding substrate molecules in the cell and produces radical anions or radical cations which may further react with the oxygen molecule to produce ROS. ROS damage the cell through the formation of oxidative stress. Type 2 pathway is based on the production of highly reactive and toxic singlet oxygen ($^1O_2$) through direct transfer of energy from the

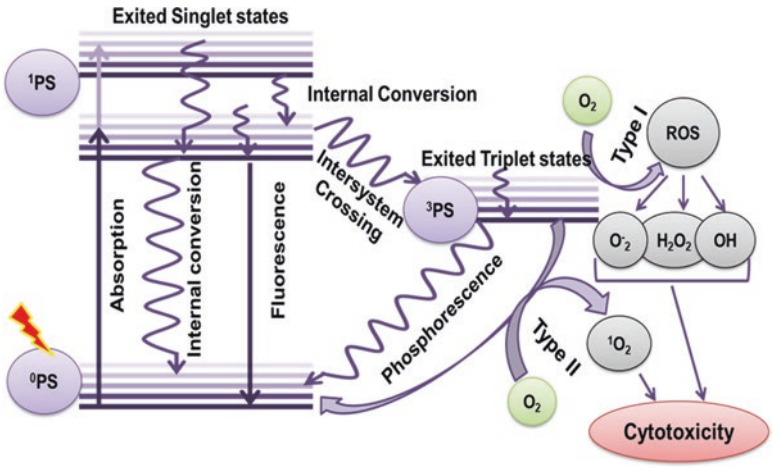

Fig. 12.2 Schematic illustration of mechanism involved in antimicrobial photodynamic therapy

triplet-state molecule to ground-state oxygen molecule (in triplet state) thereby converting it to reactive toxic singlet-state oxygen (Spagnul et al. 2015). All these ROS generated have the ability to target multiple sites at a time. They kill cells by damaging nearly all types of biomolecules (nucleic acid, protein, and lipids) which prevent microorganisms from developing resistance against aPDT making it as a promising and successful alternative to antibiotics (Hamblin 2016).

12.2.2 Recent Scope of aPDT

The recent research on aPDT is focused on multidrug-resistant pathogenic bacteria which readily form biofilms and cause dental caries, chronic wound, and skin infections. The efficacy of antimicrobial and antibiofilm effects of PDT on *Streptococcus mutans*, a cariogenic bacterium, playing an important role in dental caries was studied. The study was carried out with toluidine blue as a PS and diode laser as a light source, resulting in a significant reduction of biofilm formation (63.87%) compared to control. This can be used as an alternative methodology for treating oral diseases which is comparatively advantageous to chemical or mechanical or antiseptic agents as these methods would cause inactivation of normal microbiota and mechanical damage to oral mucosa and promote development of drug-resistant strains (Beytollahi et al. 2017). One of the most important pathogen causing clinically relevant infections within immunocompetent patients is *S. aureus*. Several studies of aPDT were performed to inactivate biofilm producers, both *S. aureus* and MRSA. An effective disruption of *S. aureus* biofilm in compact and cancellous bones (in vitro) by aPDT using 660 nm diode laser with methylene blue dye was reported. Results indicated a 10 log reduction of *S. aureus* biofilm in compact and cancellous bones compared to control group (Rosa et al. 2015).

Application of aPDT was also studied in the case of prosthetic joint infection (PJI) caused by the biofilms of MRSA and *P. aeruginosa* on prosthetic implants. PJI has become a public health concern, and due to the biofilm formation by multidrug-resistant strains, a broad-spectrum nonantibiotic antimicrobial treatment is required which is fulfilled using aPDT. The therapy was conducted with the help of a novel PS RLP068/Cl under diode laser exposure. A significant decrease in biomass volume of both *P. aeruginosa* and MRSA strains was observed, indicating the successful antimicrobial and antibiofilm activity of aPDT with RLP068/Cl compared to antibiotic treatment (Vassena et al. 2014). Studies on aPDT have been reported using natural PS like curcumin which has proven to be nontoxic to a number of cell cultures and animal tissues. But the phototoxicity of curcumin is well-known and can be used as a PS in aPDT to treat localized superficial infections on skin and mouth. Biofilms of cariogenic bacteria like *S. mutans* and *L. acidophilus* were subjected to aPDT, and their enhanced biofilm disruption due to phototoxic effect of curcumin under a light-emitting diode (LED) of 450 nm was analyzed (Araújo et al. 2012).

