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Carbon Dots

Next-generation materials for biomedical applications

**Ravindra Pratap Singh, Raju Khan, Jay Singh and Kshitij RB Singh**

# Chapter 1

# Carbon dots: an overview of their future uses in the biomedical domain

**Kshitij RB Singh, Vanya Nayak, Jay Singh, Raju Khan and Ravindra Pratap Singh**

Carbon dots belong to a group of carbon-based nanomaterials that has attracted significant attention from researchers worldwide. They exhibit diverse physicochemical properties along with high biocompatibility, cost-effectiveness, and environment-friendly synthesis, making them suitable materials for use in various domains, such as biomedicine, agriculture, environmental applications, sensing, robotics, electronics, aeronautics, food processing, packaging, etc. Furthermore, due to their high levels of fluorescence and photoluminescence, excellent water solubility, good quantum yield, enhanced biocompatibility, and low cytotoxicity, carbon dots are considered to be futuristic materials in the biomedical domain. This chapter aims to give the reader detailed knowledge about the various potentialities of carbon dots in the biomedical domain by highlighting their uses in tissue engineering, the fight against various pathogens, the fabrication of biosensors, bioimaging, the detection of biomolecules, and the treatment of inflammation and cancer.

## 1.1 Introduction

The thirst to develop novel, magnificent, and innovative techniques to make human life easy and smooth never ends. However, with the rapid increase in technological advancement, the risk of developing various diseases is also increasing. The onset of nanotechnology has taken science and technology to new heights, and nanomaterials are now hailed as futuristic materials that have the capacity to revolutionize the biomedical domain [1–7]. Currently, carbon dots are thought to be the most promising candidates for use in the biomedical domain, since they can be used as both therapeutic as well as diagnostic agents. Scrivens and his colleagues were the first to report fluorescent carbon nanoparticles in 2004, when they purified single-walled carbon nanotubes (SWCNTs) [8]. Furthermore,

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the term 'carbon dots' was coined by Sun *et al* in 2006, when they observed quantum dots in both solid and liquid forms [9]. Carbon dots are carbon-based nanomaterials whose dimensions are less than 10 nm, and are known to exhibit unique properties such as low toxicity, high biocompatibility, photostability, water solubility, and fluorescent emission and excitation. Moreover, the term 'carbon dots' includes graphene quantum dots (GQDs), carbon quantum dots (CQDs), and polymer dots. However, both CQDs and GQDs exhibit the quantum confinement effect and intrinsic-state luminescent properties due to the presence of crystal lattices and small graphene fragments made up of few-layer graphene sheets, respectively. As a result, they can easily monitor and tune the wavelength of photoluminescence. Since carbon is one of the fundamental elements of life and therefore exhibits less cytotoxicity as compared to other materials, its uses in the biomedical domain have expanded. Furthermore, owing to its small atomic size and unique properties, it is considered to be a futuristic material that can revolutionize the scientific and technological domains.

This chapter aims to provide the reader with updated and enhanced knowledge about the applications of various carbon dots in the biomedical domain, as illustrated in figure 1.1. The uses of carbon dots as anti-inflammatory agents are discussed in detail in chapter 2, and chapter 3 addresses the uses of carbon dots in the fabrication of biosensors. Chapter 4 provides a detailed discussion of the potentialities of carbon dots to detect various biomolecules. Chapter 5 thoroughly discusses applications of carbon dots in bioimaging applications. Chapter 6 comprises a detailed discussion of the potentialities of carbon dots as theranostic agents for cancer. Chapter 7 presents detailed knowledge about the significant role of carbon dots in drug and gene delivery. Moreover, recognizing the ongoing viral pandemic, chapter 8 deals with the uses of carbon dots in the fight against various pathogens. Chapter 9 provides a comprehensive understanding of the applications of carbon dots in tissue engineering, and the last chapter of this book, chapter 10, provides a detailed discussion of the biosafety and bioregulatory laws on the uses of carbon dots in the biomedical domain.

