Recent advances of carbon dots as new antimicrobial agents

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Abstract

Due to the COVID-19 pandemic, many rapid antimicrobial agents have developed intensively. Carbon dots (CDs), a new type of carbon-based nanomaterials, shows great potential against emerging infectious diseases and antimicrobial-resistant infections due to their unique optical properties, excellent biocompatibility, and easy surface modification. With the definition of the CDs structure and properties, synthesis, and characteristic technology improvement, the research on the CDs as antimicrobial agents has made significant progress. However, the lack of high repeatable and exact preparation methods, and the regular antimicrobial activity make it far from practical application. In this review, we summarize the most recent progress and challenges of CDs antimicrobial. First, an overview of the characteristics and properties is given, and the advantage of CDs applied to antimicrobial is further discussed. Then, it focuses on research progress on antimicrobial mechanisms under different conditions, the critical factors affecting their antimicrobial activity, and the practical antimicrobial applications. Finally, the main challenges and future research perspectives of antimicrobial CDs are proposed.

KEYWORDS antimicrobial, antimicrobial activity, carbon dots, photodynamic effect, reactive oxygen species

1 | INTRODUCTION

The COVID-19 pandemic caused by the novel coronavirus (SARS-CoV-2) has afflicted more than 500 million people and caused more than 6 million deaths.\textsuperscript{1,2} The action of the virus is complicated and has spread so fast that no medicine has been found to cure this disease. Even though vaccination is the best approach to prevent this pandemic, it is a long process from design to clinical practice and requires significant labor. Thus, it is difficult to combat newly-emerging infections promptly.\textsuperscript{3–5} Moreover, SARS-CoV-2 is only one new virus of many...
microorganisms (bacteria, fungi, viruses, or parasites) that cause infectious diseases that account for nearly one third of annual global mortalities. Chemical drugs and antimicrobial agents are the primary clinical treatment methods for bacteria and virus diseases. However, research show that many chemical drugs have specific biological toxicity and disrupt normal tissue cells, especially for children. In addition, many pathogens have evolved and resulted in the condition of multidrug resistant (MDR). It is reported that the number of deaths caused by antimicrobial-resistant infections is nearly 700,000 per year. We supposed that if no practical actions are taken to mitigate the rise of antimicrobial resistance by 2050, antibiotic resistance will cost an extra of US$100 trillion and affect more than 10 million people every year.

Nanotechnology can act as bacteria and virus killer to treat and prevent known and unexpected infectious diseases. Nanoparticles (NPs) have also been widely used in numerous medical applications such as biosensing, drug delivery, bioimaging, and antimicrobial therapy. NP-based therapeutic drugs can inhibit the impact of virus infection by blocking the binding of receptors to the host, inhibiting virus replication and proliferation, and directly inactivating the virus. Therefore, NP-based strategies show great potential in combating pandemics caused by viruses such as COVID-19.

Carbon dots (CDs), as a new type of carbon-based nanomaterial, have attracted broad research attentions in recent years. Based on their unique optical features, excellent biocompatibility, low toxicity, low cost, easy modification, and functionalization, CDs have been demonstrated in a wide range of applications in biomedicine. Their synthesis methods include both top-down and bottom-up routes. Top-down routes involve cutting graphene oxide, carbon fiber, or fullerene to carbon NPs with graphitic structure and less than 10 nm in size. In the case of bottom-up methods, small organic molecules or polymers as precursors are converted into CDs via dehydration, carbonization, and assembly. Different synthesis processes and precursors result in different structures and properties, but all of them can be treated as a core-shell structure. The shell can be easily passivated or functionalized by organic groups, which is particularly important for the unique properties of CDs. The surface functional groups can also have antimicrobial properties and endow CDs with targeting and selectivity. CDs were designed as multifunctional antimicrobial agents with bacteria imaging, targeting, and other functions in recent years. For example, quaternized ammonia CDs can selectively kill Gram-positive bacteria. Concurrently, after CDs are adsorbed onto Gram-positive bacteria, their fluorescence emission intensity is significantly enhanced, which allow them to be applied in imaging-guide and bacterial differentiation. In addition, the CDs also exhibit immense potential in antiviral. In 2020, Kotta et al. explored the promising applications of CDs against different types of coronaviruses and discussed the possibility of CDs to combat COVID-19. CDs can alter the attachment and penetration of viral, inhibit the virus replication, or hinder the budding and detachment to combat coronavirus infection. They may play an essential role on the frontlines of the fight against COVID-19 or other emerging infectious diseases.

Compared with traditional and novel antimicrobial agents, the CDs process better biocompatibility and lower biotoxicity. In addition, the CDs also can act as a photosensitizer to photodynamic sterilization, which provides an efficient alternative approach to cope with MDR bacteria easily.