12.2.3 Challenges in aPDT

Several studies on aPDT in vitro have reported the efficient killing of bacterial cells by the combination of PS and an appropriate wavelength of light. However, many of the potent PSs are having limitations such as low solubility in aqueous medium and tendency to aggregate. A decreased solubility results in a reduced uptake of dye by cells, i.e., bioavailability and aggregation effect efficiency of PDT (Hegge et al. 2010). Moreover, long-term storage and light exposure can also lead to degradation of PSs. Hence, to enhance the permeability and retention effect of PS, there should be a vehicle to carry PS into an appropriate target. The effectiveness of PDT depends on many factors like the degree of ROS production, concentration of PS, targeted delivery, and uptake (Rout et al. 2016).

12.3 Nanomaterial in aPDT

Although the mentioned methods are found to be the novel and alternative ways to eradicate the bacterial infection, still there is a possibility that bacteria could be able to establish a resistance mechanism to these methods. Because bacterial pathogens are evolving at a greater speed, this facilitates the bacterial species to gain resistance against antibacterial drugs by several mechanisms including modification in membrane permeability, development of the multidrug efflux pumps, degradation of the drug by enzyme, self-mutation, and covalent alteration of antibiotic molecules which result in the inactivation of antimicrobial properties (Pelgrift and Friedman 2013). These factors make the situation much complicated, indirectly boosting the resistance mechanism in pathogenic bacteria. The recent development in nanobiotechnology mediates the current research to focus on these complications to provide a proper solution for these problems, potentially elevating the efficacy of the available antimicrobial drugs by using nanoparticles as the delivery system to achieve targeted therapeutic practice (Zhu et al. 2014). In the last decade, nanobiotechnology has arrived at a magnificent growth that provides several applications in the healthcare sector. Nanomaterials are observed as multitasking agents in disease management programs, where they could appear as promising antimicrobial agents, predominantly against multidrug-resistant bacteria (Table 12.1). Furthermore, the nanomaterials were extensively used as drug carriers that could deliver the traditional antibiotics to the precise site, which promote the target-specific therapeutic approach. In addition to the several applications of nanoparticles, they are also used as promising tools in the diagnosis of infectious diseases. Currently, several nanosystem-based diagnostic tools are available to precisely detect the infectious organisms at the early stage of the infection. For example, nano-based diagnostic tools are developed to specifically detect disease-causing agents or to distinguish Gram-positive and Gram-negative microorganisms. As an advancement of this approach, a nano-ranged diagnostic tool has been developed and applied in vital

Table 12.1 List of different carbon nanomaterials and their antimicrobial efficacy

S.no.	Carbon nanomaterial	Size	Application	Targeted microorganism	Activity	References
1.	Fullerenes	10–25 nm	Antibacterial agent	*Bacillus subtilis*	Antibacterial activity	Lyon et al. (2006)
		2–200 nm	Antibacterial agent	*Escherichia coli*	Antibacterial activity	Deryabin et al. (2014)
		122–295 nm	Antibacterial agent and nanocarrier	Bacteriophage	Antibacterial and antiviral	Dostalova et al. (2016)
2.	Graphene and graphene oxide	100–200 nm	Antibacterial agent	*E. coli*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Klebsiella pneumoniae*	Antibacterial activity	Whitehead et al. (2017)
		24–4923 nm	Antibacterial agent	*E. coli* and *S. aureus*	Antibacterial activity	Gao et al. (2017)
		–	Antibacterial agent	*E. coli*	Antibacterial activity	Sharma et al. (2018)
3.	Carbon nanotubes	1–5 μm (length)	Antibacterial agent	*Salmonella enterica*, *E. coli*, and *E. faecium*	Antibacterial activity	Dong et al. (2012)
		–	Antimicrobial agent	*S. aureus*, *E. coli*, and *Candida tropicalis*	Antimicrobial activity	Venkatesan et al. (2014)
		40–60 nm (diameter) 5–15 μm (length)	Antibacterial agent	*Methylobacterium* spp.	Antibacterial activity	Choi et al. (2014)
4.	Carbon quantum dots	5–6.5 nm	Antibacterial agent	*E. coli* and *B. subtilis*	Antibacterial activity	Travlou et al. (2018)
		5 nm	Antibacterial agent	*S. aureus*, *B. subtilis*, and *E. coli*	Antibacterial activity	Li et al. (2018)
		10 nm	Antibacterial agent	*S. aureus* and *E. coli*	Antibacterial activity	Roy et al. (2015)

detection, which, along with the available analytical technology, is gaining significant attention due to its superiority (Colino et al. 2018). The overall explanation summarize, though nanoparticles contributing several applications, two of them are highly consider as promising including application of inorganic and organic nanomaterial as potential antimicrobial agents and nanomaterial based drug delivery system. The nanomaterials like metallic nanoparticles, carbon nanotubes, fullerenes, cationic peptides, or polymeric nanoparticles and chitosan nanoparticles were being considered that they were inherent with antimicrobial properties and used

widely in antimicrobial applications. On the other hand, the nanomaterials including liposomes, solid lipid nanoparticles, polymeric nanoparticles, silica nanoparticles, and gold nanoparticles are extensively employed as potential drug carrier systems (Zaidi et al. 2017; Prasad et al. 2017).