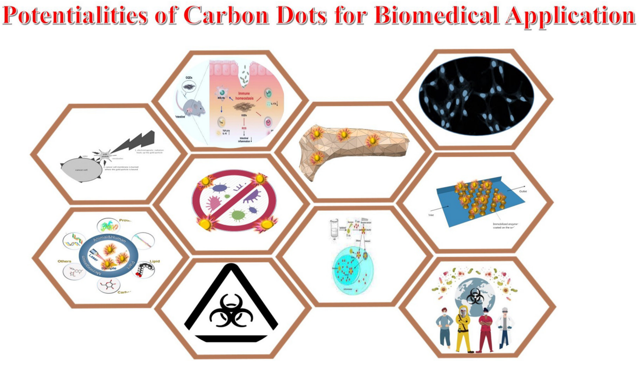

**Figure 1.1.** Potentialities of carbon dots in the biomedical domain.

## 1.2 Applications of carbon dots in the biomedical domain

Carbon dots exhibit immense potentiality in the biomedical domain, as they are composed of carbon and its compounds. Carbon is the fundamental element of life and is considered the least toxic to living beings. Moreover, the unique properties of carbon dots make them suitable materials for biomedical applications. Some uses of carbon dots in the biomedical domain are briefly discussed in this chapter.

### 1.2.1 Uses of carbon dots as anti-inflammatory agents

Inflammation is a complicated physiological reaction to injury, disease, or infection [10]. The overproduction of reactive oxygen species (ROS) is considered to be primary reason for the pathogenesis of inflammation. The term ROS includes oxygen-free radicals such as hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hydrogen radicals ($^{\cdot}OH$), and superoxide anions ($O_2{}^{\cdot -}$), which play essential roles in various biological functions. However, their overexpression can generate oxidative stress that can lead to the development of different abnormal pathobiological conditions [11–13]. Therefore, ways of eliminating excess ROS from living tissues have become top priorities for researchers today. Many techniques have been developed to eliminate excess ROS and soothe inflammation, among which, carbon-dot-based nanomaterials have been most successful in eliminating excess ROS from the body, as they participate in antioxidant catalytic activities such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) reactions. Furthermore, their high photostability, excellent biocompatibility, and the presence of various functional groups throughout the $sp^2$ hybrid carbon core help carbon dots to exhibit enzymatic catalysis properties, which can be selectively activated [14]. For instance, carbon dots have been synthesized without using any metal via hydrothermal routes, and, as an alternative to metal precursors, ethylenediamine and p-phenylenediamine have been used as carbon precursors to scavenge overproduced ROS by passive targeting and accumulation in inflamed liver tissues. Additionally, the functional groups present on their surface help them to exhibit POD, SOD, and CAT-like activities. Therefore, it has been noted that these synthesized carbon dots can be used to treat inflammation and that they possess effective therapeutic efficiency [15].

Similarly, the root extract of a Chinese herb, radix sophorae flavescentis (RSF), was used to synthesize carbon dots (radix sophorae flavescentis carbonisata-based carbon dots (RFSC-CDs)) using pyrolysis. RSF is one of the oldest Chinese medicinal herbs and is known to cure various ulcerous diseases. A common clinical type of gastrointestinal ulcer is ethanol-induced gastric ulcers, which are caused by increased inflammation and oxidative stress. This increased oxidative stress and inflammation results in damage to the stomach lining, which makes it more prone to ulcers. However, if not timely treated, this can lead to bleeding and perforation [16]. Additionally, the production of excess ROS induces oxidative damage that can result in the development of gastric ulcers. Furthermore, the excess ROS accumulate and consume endogenous antioxidants, resulting in the destruction of the immune system, lipid peroxidation, cellular death, damage to tissue, and finally gastric injury. Moreover, excess ROS production activates the NF-κB signaling pathways that induce cytokines, which are responsible for regulating the inflammatory responses in ethanol-induced gastric ulcers. Although various methods have been applied to cure these ulcers, harmful side effects always limit their use. However, these synthesized RFSC-CDs were found to be an effective treatment for this type of gastric ulcer, as observed in male adult Sprague Dawley (SD) rats. These RFSC-CDs efficiently inhibited inflammation in gastric tissues by reducing the concentration of NF-κB and downstream pro-inflammatory cytokines such as interleukin (IL-6) and tumor necrosis factor alpha (TNF-α). Simultaneously, to lessen the oxidative stress induced by ethanol, these RFSC-CDs increased the concentration of SOD, CAT, glutathione (GSH), and glutathione peroxidase (GSH-Px) and decreased the concentration of malondialdehyde (MDA) and inducible nitric oxide synthase (iNOS). Therefore, it was concluded that these excellent properties of RFSC-CDs make them a potential candidate for the treatment of ethanol-induced gastric injuries [17]. Additionally, chapter 2 provides a detailed discussion of the potentialities of carbon dots as anti-inflammatory agents.