This review highlights the antimicrobial mechanism of CDs as antimicrobial agents, discusses the effects of CDs size, surface charge, surface-functionalization, and precursors on their antimicrobial activity, and then summarizes several practical applications of antibacterial CDs. We also provide perspectives on the challenges and opportunities for practical applications (Figure 1).
2 | THE MECHANISM OF CDs AS ANTIMICROBIAL AGENTS

2.1 | CDs antimicrobial without photoexcitation

In the absence of photoexcitation, a part of CDs possess antibacterial activity. CDs can adsorb onto bacteria and fungi cell walls via diffusion and electrostatic interactions. Electrostatic effects lead to biological isolation from growing bacteria, preventing bacteria from spreading or consuming nutrients and disturbing their physiological metabolism.\(^{35,36}\) Alternately, CDs were enriched with the bacterial wall/membrane, and may infiltrate bacteria and inhibit the formation of bacterial biofilms, leading to the cellular cytoplasm leakage and apoptosis of bacteria.\(^{37,38}\) CDs can also penetrate bacterial walls and membranes and bind to DNA and RNA in bacteria and fungi via noncovalent interactions, changing the structure of DNA (secondary conformations) and RNA, causing the DNA double-helix to separate. Finally, the bacteria growth is affected by many factors to achieve an antimicrobial effect.\(^{39}\) In this process, CDs always possess good biocompatibility and are nontoxic for normal cells.\(^{40,41}\) Ma et al.\(^{42}\) used biomass as precursors to synthesize the broad-spectrum antibacterial CDs in 2020. The CDs exhibited strong antibacterial activity to Escherichia coli and Staphylococcus aureus after the concentration rose to 1000 μg/ml. The SEMs (Figure 2E) of the E. coli and S. aureus treated by CDs show that the bacterial cell wall was destroyed, and the cytoplasm leaked. That is believed to be the primary mechanism of CDs to kill bacteria.

As shown in Figure 2A,B, CDs can bind and interact with DNA and RNA, resulting in a significant increase in the hydrodynamic diameter. The PL emission intensity increases linearly with the rise of DNA concentration. In the circular dichroism spectra of DNA (Figure 2C), as the concentration of CDs increases, the negative and positive bands gradually decrease, which indicates that CDs affect the secondary conformations of the DNA, leading to the destruction of DNA structure.\(^{43}\) Li et al.\(^{43}\) used the simulations to predict the process of DNA unwinding by CDs in 2018, as shown in Figure 2D, when the terminal base-pairs bind with CDs, the DNA secondary structure is destroyed. This affects the genetic processes of bacteria and fungi. Song et al.\(^{45}\) extracted low-toxicity CDs from cigarette smoke that showed broad-spectrum antimicrobial activities in 2018. By investigating the mechanism of the antimicrobial activities, CDs could pass through the bacterial cell walls and membranes after adsorption without destroying them. Then, the bacterial DNA can be exposed. The circular dichroism spectrum (Figure 2H) shows that CDs destroyed the double-helix structure of DNA and inhibited the proliferation of bacteria. Knoblauch et al.\(^{44}\) found that brominated carbon nanodots (BrCND) displayed strong antibacterial activity under photoexcitation and dark conditions. BrCND inhibited E. coli growth via a series of mechanisms. And a new antimicrobial mechanism was proposed, which involved the pH-triggered release of reactive nitrogen species. BrCND released nitric oxide to attack bacteria through basic-acidic-basic cycling, both in the dark and under UV exposure (Figure 2F,G). It is reported by Jhonsi et al.\(^{46}\) in 2018 that the interactions between CDs derived from tamarind and calf thymus DNA (ct-DNA) were studied by absorption, steady-state, and time-resolved fluorescence techniques. UV-vis absorption (Figure 2I) revealed the binding of DNA with TCDs occurred via intercalation. The binding constant (K\(_{\text{app}}\)) value indicated that the affinity of TCDs towards DNA was 1.85 mg/ml. TCDs can interact with DNA via intercalation, replacing ethidium bromide (EthBr) in the DNA-EthBr adduct. That resulted in the fluorescence quenching of the DNA-EthBr, and the quenching effect increased with the increasing concentration of TCDs (Figure 2I). That causes the BrCND to effectively inactivate E. coli.