Among the enlisted nanomaterials, carbon-based nanomaterials have gained a lot of attention due to their exceptional structural characteristics, physical properties, tunable morphologies, relatively good biocompatibility, and eco-friendly nature. The emergence of functionalized carbon-based nanoparticles provided a new class of nanocarriers for the effective delivery of drug molecules to the appropriate sites of infections (Ibrahim et al. 2018). The application of these nanomaterials as a delivery system is widely accepted as a promising approach, since they potentially mobilize the therapeutic molecules into the bacterial cytoplasm (Arias and Yang 2009). Interestingly, these nanocarriers could be formulated with one or more bioactive molecules like therapeutic compounds, antimicrobial peptides, biosensing proteins, and nucleic acid which subsequently render the antimicrobial agents to the cells or organs (Shvedova et al. 2008). The surface functionalization on the carbon nanomaterial prevents the attachment of unwanted absorption and desorption processes. The unwanted absorption that is mentioned in this case is the adherence of substances from the biological system to the carbon nanomaterial at the time of drug administration which would minimize the original effect of the nanoformulated drugs and have greater impact on their release profile and target specificity (Mocan et al. 2017). Comparing to other nano-based therapeutic approaches, carbon-based nanoparticles are found to be more effective and cost-effective. For example, the preparation of carbon nanomaterial with antibiotics is less costly than the preparation of the nanomaterial, liposome, with antibiotics. Additionally, in both in vitro and in vivo studies, the antibiotic that was administered along with the carbon nanocarrier was found to be more effective than the free antibiotic (Prajapati et al. 2011).

It is apparent that carbon is one of the most abundant elements on the Earth's crust, and it possesses the ability to couple with other carbons to differentiate into several allotropes of carbon. This characteristic feature of carbon provides the possibility to drive several varieties of carbon nanomaterials that include fullerene, nanodiamonds, carbon dots, graphene quantum dots, and carbon nanotubes (Maas 2016). The nature of the carbon nanomaterial was altered by different hybridization states of carbon, for example, carbon nanotube, fullerene, and graphene are made with sp^2-hybridized carbon atoms. Similarly, nanodiamonds are composed of sp^3-hybridized carbon atoms; likewise, carbon dots and graphene quantum dots have mixed sp^2- and sp^3-hybridized carbon along with defects and heteroatoms. Though the orbital hybridization of all the carbon nanomaterials was found to be more likely similar due to their change in the dimensions, each carbon nanomaterial showed difference in their antimicrobial activity and mechanism of action (Xin et al. 2018). Interestingly, the similar dimensional carbon nanomaterials show change in the antimicrobial activity when their other parameters such as size, shape, number of layers, charge of the particles, and the surface material on the carbon nanomaterial have been altered. The specific physicochemical properties of the carbon nanomaterial could be mainly dependent on the method that is followed for the preparation of that particular carbon nanomaterial (Maleki Dizaj et al. 2015).

12.4 Carbon-Based Nanomaterial in aPDT

Due to the development of antimicrobial-resistant strains over the last decades, the need to design novel and efficient antimicrobial agents resulted with the integration of nanotechnology. The rise in the drug resistance among microorganisms has led to the discovery of potential and alternative therapeutic strategies including antimicrobial photodynamic therapy (aPDT). As discussed above, aPDT can be enhanced by the use of nanoparticles either as an antimicrobial agent or for the effective delivery of the photosensitizer (Perni et al. 2011). Among the different groups of nanoparticles available, carbon-based nanomaterials are of great interest and effective in aPDT. The efficient and potential use of carbon nanomedicine in aPDT is attributed to their excellent optical properties, good biocompatibility, less toxicity, and excellent mechanical strength. These carbon nanomaterials and their derivatives gained significant interest in several fields due to their excellent physical and chemical properties. They are widely used in thin-film transistors, photovoltaics, electrodes, supercapacitors, drug delivery, tissue engineering, and photothermal therapy (Al-jumaili et al. 2017).