### 1.2.2 Uses of carbon dots in the fabrication of biosensors

With the onset of nanotechnology, development in the sensor domain has scaled new heights. Moreover, carbon dots have played an important role in biosensors, as they show diverse properties such as high biocompatibility, high surface area, decreased cytotoxicity, and excellent electrocatalysis [18–20]. Therefore, owing to these properties, carbon dots are considered a propitious agent for the fabrication of next-generation biosensors. Furthermore, carbon dots exhibit robust and tunable fluorescent emission properties that can be used to design fluorescence-based biosensors [9, 21–23]. For instance, in photoluminescent biosensors, it has been observed that a low photoluminescence quantum yield is obtained from the bare carbon-dot surface. On the other hand, carbon dots with a surface passivation layer exhibited a high photoluminescence quantum yield and better crystallinity. The quantum yield increased because the diameter of the carbon dots increased to about 5–8 nm as their surface was passivated, resulting in an increased photoluminescent quantum yield [24].

Similarly, to develop and enhance the functionality of electrochemical biosensors, carbon dots are also used to immobilize various aptamers, antibodies, enzymes, peptides, etc. on the surface of an electrode. Recently, an electrochemical biosensor was developed to detect HER2 and HER2-overexpressing liver cancer cells by embedding abundant carbon dots in a bimetallic ZrHf metal–organic framework (CDs@ZrHf-MOF), which exhibited excellent anchoring aptamer strands. Furthermore, it was observed that the G-quadruplex present between HER2 and the aptamer strand was highly stable and enhanced the functionality of the electrochemical activity [25]. Likewise, an experiment was performed in which banana peels were treated using microwaves to synthesize carbon dots without utilizing any surface passivation agents. These synthesized carbon dots were used as reducing and stabilizing agents to synthesize Pd–Au@CDs nanocomposites used to modify glassy carbon electrodes (Pd–Au@CDs/GCE) to develop a biosensor that was able to detect a target sequence related to colitoxin DNA from human serum [26]. The prepared biosensor exhibited high sensitivity and high selectivity and a limit of detection of $1.82 \times 10^{-17}$ mol $L^{-1}$ was recorded [26]. As a result of the wide-ranging properties of carbon dots, biosensors based on carbon dots have found many uses in the biomedical domain. Chapter 3 of this book provides further details about the various biosensor fabrication methods that use carbon dots.

### 1.2.3 Detection of biomolecules using carbon dots

The introduction of carbon dots into the biomedical domain has generated much excitement among researchers worldwide, as they exhibit unique properties, such as high biocompatibility, high aqueous solubility, tunable luminescence, excellent electrical and conductive properties, etc [22, 27–30]. However, most of the applications of carbon members are generally based on their stable and intense luminescence. Therefore, owing to this property, the bioimaging domain and the development of optical sensors have taken a great leap. In addition, due to the presence of surface functionalities on carbon dots, they can interact efficiently with various analytes, such as biomolecules, metal ions, etc. which enhance the luminescence and help in the detection of analytes. The detection of biomolecules has gained a lot of importance, as it is more helpful than traditional diagnosis methods in the early diagnosis of various diseases, and carbon dots are often used as fluorescent probes to detect various biomolecules. For instance, bilirubin from human serum and urine samples was detected by carbon dots doped with sulfur and nitrogen (S, N-CDs) to create a paper-strip-based fluorescent probe. Bilirubin detection is vital in diagnosing neonatal jaundice. Bilirubin is generated by heme catabolism and is excreted from the body through bile. However, its accumulation in the blood causes neonatal jaundice and yellowing of the skin and sclera. In addition, high levels of bilirubin can result in hearing impairment, athetosis, etc. In this experiment, a microwave-assisted route was used to synthesize S, N-CDs in which citric acid and L-cysteine were utilized as the sources of carbon, nitrogen, and sulfur. It was observed that the S, N-CDs showed a bright blue fluorescence emission peak of 452 nm, in which fluorescence was quenched by Fe (III) and was selectively restored by bilirubin. These quenched fluorescent probes exhibited high sensitivity and selectivity for bilirubin and showed a detection limit of 0.12 nM. This experiment was further used to develop a paper-based test strip to detect bilirubin. This gives an insight into a way of designing novel point-of-care diagnostic devices [31].

Similarly, Raveendran *et al* (2019) synthesized carbon dots at a size of 4 nm using mint leaves. These synthesized carbon dots provided a high-ionic-strength environment, a hydroxyl-rich surface, and stability. These synthesized carbon dots were used to prepare an on–off–on fluorescent probe that could detect dual bioanalytes: ferric ion ($Fe^{3+}$) and ascorbic acid. The $Fe^{3+}$ quenched the carbon dots through the coordinate bond present between them, but with the addition of ascorbic acid, the fluorescence was restored, as the ascorbic acid removed the coordinate bond between the carbon dots and the ferric ion. The ascorbic acid and ferric ion detection limits were observed to be 0.079 μM and 374 nM, respectively.