2.2 | CDs antimicrobial under photoexcitation-photodynamic

In the other part of the antimicrobial process of CDs, the lighting is necessary to exert an antibacterial effect. When CDs act as photosensitizers (PS), the process is called antimicrobial photodynamic therapy (APDT), which possesses great potential for fighting antimicrobial resistance.\(^{47-52}\) Figure 3 shows the Jablonski diagram depicting the mechanism of APDT that contains physio- and photochemical processes: The PS is excited after absorption (A) photons, electrons transition from the ground singlet state (S\(_{0}\)) to the excited singlet state (S\(_{1}\)). Subsequently, the excited state electrons can release energy to return to the S\(_{0}\) through radiative transition (fluorescence, F) and non-radiative transition (internal conversion, H). At the same time, the excited state electrons can also be converted to the triplet excited state (T\(_{1}\)) by inter-system crossing (ISC). Then, it can return to S\(_{0}\) from T\(_{1}\) by phosphorescence emission (P) or internal conversion and generation of reactive oxygen species (ROS) through two photochemical processes. In the type I processes, the electrons can transfer to the surrounding substrates (H\(_{2}O\), O\(_{2}\)), leading to the substrates being oxidized to produce superoxide radicals (O\(_{2}^{-}\)). Additionally, in the type II mechanism, the energy is directly
FIGURE 2  (A) Size distribution of DNA before and after the addition of CDs (DNA/CDs). (B) Fluorescence spectra of the CDs (200 μg/ml) toward the addition of various concentrations of DNA in the PBS buffer (10 mmol/L, pH = 7.2) under excitation of 380 nm; inset: fluorescence intensity ratio versus the different concentrations of DNA. I₀ and I stand for the emission peak intensity of the CDs before and after the addition of DNA. (C) The circular dichroism spectra of DNA treated by CDs with different concentrations. (D) Some representative configurations were taken from crucial time points to show the terminal base pair denaturation process. The terminal base pair was shown with sticks, where red, yellow, blue, and tan sticks represent oxygen, carbon, nitrogen, and phosphorus atoms, respectively. The purple ribbon was the DNA hairpin. The cyan, red, and white spheres were the CDs’ carbon, oxygen, and hydrogen atoms. Reproduced with permission: Copyright 2018, American Chemical Society.43  (E) The SEM images of *Escherichia coli* (left) and *Staphylococcus aureus* (right) after incubation without and with OCDs at 600 μg/ml for 4 h. Reproduced with permission: Copyright 2020, Elsevier.42  (F) Photograph of *E. coli* after 4-min exposure to brominated carbon nanodot (BrCND) solutions with varying concentrations. (G) Graphical representation of one possible mechanism for acid-mediated nitric oxide (NO•) donation characteristics from a diazeniumdiolate form of BrCND. Reproduced with permission: Copyright 2021, RSC Pub.44  (H) Circular dichroism spectra of DNA with and without CDs treatment. Reproduced with permission: Copyright 2018, American Chemical Society.45  (I) Absorption study of DNA with TCDs in the concentration range of 0–0.05 mg/ml. (J) Fluorescence study of EthBr-DNA system in the absence and presence of TCDs in the concentration range of 0–0.05 mg/ml. CD, carbon dot. Reproduced with permission: Copyright 2018, Elsevier.46
transferred to the ground state with singlet oxygen generation ($^1\text{O}_2$). After such a cycle of photophysical and photochemical processes, the PS returns to the $S_0$ and is ready for the next cycle to generate additional ROS.\textsuperscript{53}

Similarly, photoexcited CDs can produce ROS, which exerts a photodynamic effect on cancer cells.\textsuperscript{54,55} ROS generation is in direct correlation to antibacterial activity. As depicted in Figure 4, the mechanism of action is the adhesion of CDs to bacterial surfaces, which alters the bacterial cell wall/membrane structure/permeability. After the photoexcited by visible/natural light, CDs generate ROS (OH• or $^1\text{O}_2$) efficiently by activating oxygen in air or water. The ROS induce the oxidative damage of critical intracellular biomolecules, leading to changes or the inhibition of the expression of essential genes and eventually leading to cell death.\textsuperscript{30,56,57} There appear to be two requirements for efficient visible/natural light-activated antimicrobial agents. First, the
CDs must be close to interacting with bacteria. Second, they must show strong absorption over the visible spectrum and highly reactive photo-generate ROS.\(^{51,58,59}\)

CDs’ bactericidal functions were reported for the first time in 2016, Meziani et al.\(^{60}\) demonstrated that CDs could be readily activated by relatively weak visible light. The CDs treatment coupled with visible-light illumination was effective for bacteria (Figure 5A). About four logs of \(E.\ coli\) cells were killed under the visible-light illumination, that is \(10^4\) times for the same treatment in the dark (Figure 5B). Abu Rabe et al.\(^{27}\) prepared relatively simple chemical compositions and nanoscale structures of CDs using oligomeric polyethyleneimine for surface functionalization–passivation in 2020 (Figure 5C). The CDs could be readily activated under visible light for the highly effective killing of \(MDR\) bacteria. And the antibacterial activity gradually increased with the enhancement of light intensity, as the light intensity up to 7.6 mW/cm\(^2\), the CDs completely inactivated both \(Enterococcus\ faecalis\) and \(Enterococcus\ faecium\) (Figure 5D). Sidhu et al.\(^{47}\) synthesized penicillin carbon dots (PCDs) with penicillin as a precursor, which retained the penicillin antibacterial activity and made the CDs more aggressive toward pathogenic microbes in 2017. In the absence of visible light, the PCDs (20 \(\mu g/ml\)) significantly reduce the growth of \(S.\ aureus\) and \(E.\ coli\) by 15% and 33%, respectively. In visible light, the PCDs exhibit a more pronounced effect against \(S.\ aureus\) and \(E.\ coli\). More importantly, it also possesses a special effect against resistant bacteria (\(MDR\ E.\ coli,\ methicillin-resistant S.\ aureus\ [MRSA]\)). At a 100 \(\mu g/ml\) concentration, PCDs inhibited the growth of \(MDR\ E.\ coli\) and MRSA by more than 50% under illumination. Under the inverted scanning electron microscope, nontreated cells possessed smooth and clear cell walls, while treated cells had damaged and wrinkled cell walls. Damaged cell walls resulted in the leakage of cellular contents (Figure 5E). Furthermore, visible light-activated CDs diminished the infectivity of bacteriophages MS2 into the host \(E.\ coli\) cells. CDs use the photodynamic effect to carbonylate proteins and degrade viral genomic RNA to kill viruses.\(^{61}\) The specific antivirus mechanism of CDs was discussed in the next section.