Carbon nanomedicine is an emerging field which combines antimicrobial photodynamic therapy for the treatment of notorious pathogens. These nanoparticles are employed in PDT either as photosensitizers or drug vehicles. The ability of the carbon-based nanomaterials to carry photosensitizers or to exert antimicrobial action can be enhanced by the functionalization methods. Covalent and noncovalent modes of functionalization strategies are available which render the efficient application of carbon in nanomedicine (Albert and Hsu 2016). The covalent functionalization strategies include the conjugation of polymers to NPs using polyethylene glycol and hydrophobic functional groups using carboxyl and hydroxyl groups. The covalent functionalization also targets ligands for effective conjugation with carbon-based nanoparticles. Weaker interactions such as van der Waals forces, hydrophilic interactions, hydrophobic interactions, electrostatic forces, and pi–pi stacking are the noncovalent functionalization strategies. Among these methods, covalent functionalization produces more stable carbonaceous nanoparticles due to their ability to form covalent linkages between NPs and biomolecules (Chen et al. 2012; Zhu and Xu 2010). Carbonaceous nanomaterials such as carbon nanotubes, fullerenes, graphene oxide, carbon nanodots, etc. are widely used as photosensitizers or employed to improve the delivery of PS in aPDT (Fig. 12.3).

12.4.1 Carbon-Based Nanomaterials and Their Properties

Carbon-based nanomaterials exhibit high microbicidal activity along with their photosensitizing capacity. The antimicrobial properties of carbon-based nanoparticles are dependent on their small size and high surface-to-volume ratio with an excellent interaction with bacterial pathogens. As the size and surface area of

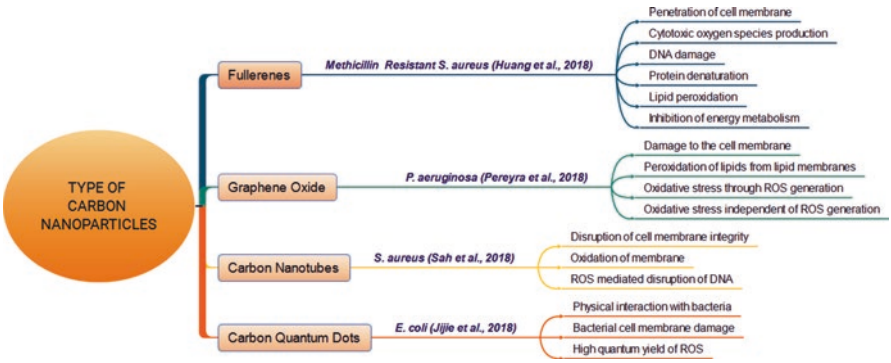

Fig. 12.3 Different types of carbon nanostructures employed in antimicrobial photodynamic inactivation

nanoparticles are the important parameters affecting microbicidal action, other factors such as the type of target microorganisms, functionalization yielding surface modification, intrinsic properties, and composition of carbon-based nanomaterials also matter (Buzea et al. 2007; Kang et al. 2008). The death of bacteria is mainly due to the physical interaction of bacteria rather than the phototoxicity. The carbon-based nanomaterials which make contact with the bacterial membrane cause the production of free radicals and lead to the oxidative death of cells. The oxidative stress of bacteria is enhanced by the use of photoactivated carbon nanoparticles (Manke et al. 2013; Abrahamse et al. 2017). Briefly, the antimicrobial action of carbon-based nanoparticles can be grouped as physical and chemical mechanisms. The interaction with microorganism and significant structural damage are known to be the physical mechanisms of bactericidal action. Chemical interaction leads to the production of reactive oxygen species or cause an ROS-independent cell death due to the electron transfer from the outer surface of the microorganism (Li et al. 2015). Other advantages of carbonaceous nanomaterials in aPDT include easy design, synthesis, fast elimination, less cytotoxicity, and good penetration (Abrahamse et al. 2017).

12.4.2 Fullerenes

Researchers are engaged in the design of new and efficient photosensitizers in order to enhance the efficacy of aPDT. Fullerenes came into field due to their high drug carrier or loading capacity. Fullerenes have a unique structure with biologically inert molecules of 60–100 carbons arranged in a soccer ball shape. The fullerenes are characterized by the extended and conjugated molecular orbitals (Constantin et al. 2010). Fullerenes have huge applications in nanoelectronics, nanocomposites, and drug delivery because of their tensile strength, high electroconductivity, and unique optical and thermal properties. C_{60} and C_{70} fullerenes are insoluble in water

and made soluble by preparing their derivatives. These fullerene derivatives especially of C_{60} have shown strong antibacterial effects on prokaryotes through the direct oxidation of cells (Li et al. 2008). The antimicrobial properties of nano-C_{60} fullerenes were studied previously on laboratory bacteria, *E. coli* and *B. subtilis* (Yacoby and Benhar 2008). Nano-C_{60} has UV/visible absorption properties in solutions which cause the generation of free radicals. Some of the fullerenes showed the ability to cause free radical damage to bacterial cells. Free radicals-induced damage to bacteria was marked by lipid peroxidation and DNA damage (Nakanishi et al. 2002).