Therefore, it can be concluded that this method can be used for the selective sensing and detection of various natural products such as butanolides, etc [32]. Furthermore, water-soluble N-doped carbon dots (NCDs) were synthesized using an aqueous urea and citric acid mixture via hydrothermal treatment. To improve and increase the emissive properties of the prepared NCDs, they were encapsulated in L-GSH via a carbodiimide-activated coupling reaction, which resulted in intense blue emission at 440 nm. This enhanced property was then utilized to detect dopamine in a human urine sample. It was observed that the dopamine selectively quenched the fluorescence of GSH-NCDs, which can be attributed to the presence of a photoinduced electron-transfer-assisted inner filter effect mechanism, as confirmed by the average lifetime values and spectral profiles of GSH-NCDs with and without dopamine. The GSH-NCDs exhibited high sensitivity and stability and a detection limit of 1.01 nM. This experiment was successful, and this approach can be used for real sample analysis of human urine [33]. As a result of these properties of carbon dots, they are now widely known to detect various biomolecules, as discussed in detail in chapter 4.

### 1.2.4 Uses of carbon dots in bioimaging

Carbon dots have attracted massive attention in the bioimaging domain, due to their excellent fluorescence properties. Moreover, their high biocompatibility, photobleaching resistance, tunable surface properties, and low toxicity have made them notable biomedical agents. These properties have been further utilized in the development of various bioimaging agents and fluorescence sensors used to detect biomolecules and help diagnose diseases and in the development of several point-of-care devices. Their tunable optical properties are related to particle size, surface functional groups, and radiative excitation recombination, which have broadened their uses in bioimaging applications. For instance, baicalein (5,6,7-trihydroxyflavone) was used to synthesize carbon dots (B-CDs) at a size of 3.82 nm by the hydrothermal method, in which L-cysteine, trisodium citrate dehydrate, and ethylenediamine were used as precursors. These synthesized B-CDs exhibited high ionic strength, high blue fluorescence, high stability, excellent biocompatibility, and their fluorescence yield reached 66%. Moreover, these B-CDs also demonstrated good fluorescence imaging capability in imaging human ovarian cancer cells and also detected baicalein with a linear range of 0–30 μM. In addition, these carbon dots were recovered from human urine and blood samples, with recorded recovery rates from 97.10% to 98.19% and from 101.93% to 103.49%, respectively. These results showed that these carbon dots could be a potential agent for bioimaging [34].

Additionally, it is challenging to synthesize red/near-infrared (NIR)-emissive carbon dots that can be utilized in bioimaging applications. Therefore, a solvothermal approach was used to synthesize near-infrared carbon dots (NIR-CDs) using sulfonated tetraphenylporphyrin as a precursor. These NIR-CDs exhibited excitation-independent characteristics at the wavelength corresponding to the strongest emission, namely 692 nm. A further analysis confirmed that aggregated molecular states of the carbon dots were responsible for developing the NIR fluorescence properties. Moreover, these NIR-CDs also exhibited great water stability, high biocompatibility, and excellent cellular labeling properties with low toxicity, from which it was concluded that these NIR-CDs could be efficiently used as NIR emission bioimaging probes for both *in vivo* and *in vitro* applications [35]. Based on these studies, carbon dots can justifiably be called futuristic materials that can completely revolutionize diagnostic methods. Chapter 5 discusses the uses of carbon dots in bioimaging.

### 1.2.5 Carbon dots as cancer theranostic agents

As a result of the fast pace of life and poor eating habits, the risk of developing lifestyle diseases is also rising. Cancer has become one of the major causes of death globally, accounting for almost 10 million deaths worldwide in 2020 [36]. Moreover, the lack of proper treatment and diagnostic tools limits the appropriate treatment of cancer. However, nanotechnology will be able to improve and enhance cancer theranostics in the near future. Carbon dots constitute a promising class of nanomaterials for cancer theranostics. They exhibit enhanced and tunable photoluminescence, fluorescence, and surface properties, making them a potential candidate for diagnostics, imaging, and therapeutics. Furthermore, it has also been observed that research into the combination of nanomaterials with enzyme-like catalysts, known as nanozymes, has accelerated the development of cancer therapeutics. Owing to the high biocompatibility and low toxicity of carbon dots, it has been found that they can mimic enzyme-like activity. However, their high agglomeration susceptibility, non-uniform size, and morphology degrade their enzyme-like activity [37]. Therefore, to overcome this limitation, the encapsulation of carbon dots in a suitable template, such as a zeolite imidazolate framework (ZIF-8), has been proposed, as it is a metal–organic framework (MOF) material with a stable skeleton and a uniform internal pore size of ~1.9 nm [38]. Keeping this idea in mind, an experiment was conducted in which carbon dots were confined with N-doped carbon (CDs@NC) and further fabricated through two-step carbonization of glucose-filled ZIF-8 (G@ZIF-8). Experimental results showed that the prepared CDs@NC could efficiently mimic peroxidase activity that can be modified by regulating the glucose content. Additionally, it was noted that the catalytic properties of carbon dots were enhanced by doping carbon dots with N-doped carbon (NC). Building on these results, the synthesized CD@NC was used for the colorimetric detection of D-proline and D-alanine obtained from human saliva, which can be used for the early detection of gastric cancer, making it a potential candidate for developing point-of-care devices for early diagnosis of gastric cancer [39].