### 2.3 Antiviral mechanism of action of CDs

Virus infection must complete the four flowing stages: attachment, penetration, replication, and budding.
FIGURE 6  (A) Schematic representation of virus infection process. Reproduced with permission: Copyright 2019, John Wiley and Sons.65  (B) Identification of polyamine CQDs antiviral ability in vitro by real-time PCR. (C) Comparison of the number of polyamine CQDs around control and polyamine CQDs-containing virus. (D) Identification of CQD antiviral ability in vivo. Reproduced with permission: Copyright 2020, Springer Nature.62  (E) C-dots interact in the early step of HSV-1 infection of cells: schematic representation of CDs treatment. (a) Cells were treated with CDs before inoculating with HSV-1; (b) cells were inoculated with HSV-1 mixed with CDs; (c) cells were inoculated with HSV-1 afterward C-dots were added. (F,G) Viability of A549 cells (F) and Vero cells (G) (6× 10^3 cells per well) infected with HSV-1 (10^3 TCID50 mL^-1) treated with 10 μg/mL CDs. Reproduced with permission: Copyright 2016, American Chemical Society.63  (H) Influence on JEV infection by cell- or virion-treated with 75 μg/ml BZM-CDs. (I) The cell-binding assay detected viral titers in the cell lysates. (J) TEM images of BZM-CDs-treated virus. JEV was treated with DMEM (left), BZM-CDs (right). CD, carbon dot. Reproduced with permission: Copyright 2019, Elsever.64
(Figure 6A). The CDs can destroy virus infection in different stages via inhibiting virus attachment, blocking virus penetration, interfering with viral replication, and preventing viral budding.\textsuperscript{13} Viral attachment and penetration to a host cell are essential to be infected. Thus, inhibiting the first two steps will effectively inactivate the virus. Huang et al.\textsuperscript{62} found that the polyamine CQDs can inhibit the virus infection by attaching to the white spot syndrome virus (WSSV) envelope in 2020. The inhibitory effects are dose-dependent (Figure 6B). Many polyamine CQDs were observed attached to the envelope of polyamine CQD-treated virus by cryo-electron microscopy, which was higher than the control group (Figure 6C). Then the shrimps infected by WSSV were fed with polyamine CQDs-containing feed, and the mortality rates were significantly lower than those of the control group (Figure 6D). These demonstrated CQDs could inhibit the WSSV infection of shrimp, improving survival prospects. This antivirus mechanism of the CDs is considered as envelop structure of the virus was destroyed, and the penetration was blocked. Actually, CDs can bind with virus envelope or host-cell member to inhibit virus attachment and block penetration. Barras et al.\textsuperscript{63} reported that benzoxazine monomer-derived CDs could effectively inhibit the infection of the Japanese encephalitis virus and other flaviviruses in 2016. As shown in Figure 6F, to certify the cause of CDs inhibiting virus attachment, the CDs were added before, during, and after the infection by HSV-1. The cell viability of A549 (Figure 6F) and Vero cells (Figure 6G) are the highest when the CDs are first incubated with the virus. These indicate that the CDs directly contact the virus membrane, interfering with host cell invasion. That is the leading cause of inhibition infection rather than the host cells mounted an antiviral reaction. Huang et al.\textsuperscript{64} provided evidence that biocompatible CDs affected the cell-binding capability of viral particles in 2019. The BZM-CDs pretreat the virus and host cell, respectively. Subsequently, the viral titers data shows the virus-treated group more extraordinarily reduced the viral titers than cell treated group (Figure 6H). From the TEM images (Figure 7I) of Japanese encephalitis virus (JEV) virions treated by Dulbecco's modified Eagle's medium (DMEM) and BZM-CDs, the BZM-CDs can be absorbed by the JEV. After the virus treated by BZM-CDs enters the host cell, the viral titers of cell lysates are significantly lower than those of the untreated group (Figure 7I). These indicate that BZM-CDs can bind with the virus, have the capability of weakened virus-cell binding, and block the pre-entry step of the viral lifecycle.