One of the fullerene derivatives, fullerol, showed higher phototoxicity toward MS2 bacteriophage than other nanoparticles. MS2 bacteriophage was efficiently inactivated by UVA light-activated fullerol (Badireddy et al. 2007). In a study, it was showed that carbonaceous nanoparticles exhibited more photoinactivation than TiO_2 as they produce more singlet oxygen (Brunet et al. 2008). Fullerenes show a large absorption in the visible light and good quantum yield in the triplet electronic states. Different functionalizations using hydrophilic and amphiphilic groups or fused ring structures render the fullerenes with water solubility and protect from aggregation. The attachment of groups which provide them cationic charges offers several roles of broad-spectrum bacterial targeting and water solubility. This also imparts them with the ability to produce higher amounts of free radicals such as superoxide anion, hydroxyl radicals, and singlet oxygen for effective aPDT. Fullerenes have recently gained interest in the research of photodynamic treatment for infections as they possess higher degree of photostability compared to traditional tetrapyrrole ring-based PSs (Mroz et al. 2007). Generally the absorption range of fullerenes is in the range from UVA (360 nm) to red (635 nm), which makes them effective candidates for aPDT (Mizuno et al. 2011; Sharma et al. 2011). Yin et al. (2015) investigated the aPDT of broad-spectrum microbial strains using new decacationic fullerene derivatives such as $C_{60}[_4M(C_3N6þC_3)_2]–(I)_{10}$(LC14), $C_{60}[_4CPAF–(MN_6þC_3)_2]–(I)_{10}$ (LC15), and L16 (malonate bisadduct derived from C14), illuminated with white light and UV light. They showed that the LC15 derivative was the most powerful broad-spectrum antimicrobial PS followed by LC16. LC14 was found to be with the least antimicrobial action. The molecular mechanism behind the enhanced ROS production was through type 1 electron transfer method which increased the ability of monoadduct fullerene derivative to kill microorganisms (Yin et al. 2015).

The mechanism of fullerenes and their derivatives under irradiation includes the penetration of cell membranes and destruction of nucleic acids, proteins, and lipids resulting in strong antibacterial, antiviral, and antioxidant activities. The photodynamic inactivations of viruses using photoactivated fullerenes are widely studied. Rud et al. (2012) suggested the use of photoactivated C_{60} fullerenes for the photoinactivation of mosquito-iridescent virus (MIV) *Aedes flavescens* under 30 min of illumination in biological systems. The photoexcited C_{60} reduced the infectious viruses by 4.5 lg ID_{50}/mL units (Rud et al. 2012).

Photoactivated fullerenes, functionalized with three dimethyl pyrrolidinium groups (BF6) using white light, cured fatal infections caused by *Proteus mirabilis*

and *Pseudomonas aeruginosa* in mouse wound models (Lu et al. 2010). Recently, water-borne bacteria *Enterococcus faecalis* was photoinactivated by C_{60} and its derivative immobilized onto a macroporous silicone (a support polymer) (Manjón et al. 2014). Aoshima et al. (2009) used water-soluble fullerenes encapsulated into different carriers and three types of fullerenols for the photoinactivation of *Propionibacterium acnes*, *Staphylococcus epidermidis*, *C. albicans*, and *Malassezia furfur*. In the study fullerenols showed stronger activity against fungi than bacteria which further explains that water-soluble nanoparticles could interact more with the fungal cell wall components (β-glucan and chitin) than the peptidoglycan layer of the bacterial membrane (Aoshima et al. 2009). The possible targets of fullerenes include cell membrane, lipids, and DNA. The damage to the biological constituents is either caused by type I or type II mechanism. Recent reports are available with the effective broad-spectrum antimicrobial photodynamic therapy using fullerenes functionalized with methylpyrrolidinium groups. Grinholc et al. (2015) employed C_{60} functionalized with methylpyrrolidinium groups to kill Gram-negative, Gram-positive, and fungal cells following the irradiation with white light (Grinholc et al. 2015).