With the arrival of organic–inorganic nanohybrids, better theranostic agents have been made possible, as they are a combination of both inorganic and organic compounds. These nanohybrid materials integrate excellent properties, such as the strong optical, magnetic, electric, physical, and chemical properties of inorganic compounds, combined with the enhanced stimulus responses, precise targeting, and controlled release of organic compounds, which helps them gain diverse potentialities in the biomedical domain [40]. For this reason, an experiment was conducted by Luo *et al* (2021) in which carbon dots were doped with iron nanoparticles (Fe@CDs) that could act as an ‘all-in-one’ theranostic agent. They chose carbon dots due to their excellent fluorescent and photothermal activities and Fe because of its excellent magnetic targeting, MRI imaging, and synergistic magnetic hyperthermia properties. Using self-assembly, these Fe@CDs were functionalized with DSPE-mPEG2000 (amphiphilic polymer) and RLS (a dendritic arginine and disulfide-bond-containing cationic lipopeptide) (PEG-RLS/Fe@CDs). PEG-RLS/Fe@CDs were used to screen breast cancer cells by studying irradiation conditions and calculating the optimal mass and N/P ratio for gene transfection. These PEG-RLS/Fe@CDs exhibited high stability in water and a photothermal conversion efficiency of 63.4%, along with good biocompatibility. It was also observed that these PEG-RLS/Fe@CDs promoted both *in vivo* and *in vitro* gene transfection efficiency by a factor of two in 4T1 tumor tissue and by a factor of 3.5 in 4T1 cells. These PEG-RLS/Fe@CDs can also be used as a tetramodal contrast agent that can be used as an imaging agent for gene delivery diagnosis in absorption and emission spectra, photothermal conversion efficiency, photostability, multi-modal imaging. Additionally, it was observed that the POD-like activity increased in both *in vivo* and *in vitro* tumor cells, resulting in photothermal/chemodynamic activity and synergistic tumor inhibition, which inhibited the growth of the tumor by 83% with 80% survival for at least 50 days, as illustrated in figure 1.2. Therefore, it was concluded that these nanohybrids could be a breakthrough in the biomedical domain, as they can be used to diagnose and treat cancer simultaneously [41]. Chapter 6 comprehensively lists the up-to-date literature on this topic and provides