After the virus enters a host cell, the most effective strategies for inhibiting infection are to block replication or prevent budding. CQDs synthesized from curcumin (Cur-CQDs) are effective antiviral agents against enterovirus 71 (EV71). It can bind with the virus to prevent the attachment of the virus and inhibit the viral replication process, terminate the EV71- of host translation, and inhibit the expression of phosphorylated p38 kinase.\textsuperscript{65} Tong et al.\textsuperscript{66} used glycyrrhizin acid as a precursor to synthesize Gly-CDs in 2020, which exhibited high antiviral activity against the respiratory syndrome virus (PRRSV). By analyzing their effects on the PRRSV proliferation at the adsorption, invasion, replication, and release stages, they suggested that Gly-CDs did not inhibit PRRSV adsorption and the release of progeny PRRSV (Figure 7A–D). Gly-CDs may suppress PRRSV mainly by inhibiting the PRRSV invasion and replication stages. Curcumin cationic dots (CCM-CDs) was synthesized by a one-step method, and it was found to inhibit the proliferation of the porcine pandemic diarrhea virus (PEDV) (Figure 7E). When the PEDV was added to the CCM-CDs solution, the zeta potentials changed from positively charged to electrically neutral (Figure 7F). The PL intensity decreased, and the maximum emission wavelength was red-shifted (Figure 7G). These suggested PEDV can interact with CCM-CDs by electrostatic interaction. When the CCM-CDs were treated after attaching PEDV, the viral titers were not noticeably decreased with the concentration increase of CCM-CDs (Figure 7H), indicating that the CCM-CDs did not block virus penetration. At the different postinfection times, the negative-strand RNA level of PEDV in CCM-CDs-treated cells is significantly lower than that of the untreated control (Figure 7I). That indicated that CCM-CDs inhibited replication of PEDV in the host cell. In the intracellular, the PEDV intracellular titers of CCM-CDs-treatment groups showed significant downregulation with the untreated control groups after various CCM-CDs treatment times (Figure 7J). But in the supernatant, the virus titer of CCM-CDs-treatment groups was slightly lower than those in the control group (Figure 7K). These indicated that the CCM-CDs could prevent PEDV viral budding.\textsuperscript{67} Even though these processes as effective antiviral mechanisms of CDs have been demonstrated, the exact mode of the action has never been mentioned, and it remains ambiguous until now. Therefore, it is necessary to explore the concrete process of inactivating and killing viruses by CDs.

3 | IMPACT FACTORS OF CDs ANTIMICROBIAL ACTIVITY

In addition to environmental factors such as light intensity and time and concentration of CDs, the nature of the CDs also affects their antibacterial performance, such as size, surface charge, and surface charge.
3.1 Effect of CDs size

Both the internalization in the cells and the distribution of CDs in membranes depend on CDs’ size. Small particle size CDs are easier to run through the lipid bilayer and show more excellent antibacterial activity (Figure 8C). Sun et al. separate CDs into small-scale, middle-scale, and large-scale particle size groups in 2021 (average diameters are 2 nm (s-CDs), 4 nm (m-CDs), and 5 nm (l-CDs), respectively; Figure 8A) through 1k Da, 3.5k Da, 8k Da MWCO dialysis bags (Figure 8B). Figure 8D,E shows the survival rates of *E. coli* and *S. aureus* under the different concentrations of the three types of CGCDs treatment. The diffusion disk method and spread plate tests show that these CDs exhibit excellent antibacterial activity to *E. coli* and *S. aureus*. With the increasing diameters of CDs, the antibacterial activity significantly weakens at different concentrations of CGCDs. The measurement of O.D. 540 was used to summarize the MICs treated with s-CDs, m-CDs, and l-CDs, the corresponding MIC values are 75, 100, and 100 μg/ml against *E. coli*, and 50, 75, and 100 μg/ml for against *S. aureus* (Figure 8F,G). The MIC values more directly reflect that the small-size CDs possess more potent antibacterial activity, and these CDs are more effective for *S. aureus*. The smaller scale makes the CDs easier to cross the lipid bilayers and interact with the DNA molecules.

3.2 Effect of surface charge

During the application of antimicrobial CDs, contact between CDs and microbes is the first necessary step. It is well known that bacterial cell membrane is composed of the phospholipid bilayer. The phosphoryl and carboxylate substituents on their outer cell envelope can be ionized, which causes the exposed surfaces of bacteria to possess a net negative charge. Thus, positively charged CDs should be easily absorbed on bacterial cell membranes via electrostatic interactions, resulting in better antimicrobial activity. A previous study by Abbazadehgan
et al. has shown that the antimicrobial activity of positively charged, neutral, and negatively charged silver NPs exhibited sequential decreases in antibacterial activity. Bing et al. prepared the positively charged SC-dots, negatively charged CC-dots, and neutral charged GC-dots with spermine, candle-soot, and glucose as precursors in 2016. As shown in Figure 9A, the SC-dots exhibit strong bactericidal activity, but the CC-dots and GC-dots almost cannot affect the E. coli survival. The growth curve of strain treated by three types of CDs indicates that the SC-dots exhibit bactericidal activity, the CC-dots can inhibit bacterial growth, and the GC-dots had no effect on bacterial growth (Figure 9B). And SC-dots possess good biocompatibility and low toxicity compared with CC-dots and GC-dots (Figure 9C). In addition, the SC-dots generate more endogenous ROS than CC-dots and GC-dots (Figure 9D), which also is one of the antibacterial mechanisms of SC-dots. Verma et al. synthesized positive CDs (PCDs), negative CDs (NCDs), and neutral CDs (UCDs) by a microwave method with diethylene glycol (DEG) as the carbon source and an amine-containing molecule as the surface passivating agent in 2020. The bactericidal activity of these CDs was investigated using E. coli as the model bacteria. The results suggested that NCDs were the least effective, while positively charged and neutral CDs were the most effective against E. coli (Figure 9G). The PI base FACS was used to probe the primary bacterial cell mechanism, which shows the maximum number of compromised cells treated by UCDs (Figure 9E). Different from previous reports, UCDs were more effective against E. coli than PCDs, caused by their different chemical composition, UCDs produced more ROS (Figure 9F), leading to the deformations of the bacterial cell wall (Figure 9H).