12.4.3 Graphene and Graphene Oxide (GO)

Graphene is generally called as the simplest structure among all carbon nanomaterials and possesses structural similarities with the 3D closed cage of fullerenes, 3D rolled tube of carbon nanotubes, and 3D stack sheets of graphite. Graphene is structurally characterized as a 2D sheet of carbon atoms with sp^2 and sp^3 hybridization. Graphene oxide has a 2D honeycomb lattice structure (Wang et al. 2011). Graphene is known to be the simplest form and thin material of carbon so far. Few layers of graphite are arranged in the closely packed honeycomb structure. The different members of graphene family include reduced graphene oxide, graphene oxide, graphene sheets, few-layered graphene, and multilayered graphene (Priyadarsini et al. 2018).

Many scientists have been attracted toward GO because of its water solubility, chemical inertness, optical transmittance, density, biocompatibility, large surface area, and stability in aqueous solutions. The large specific surface area of GO has enhanced its ability to carry and deliver hydrophobic drugs such as doxorubicin. This is because of its ability to solubilize hydrophobic moieties between the graphene sheets due to the pi–pi stacking (Sun et al. 2008; Huang et al. 2012). Graphene is known as the rising star of carbon nanomaterials due to its superior properties. Hence, it is widely studied in near-infrared photoinactivation of broad-spectrum microorganisms (Deokar et al. 2017). Mesquita et al. (2018) reviewed the improved delivery of drugs through nanographene oxide for root canal disinfection. The study showed more antimicrobial (2.81 log) and antibiofilm (94%) activities against *E. faecalis* than the free drug under illumination (Mesquita et al. 2018). Gholibegloo et al. found that multifunctionalized graphene oxide could be used as a nanocarrier

for efficient loading of indocyanine green and enhanced antibacterial activity against *S. mutants* for the treatment of local dental infections (Gholibegloo et al. 2017).

Graphene quantum dots (GQDs) are synthesized with special physicochemical features of graphene. The photoinactivation ability of GQDs is mediated by the production of ROS through energy or electron-transfer mechanism upon photoexcitation which results in microbial death (Ristic et al. 2014). In a study, GQDs were found to be toxic to MRSA and *E. coli* when photoactivated with a green laser but nontoxic to mouse spleen cells indicating their specific antibacterial photodynamic inactivation property (Ristic et al. 2014). Another advantage of using graphene-based nanomaterials for aPDT is their NIR (700–1100 nm) absorption. This allows targeted and deeper cell penetration of drugs for effective photothermal therapy (Weissleder 2001). Akhavan and Ghaderi (2009) described chemically stable reduced graphene oxide/TiO_2 thin films for the photoinactivation of *E. coli* under 4 h of solar light irradiation, and it resulted in an improved antibacterial activity by a factor of 7.5 (Akhavan and Ghaderi 2009). Graphene oxide with a high loading and release of antibiotics is utilized for near-infrared photothermal therapy of pathogens. Altinbasak et al. (2018) disclosed that reduced graphene oxide integrated with polyacrylic acid with high drug loading controlled and released antibiotics on demand for the photoinactivation of pathogens. The nanofiber platforms loaded with different antibiotics were found to be potential candidates for medical applications with excellent wound-healing activity in mice infected with *S. aureus* (Altinbasak et al. 2018).

12.4.4 Carbon Nanotubes

The minuscule of CNT is reported to be significant for many biomedical applications due to their unique physicochemical properties. CNTs were categorized as single-walled and multiwalled carbon nanotubes based on the number of graphene sheets. CNTs have either open or closed ends with sp^2-hybridized carbon atom sheets. Carbon nanotubes have an axial symmetry and tubular shape with a nanoscale diameter which make them effective for the targeted release of drugs (Albert and Hsu 2016). Carbon nanotubes (CNT) may open new windows for eliminating the biomedical problems related to the bacterial virulence and infections. The unique properties of CNTs make them toxic to microorganisms. The antibacterial activity of CNTs has been widely used in the production of bacterial filters. The high binding affinity and spontaneous internalization of CNTs by bacteria have exploited them for gene/drug delivery. Further, the absorption of NIR radiation by CNTs suggested their application as efficient photosensitizers in aPDT (Kim et al. 2007). The functionalization of CNTs with various molecules can enhance the biocompatibility and cellular uptake. The conjugation or encapsulation of CNTs with other photosensitizers is a potential tool for PDT toward infectious diseases.

As CNTs are insoluble in aqueous solutions, various surface modifications especially noncovalent modifications are employed widely to maintain their original properties and improved biological applications. The enhanced interaction between

CNT and bacteria is attributed to their high surface–volume ratio and thereby decreased size. Single-walled carbon nanotubes (SWCNTs) showed more phototoxicity toward bacteria than multiwalled carbon nanotubes (MWCNTs) indicating that their nanodiameter and nanosize are two important factors in PDT treatment. The interactions between the bacterial cell and CNT result in membrane perturbation and eventually bacterial death due to the increased ROS generation (Chen et al. 2013). The mechanism of damage of microbial cells when they come in contact with the CNT is well explained by the quantum yield of reactive oxygen species. Among SWCNTs and MWCNTs, the former is found to be producing high quantum yield of singlet oxygen than the other through an energy-transfer mechanism (Aboofazeli et al. 2011).