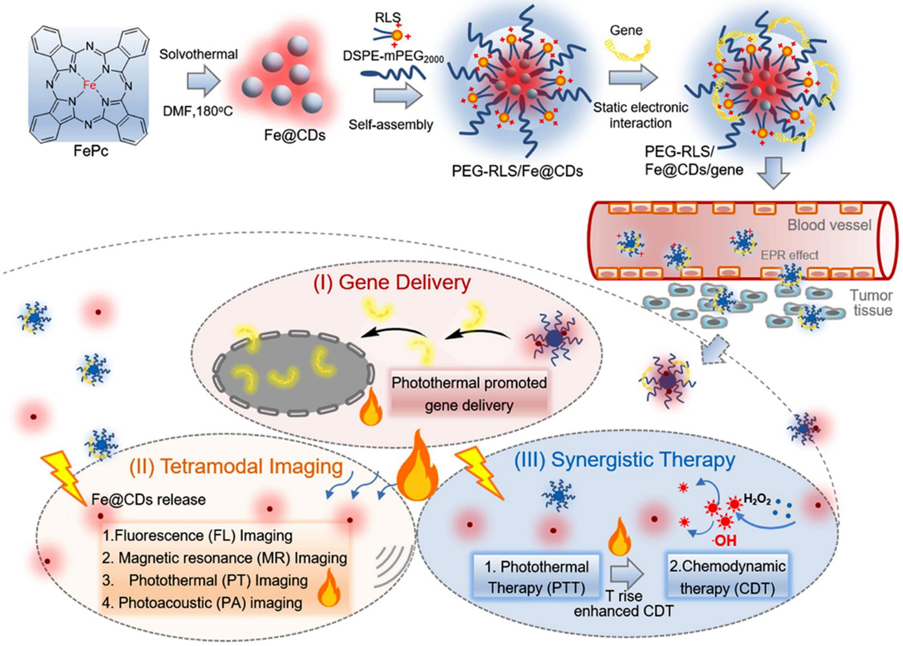

**Figure 1.2.** Applications of PEG-RLS/Fe@CDs in cancer nanotheranostics for: (I) gene delivery, (II) synergistic therapy and (III) tetramodal imaging (fluorescence imaging, magnetic resonance imaging, photothermal imaging, and photoacoustic imaging) (reproduced with permission from Luo *et al* (2021) [41]).

an insight into the applications of carbon dots as theranostic agents for cancer treatment.

### 1.2.6 Carbon dots for gene and drug delivery

Gene therapy has been considered to be an effective therapy, which has been known to cure many diseases for decades; it has appeared as a powerful approach for treating various illnesses, such as cancer, cardiovascular ailments, etc. Non-viral vector-based gene therapy is regarded as a promising therapy for the treatment of malignant cancer. The success of gene therapy mainly depends upon the specific delivery of a gene to the targeted cell or tissue; therefore, vectors are used as carriers. Compared to viral vectors, vectors made of carbon dots are a safer and simpler way to deliver therapeutic genes. For instance, carbon dots that exhibited strong blue fluorescence were synthesized using hyaluronic acid (HA) as a carbon source and polyethyleneimine (PEI) as a passivant (HA-CDs). It was found that the remnants of PEI and HA caused the emission of strong blue fluorescence. Furthermore, due to the high level of fluorescent activity, these HA-CDs can be used in gene transfection, as they efficiently release DNA cargo into the cytosol, suggesting that they may be suitable gene carrier agents for targeted gene delivery and suitable agents for gene therapy [42].

Furthermore, carbon dots have also been utilized as traceable multicolor photoluminescent gene delivery nanocarriers synthesized through a microwave approach using a pyrolysed mixture of branched PEI and citric acid. This was the first study that transferred pCRISPR to cells with a high quantum yield and low cytotoxicity using CD-PEI, as observed in HEK 293 cell lines. The PEI assisted in the condensation and passivation of the DNA on the surface of the carbon dots. This approach was found to be helpful in target gene delivery [43]. Similarly, carbon dots are also known for their potential for target drug delivery. They exhibit tunable and excellent luminescent, photocatalytic, physical, chemical, and surface properties, making them a futuristic nanomaterial in the biomedical domain. Since hollow carbon dots exhibit strong fluorescence properties, they were used as a vehicle for delivering doxorubicin (DOX) by synthesizing it from bovine serum albumin (BSA) via solvothermal treatment. The results showed that the DOX was rapidly taken up by cells, it was released in a pH-controlled manner, and it did not hamper the release of drug activity. Additionally, it was noted that these hollow CDs (HCD) also exhibited high biocompatibility, cell viability, and antibacterial activity [44]. Chapter 7 discusses the uses of carbon dots as drug and gene carriers that can be utilized for target delivery. However, proper research into carbon dots as gene and drug carriers is still emerging. This field needs a detailed and elaborated study of the development of suitable drug and gene carriers that focuses on the biocompatibility, biotoxicity, and biosafety of carbon dots as carrier agents.

### 1.2.7 The use of carbon dots to combat various pathogens

Recent outbreaks of infectious diseases caused by microorganisms such as viruses, bacteria, fungi, etc. have severely affected the global population and public health. Moreover, they have also triggered an urgent requirement to develop efficient anti-microorganism materials to prevent their spread in the near future. In recent times, the most fashionable materials for combating microorganism-based infections have been nanomaterials, among which carbon dots have attracted a lot of attention, as they exhibit unique properties. Previous reports have stated that CQDs interact directly with bacteria by starting an electrostatic interaction between the cationic residues present on the surface of CQDs and the anionic microbial membranes, thereby inhibiting bacterial growth. CQDs can be used as conventional antibacterial agents. When they enter bacterial cells, CQDs interfere with bacterial enzymes and prohibit cellular proliferation [45]. The various mechanisms whereby CQDs inhibit the growth of bacterial cells are depicted in figure 1.3 [46]. Various bacterial species such as *Staphylococcus aureus, Pseudomonas aeruginosa*, *Vibrio cholera*, and *Escherichia coli* are inhibited by several carbon dots [46–49].