3.3 Effect of surface-functionalization

Due to the special core-shell structures of CDs, the properties can be regulated via tuning the shell composition. Thus, CDs’ shell passivation/functionalization is particularly important for antibacterial activity. Spermine and putrescine play an essential role in bacterial cell metabolic processes. They are tightly regulated by the influx/efflux mechanisms and cellular metabolism and protect porin and polyamine transport functional and structural stabilities. Thus, the functional groups of CDs directly affect the CDs’ permeability of the bacteria membrane, which acts the antibacterial activity. Gagic et al. prepared functionalized CQDs (HCQDs, PCQDs, CCQDs, and SCQDs) by capping with histamine, putrescine, cadaverine, and spermine in 2020 (Figure 10A). In the different

FIGURE 8 (A) Schematic diagram separates different particle sizes through dialysis membranes with different cut-off molecular weights. (B) Particle size distribution statistics of s-CGCDs, m-CGCDs, and l-CGCDs. (C) Schematic diagram of possible antibacterial differences of s-CGCDs, m-CGCDs, and l-CGCDs against bacterial cells. (D,E) The bacterial survival rates of Escherichia coli and Staphylococcus aureus after treatment with different concentrations of s-CGCDs, m-CGCDs, and l-CGCDs on LB nutrient agar medium for 24 h. (F,G) The O.D. 540nm value of s-CGCDs, m-CGCDs, and l-CGCDs treated with E. coli and S. aureus (5 × 10^6 CFU/ml) in LB broth medium for 24 h. Reproduced with permission: Copyright 2021, Elsevier.
concentrations of these CDs, all the CDs exhibit excellent 100% antibacterial activity for *S. aureus*. The antibacterial activity of PCQDs and SCQDs is more vigorous than HCQDs and CCQDs against Gram-negative bacteria (Figure 10B). That could be because the PCQDs and SCQDs can disturb polyamine transport by interacting with porin proteins on the bacterial membrane surface (Figure 10A). Strong interactions destabilize the cell membrane’s function and inhibit membrane synthesis. Thus, these CDs also possess potent antibacterial activity (Figure 10C). Travlou et al.\(^77\) obtained sulfur and nitrogen-doped carbon quantum dots (S-CQDs and N-CQDs) by hydrothermal treatment in 2018. Bacterial tests showed that the N-CQDs were more effective than S-CQDs because amides and amines played the most crucial role in enhancing bactericidal function (Figure 10D–F). The nitrogen functional groups could be protonated and engage in electrostatic interactions with the negatively-charged cell membranes.

Abu Rabe et al.\(^58\) synthesized PEI/CA-CDs with pH values of 7.5 (CDs-1), 8.6 (CDs-2), and 9.7 (CDs-3) by tuning the composition of the PEI and CA mixture and changing the processing conditions in 2019 (Figure 11A). As shown in Figure 11B, PEI/CA-CDs-3 exhibit the best antibacterial activity under the light illumination, wholly killing the *Bacillus subtilis*, but the PEI/CA-CDs-1 and PEI/CA-CDs-2 are no or minimal effect on *B. subtilis* survival rate. In the mixture of PBS buffer solution of bacterial cells and CDs, the pH was around 7.4, and the NH\(_2\) terminal groups were protonated as \(\text{−NH}_3^+\). Thus, PEI/CA-CDs-3 had a more significant positive charge, was favorable to bacterial surface adhesion, and possessed the highest antibacterial activity. In addition, they also
regulated the thickness of the surface passivation layer by changing the molecular weight of PEI (~1200 in PEI Cdots and ~600 in PEI 600 Cdots), and PEI 600 Cdots possess a thinner passivation layer than PEI Cdots. The antibacterial experimental results show that the thinner surface passivation layer CDs possess more effective antibacterial activity (Figure 11C), and the PEI 600 Cdots completely inactivate the cell at lower concentrations than PEI Cdots (Figure 11D), that due to the photogenerated ROS being more active to the bacterial cell.

3.4 | Effect of precursors

In addition to the properties of the CDs, the precursors of CDs appreciably impact the antibacterial activity and bacteriostatic species. CDs could preserve some carbon source characteristics during the carbonization process. Thus, antibiotics, medicinal, and some biomass become the ideal precursors for synthesizing antibacterial CDs. Table 1 summarizes the antibacterial activity, bacteriostatic species, and antibacterial conditions of different precursor synthetic CDs. The precursors are mainly natural biomass, antibiotic drugs, and chemicals.

From Table 1, the antimicrobial effect of CDs synthesized by biomass is not outstanding. Most MIC values are between 100 and 1000 μg/ml, but they always possess excellent biocompatibility. When the chemicals as the carbon source, CDs synthesized from quaternary ammonium salts, and polyamines exhibit high antibacterial activity, the MIC value is below 10 μg/ml. And the MIC value of halogen-doped CDs is below 1 μg/ml, which even better than that of the antibiotic. Antibiotic types of CDs also have good
antibacterial activity but are always lower than their precursors.