There are several studies with regard to the antimicroial activity of CNTs. In a study, CNT scaffolds inhibited cell cycle division and reduced the cell number of *Tritrichomonas foetus*, a eukaryotic parasite after PDT. The study concluded that the production of ROS caused programmed cell death in protozoa due to the release of lysosomal protease which activates procaspases (Machado et al. 2014). Photoactivated MWCNT/TiO_2 was used for the removal of enterohemorrhagic *E. coli* (EHEC) (Oza et al. 2013). The mechanism behind the pathogen removal was explained by the generation of free radicals such as superoxide radicals and hydroxyl radicals. Visible light-activated porphyrin–MWCNT conjugates were found to be efficient antiviral therapeutics for the inactivation of influenza A virus which infects mammalian cells (Banerjee et al. 2012). SWCNT–lysozyme coatings prepared by a layer-by-layer assembly showed strong antibacterial activity against *S. aureus* and *Micrococcus lysodeikticus* (Nepal et al. 2008). Both the single-walled and multiwalled carbon nanotubes are efficient drug delivery agents. These two CNTs exhibited excellent antibacterial activity as they serve as NIR-clustering photothermal agents. Carboxyl-functionalized mutiwalled carbon nanotubes were used as a carrier of rose bengal for the effective photodynamic inactivation of *E. coli* with green laser exposure (Vt et al. 2018). Phototoxicity of CNT/agar composites was higher against *S. mutants* under NIR irradiation than graphite/agar and activated carbon/agar composites (Akasaka et al. 2010). In the study, denaturation of bacterial proteins was the mechanism of death after PDT treatment but not targeting of the cell wall.

Conjugation of PS with CNT causes an increased bacterial uptake of PS, reduced efflux of PS, enhanced metabolic intervention, and altered delivery of drugs with controlled release. Photoinduced porphyrin–SWCNT conjugates were proposed for the visible-light inactivation of drug-resistant pathogen, *S. aureus*. The interactions between the conjugate and cell wall result in the membrane damage and can be used as a potential antibacterial agent (Sah et al. 2018).

12.4.5 Carbon Quantum Dots

The extended use of semiconductor-based quantum dots has limited their application in biomedicine due to their heavy metal release and cytotoxicity. Surface functionalization of quantum dots resulted in the development of new nanocarbons such

as graphene quantum dots (GQDs) and carbon quantum dots (CQDs). CQDs are very small carbon nanomaterials with several surface passivation schemes. Among the surface passivation schemes, the numbers of organic molecules used to chemically functionalize the carbon quantum dots are most effective. CQDs have special structures of core shell nanodot structures and thin shell of soft materials (Al Awak et al. 2017). These quantum dots especially carbon-based QDs have gained much interest in biomedicine due to their high solubility, low toxicity, good compatibility, modification, strong photoluminescence, and chemical inertness. The wide applications of CQDs in biomedicine make them as a rising star in the field (Y.-P. Sun et al. 2006). The surface defects of CQDs affect the yield of ROS which can be eliminated by functionalization with the amine, carboxyl, and carbonyl groups. Along with the high yield of ROS, functionalization makes CQDs more water-soluble and prevents the carbon particle aggregation (Liu et al. 2007).

Researchers showed a high quantum yield of 27% from 7.5% after the functionalization of CQD with 2, 20-(ethylenedioxy)bis(ethylamine). These photoactivated EDA-CQDs were observed with high antibacterial activity against *E. coli* and *B. subtilis* under varying conditions of treatment (Al Awak et al. 2017). Sattarahmady et al. (2017) suggested photo-ablation strategy as a minimally invasive method to fight with pathogens using carbon dots. The authors showed the reduction in the viability of wild and MRSA upon irradiation in the near-infrared range. The main reasons for the bactericidal effect exerted by CQDs were due to the ROS production and the subsequent cell wall disruption which interferes with the structure and functions of proteins, enzymes, and lipids (Sattarahmady et al. 2017). Multifunctionalized CQDs were prepared as antimicrobial agents and/or as carriers of ampicillin for effective killing of *E. coli* under visible-light irradiation. The enhanced antibacterial activity was observed due to the improved stability of antibiotic and high amount of ROS production. The ability of CQDs to attach with the bacterial cell wall exposes the bacteria to more drugs and ROS disrupting the integrity of the cell membrane (Jijie et al. 2018).