Carbon dots are also known to possess antiviral properties; their antiviral mechanisms are unexplored, but studies have been conducted that could predict their antiviral mechanisms. In pursuit of this objective, a study was conducted in which carbon dots were prepared from an active ingredient, glycyrrhizic acid (Gly-CDs), obtained from Chinese herbal medicine, as it has been known to have various biological effects, such as antiviral, anti-inflammatory, and anti-oxidation characteristics. These Gly-CDs were then further tested against porcine reproductive and respiratory syndrome virus (PRRSV), which is an enveloped single-positive-strand RNA virus that mainly infects pigs and has significantly affected the global pig industry for more than two decades [50, 51]. To date, no vaccine or treatment has

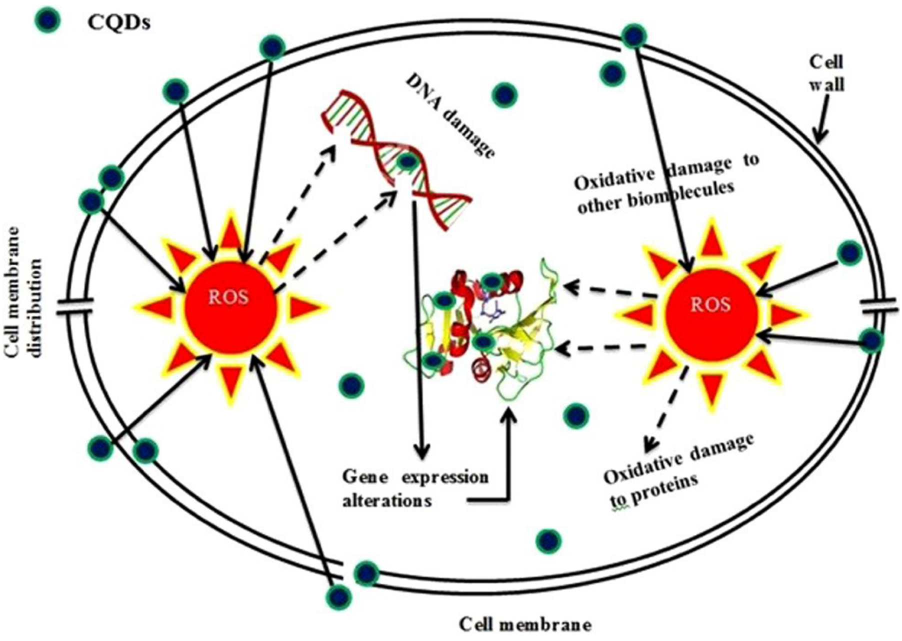

**Figure 1.3.** Antimicrobial activity mechanism exhibited by CQDs (reproduced with permission from Pandiyan *et al* (2020) [46] (distributed under CC-BY-NC-ND)).

been developed that can stop its occurrence or spread [52]; however, some microbiocides have been shown to have a minor inhibitory effect of ≈3 orders on PRRSV [53–56]. In this experiment, plaque assay was used to detect the infectivity of the virus, and the number of viral particles was denoted in titers. It has been previously reported that glycyrrhizic acid alone exhibited an inhibitory effect of ≈2 orders of magnitude [57]. However, this study showed that combining Gly with carbon dots efficiently enhanced its antiviral activity by increasing the inhibitory effect by ≈5 orders of magnitude. Furthermore, the antiviral mechanism exhibited by Gly-CDs was predicted by inactivating PRRSV *in vitro,* retarding its replication and invasion, and promoting the production of ROS. Finally, the proteomics data showed that these synthesized Gly-CDs could promote the upregulation and downregulation of intracellular antiviral proteins and proviral proteins, respectively [58]. Given the events of recent times and the increase in globalization, it is said that the occurrence of microbial infection will also increase. Therefore, it has become the need of the hour to develop antimicrobial agents that can effectively combat various hazardous pathogens. Some of the carbon dots that can act as anti-microorganism agents are discussed in chapter 8.