4 | THE PRACTICAL APPLICATIONS

4.1 | Biomaterials for healing wounds

CDs were widely used as antimicrobial candidates for treating wounds and skin infections due to their excellent water solubility, biocompatibility, low cytotoxicity, and significant antimicrobial properties. They have been made into various dressing materials to cover wounds, prevent bacterial infections, and accelerate wound healing. Upon visible light irradiation, the ROS generated by CDs enhances the antibacterial effects and speeds up the sterilization, thereby promoting wound healing. Furthermore, minimal amounts of ROS can act as essential mediators of cell signaling and accelerate wound healing, promoting the skin cells to participate in skin regeneration actively. Zhang et al. prepared BPs@CDs composite material through situ growth of cationic CDs on decorated black phosphorus nanosheets (BPs) in 2021 (Figure 12A,B). The vitro investigations demonstrated that BPs@CDs possessed contact-responsiveness and photo-responsiveness. It also exhibits high antibacterial activity, with or without laser illumination. In vivo, BPs@CDs significantly accelerated wound closure, promoted skin tissue regeneration (Figure 12C,D), and prevented bacterial infection in the wound (Figure 12E). Cui et al. used cationic CDs, pectin, and acrylic acid as precursors to developing an antibacterial hydrogel in 2021, which possesses excellent fluorescent properties, favorable biocompatibility, high adhesiveness, and mechanical properties strength. The
| CDs label | Type of precursor | Precursor | Light (yes/no) | Microorganisms | Antimicrobial effect | Refs. |
|-----------|-------------------|-----------|----------------|----------------|----------------------|-------|
| Ag@CDs    | Biomass           | Cannabis sativa leaf | No             | E. coli, S. aureus | MIC: 42 μg/ml        | 79    |
| CQDs      | Sugarcane bagasse pulp | No         | E. coli, S. aureus, B. cereus, V. cholera, P. aeruginosa | Zone of inhibition diameter: 14, 22, 30, 25, 24 mm, respectively. | 39    |
| NS-CQDs   | Natural honey     | Yes        | S. aureus, B. cereus, S. flexneri, P. aeruginosa, V. cholera, and E. coli | Zone of inhibition diameter (10 μg/ml): 22, 30, 15, 25, 31 mm, respectively. | 80    |
| Degradable CDs | Cigarette smoke | No | E. coli, KREC, S. aureus, AREC, | MIC (E. coli, KREC): 1000 μg/ml | 45    |
| Hydrophilic CQDs | Guar gum | No | B. cereus, E. coli, C. albicans | MIC: 350, 450 μg/mL | 81    |
| T-CDs     | Turmeric leaves   | No         | E. coli, K. pneumoniae, S. aureus, S. epidermidis | MIC: 250, 1000, 250, 1000 μg/mL, respectively. | 82    |
| ACDs      | Artemisia argyi leaves | No           | E. coli, P. aeruginosa, P. vulgaris, S. aureus, B. subtilis | MIC (E. coli, P. aeruginosa, P. vulgaris): 150 μg/mL | 83    |
| OCDs      | Osmanthus leaves  | No         | E. coli, S. aureus | Inhibition rate (1000 μg/ml): 86.91% and 96.63% | 42    |
| TCDs      | Tea leaves        | No         | E. coli, S. aureus | Inhibition rate (1000 μg/ml): 62% and 90% | 84    |
| MCDs      | Milk vetch       | No         | E. coli, S. aureus | Inhibition rate (1000 μg/ml): 30% and 30% | 85    |
| O-CDs     | Oyster mushroom  | No         | K. pneumoniae, P. aeruginosa. | MIC: 30 μg/ml | 84    |
| L-CDs     | L. inermis leaves | No         | E. coli, S. aureus | MIC: 5000 μg/ml | 85    |
| Negative charge CDs | Chemicals | Citric acid and urea | No | MRSA, VISA | MIC (MRSA, VISA): 630 mg/ml | 86    |
| PEI-CD₈  | Glucose and PEI  | No         | E. coli, S. aureus | MIC: 32 and 64 μg/mL, respectively. | 87    |
| PEI-CD₁  | Glucose and PEI  | No         | E. coli, P. aeruginosa, S. aureus, B. subtilis | MIC: 375 μg/ml | 88    |
| Water-solvable CDs | Curcumin and citric acid | No | E. coli, P. aeruginosa, S. aureus, B. subtilis | MIC: 375 μg/ml | 88    |
| PS-CDs    | Protamine sulfate | No         | S. aureus, B. subtilis, MRSA, P. aeruginosa, E. coli, | MIC: 25, 100, 37.5, 150, 2000 μg/mL, respectively. | 89    |
| PEI-CDs   | Glucose and PEI  | No         | S. aureus | MIC: 4.7 μg/ml | 35    |