12.5 Application and Significance of Carbon Nanomaterials in aPDT

There are several studies regarding the effective and enhanced antimicrobial and antibiofilm activities of carbon nanostructures. The size and diameter of nanoparticles in nanodimensions are the two main characters determining the efficacy of aPDT. Fullerenes, single-walled carbon nanotubes, and graphene oxide were reported as potential antibacterial agents. The promising mechanism of action of these nanomaterials includes inhibition of bacterial growth, harming the electron transport chain, interruption of the energy metabolism, disruption of the cell membrane, and development of bacterial cell–nano aggregates (Maleki Dizaj et al. 2015) (Fig. 12.4). The sole properties of carbonaceous nanomaterials such as their lack of

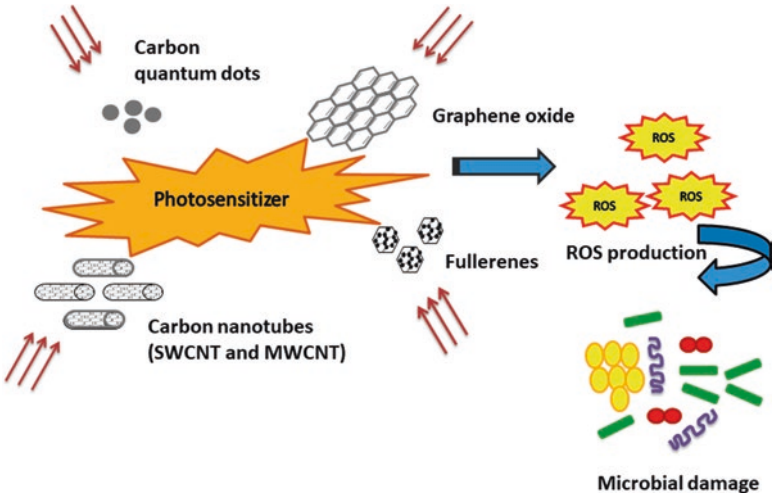

Fig. 12.4 Antimicrobial photodynamic inactivation and mechanism of action of carbon nanostructures causing phototoxicity

toxicity, good biocompatibility, large surface area, tunable morphology, water solubility, photothermal activity, and absorbance at near-infrared range make them effective candidates as photosensitizers or PS carrier molecules. Among these, the ability of carbon nanotubes to photogenerate ROS has attracted their applications toward antimicrobial therapy against infectious diseases (Mesquita et al. 2018). Carbon nanostructures are developed in a highly purified manner and functionalized with different groups for their successful application in biology and medicine. Carbon nanostructures showing an enhanced solubility in physiological solutions are considered as an encouraged methodology for the photothermal therapy of infectious diseases.

12.6 Future Perspectives and Conclusions

This chapter is mainly focused to provide scientific developments on the type of carbon nanomaterials and their application as antimicrobial and antibiofilm agents. Carbon in nanomedicine has been widely accepted by the scientific world, but the clinical application is yet to be validated. Tunable morphologies of carbon nanomaterials help to overcome the hindrance to access biological systems due to their insoluble nature. The intrinsic ability to photogenerate different types of cytotoxic species allows the integration of nanostructures into PDT as photosensitizers or carriers. Recently, carbon nanomaterials found a unique place in the ablation of cancer. Scientists expect the aPDT using carbon nanomaterials will be replaced with other modalities and theranostics for the treatment of infections and other forthcoming

diseases. Contemporary evolution in carbon nanomedicine opens extraordinary opportunities for the development of new biomaterials. In particular, the proposed mechanisms of action of these carbon nanomaterials in aPDT are defined as damage to the cell membrane, separation of cytoplasm, ROS generation, and oxidative death. The antimicrobial effect is not purely dependent on the type of nanostructures, but various parameters such as light, temperature, functionalization, size, shape, electronic structure, and the nature of pathogens also influence the therapy. In general, carbon nanostructures are potential antimicrobial candidates with several biological applications because of their capacity to kill and prevent the adhesion and biofilm formation of pathogenic bacteria. The story of carbon nanostructures for antimicrobial photodynamic therapy is not fully unfolded and further research is required in this direction. Research on the exploitation of carbon nanostructures for effective photodynamic therapy against different groups of microorganisms with antibiotic resistance and their clinical application in medicine is the need of the hour. Carbon nanostructures as photosensitizers as well as reliable carriers of photosensitizers in photodynamic therapy to prevent the growth of antibiotic-resistant microorganisms and their biofilms can be considered as an effective alternative to the conventional antibiotics.

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