### 1.2.8 Uses of carbon dots in tissue engineering

Tissue engineering is a unique integration of engineering procedures with biological sciences to develop novel methods to fabricate and prepare artificial biological replacements such as scaffolds, bioactive molecules, etc [59–61]. Hydrogels are 3D networks composed of cross-linked hydrophilic polymer chains that have the potential to absorb a large amount of water, which can be more than a thousand times their dry mass. They are considered to be one of the elementary types of biological materials used to fabricate scaffolds, as they exhibit enhanced mechanical behaviors in the extracellular matrix (ECM) and better interaction with cells. Additionally, they generate signals used to maintain cell adhesion, generation, and differentiation [62, 63]. Due to these properties of hydrogels, they are widely considered for cartilage repair and can also help hyaline cartilage tissue regeneration by modifying cellular activities [64, 65]. Therefore, an injectable hydrogel composite was prepared that was composed of oxidized alginate/gelatin and nitrogen-doped carbon dots (OA/GEL/NCDs) by cross-linking the amino groups of GEL and the aldehyde groups of OA. *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDC) were used as cross-linkers. The OA and GEL were selected because they are both natural compounds; in addition, OA can efficiently imitate the extracellular matrix, and GEL is commonly used in medical treatments due to its high biocompatibility. On the other hand, NCDs were used as reinforcements. This synthesized scaffold was found to be durable, environment-friendly, highly biocompatible, cost-effective, and is present on the Earth in high abundance. Due to the presence of carbon dots, the hydrogel exhibited a water uptake of about 81.3%, excellent mechanical properties, high biodegradation, and a smaller pore size of about 102 μm, as the interaction between the hydrogel system and the NCDs was found to be strong. However, it was noted that the concentration of carbon dots along with other factors like body temperature, pH, etc. could change the fluid to gel; therefore, these hydrogels were synthesized in an injectable form and were found to have excellent biological functions in cartilage tissue engineering, offering a promise of hope to the future of tissue engineering [66]. With the introduction of carbon dots into tissue engineering, the applications of tissue engineering have also increased, which is elaborated in chapter 9.

### 1.2.9 Biosafety and bioregulation of carbon dots

Biosafety is defined as the implementation of safety parameters that decrease a researcher's risk of exposure to a potentially infectious microbe or other biological agents and simultaneously prevent contamination of the environment and community. Biosafety consists of four levels, and each level governs a specific control of contamination by microbes or other biological agents. Different contamination levels are determined by considering infectivity, transmissibility, the type of work conducted, the microbe's origin, and the exposure route. In addition, specific contaminant controls, such as the facility's construction, laboratory practices, and safety equipment, are used to determine biosafety levels. The biosafety levels range from BSL-1 to BSL-4, and each level is based on the control of the level before it. As a result of the unique properties of carbon dots, it is clear that they carry immense potentialities in the biomedical domain and can be considered to be futuristic materials that can bring about a revolution in medical science. Therefore, the evaluation of carbon-dot-related biosafety has become essential. Carbon has low toxicity, as it is one of the fundamental elements of life, but it has still become crucial to study and evaluate the biological safety of carbon-based nanomaterials. Various studies have explored and evaluated carbon dots' *in vivo* and *in vitro* behaviors, such as their biodistribution in organs, cell viability, pharmacokinetics, and biodegradability. For instance, it was observed that polyethyleneimine-modified carbon dots showed enhanced luminescence and were found to be stable at different pH values. Additionally, these synthesized PEI-CDs were found to target HeLa cells when successfully conjugated with CEA8 antibody. Cytotoxic assessment revealed that the PEI-CDs exhibited high biocompatibility and reduced viability on HeLa cells, confirming that these PEI-CDs were safe for cell labeling [67]. To launch a new product from lab to market is a tedious job, as it requires various biosafety procedures to be carried out; simultaneously, any new product should also satisfy bioregulatory laws. Therefore, the biosafety and bioregulation of carbon dots has become a significant limitation that restricts their commercial application. However, the biosafety and bioregulation of any material is an important topic that should always be elaborated. Chapter 10 of this book is dedicated to discussing the various biosafety and bioregulatory rules that relate to carbon dots.

## 1.3 Conclusions

The arrival of nanotechnology has given rise to new opportunities and has made it possible to work on nano-ranged materials. These nanomaterials offer a wide platform for the exploration and use of their properties, supporting the development of novel materials that are beneficial to living beings and the environment. There are different types of nanomaterials, among which carbon-based and metal-based nanomaterials have gained much attention. Carbon dots are extremely small nanomaterials whose dimensions are less than 10 nm; they are classed as carbon-based nanomaterials. Since carbon is one of the fundamental elements of life on this planet, carbon-based nanomaterials have gained popularity in almost all domains. Carbon dots have extremely small sizes and exhibit excellent quantum yields and unique surface, photoluminescent, and fluorescent properties. Therefore, they are considered to excel in the biomedical domain, as they are known to be futuristic materials that can be used in diagnostics and therapeutics. This chapter briefly elaborated the different potentialities of carbon dots in the biomedical domain by highlighting their uses in the diagnosis of various diseases and in combating inflammation, multiple pathogens, and cancer.

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