(Continues)
| CDs label | Type of precursor | Precursor | Light (yes/no) | Microorganisms | Antimicrobial effect | Refs. |
|-----------|------------------|-----------|----------------|----------------|----------------------|-------|
| CDs-3     |                  | Tartaric acid and m-aminophenol | No            | *B. subtilis*  | MIC (*B. subtilis*): 250 μg/ml | 42    |
| Cl/N-PGQDs Br/N-PGQDs I/N-PGQDs | Spermidine, HCl, HBr, HI | Yes | *E. coli, S. aureus* | MIC: 0.005, 0.05 μg/ml, respectively. | 90    |
| N-CQDs    |                  | Bis-quaternary ammonium salt | No | Ampicillin-resistant *E. coli*, *S. aureus*, MRSA | MIC: 8.0, 4.0, 8.0, 2.0 μg/ml, respectively. | 91    |
| NCQDs     |                  | d-glucose and DETA | No | *S. epidermidis* | MIC: 500 μg/ml | 92    |
| CNDs-250  | Antibiotic       | Metronidazole | No | *P. gingivalis* | Inhibition rate (1.25 μg/ml): 71.7% | 93    |
| PCDs      | Penicillin       | Yes | *E. coli, S. aureus, MDR E. coli, MRSA* | Inhibition rate (20 μg/ml): 71%, 85%, 65%, 70%, respectively. | 47    |
| g-CDs     |                  | Ciprofloxacin hydrochloride | No | *E. coli, S. aureus* | MIC: 1.0 and 0.025 500 μg/ml, respectively. | 94    |
| CDs-AMP   |                  | CA, EDA, ampicillin | Yes | *E. coli* | MIC: 14 500 μg/ml | 95    |
| AMP-CDs   | Ampicillin       | Yes | *S. aureus* | MIC: 700 500 μg/ml | 96    |
| CQDGents-180 |                  | Gentamicin sulfate | No | *E. coli, S. aureus* | MIC: 203.4, 1.09 500 μg/ml, respectively. | 97    |

Abbreviations: AREC, ampicillin-resistant *E. coli*; AMP, ampicillin; CA, citric acid; DETA, diethylenetriamine; EDA, ethylenediamine; KREC, kanamycin-resistant *E. coli*; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; PEI, polyethylenimine; VISA, vancomycin-intermediate *S. aureus*. 
FIGURE 12  (A) A cartoon illustration of the preparation process of CDs@BPs (black phosphorus nanosheets) antibacterial nanomaterial. (B) Schematic diagram of the antibacterial principle of CDs@BPs. (C) Thermal images of the control group and the BPs@CDs group using NIR irradiation at 808 nm and 1.5 W/cm². (D) Control group and BPs@CDs group wound healing photographs on Days 0, 2, 5, and 7. (E) The number of Staphylococcus aureus in the wound tissue on the 7th day. Reproduced with permission: Copyright 2021, Springer Nature.101 (F) Schematics of the fluorescent antibacterial CDs hydrogel antibacterial in vivo. (G) Images of histological examination of the tissues surrounding the hydrogel, (a) control group, (b) SCDs-AP group. Reproduced with permission: Copyright 2021, Elsevier.102 (H) Schematic representations of the synthesis of spermidine-modified carbon quantum dots (Spd-CQDs) from ammonium citrate and spermidine and their effective antimicrobial activity for wound healing. Reproduced with permission: Copyright 2016, John Wiley and Sons.103 (I) Schematic representation of the one-step dry heating synthesis of fluorescent carbon quantum dots (CQDspds) from spermidine and their treatment for S. aureus-induced bacterial keratitis. (J) The slit-lamp biomicroscopic images of time-course in vivo therapeutic efficacy of Ag NPs, CQDspds, or SMX eye drops (4% or 0.4%) after topical instillation in rabbit eyes with experimentally induced bacterial keratitis. CD, carbon dot. Reproduced with permission: Copyright 2017, American Chemical Society.75
bacteria growth causes the change in the ambient environment, resulting in the broken hydrogen bond between the CDs and hydrogel. Subsequently, the CDs were released and damaged the cell membrane to kill bacteria. Thus, this material possesses strong antibacterial activity (also contains multidrug-resistant bacteria). In this antibacterial process, the release CDs are slow, and only occur in bacterial infection environment. The SCD-AP hydrogels can inactive S. aureus and inhibit inflammation, and repair the skin tissue as well (Figure 12F). Combined with multicolor fluorescence property, this hydrogel can be developed to a dual-function material for the in situ monitoring and prevention of bacterial infections to treatment of wounds (Figure 12F). Li et al. synthesized antibacterial spermidine-capped fluorescent carbon quantum dots (Spd-CQDs) by a two-step method in 2016. Spd-CQDs exhibited strong antibacterial ability by disrupting the bacterial membrane, and the antibacterial activity of Spd-CQDs is much higher than free spermidine. The Spd-CQDs were used as an antibacterial dressing to treat infected wounds. The results show Spd-CQDs can speed wound healing by promoting the production of collagen fibers and epithelialization. Based on their excellent properties, Spd-CQDs have become a promising antimicrobial candidate for treating wounds and skin infections in preclinical (Figure 12H). Jian et al. used CDs solution from biogenic polyamines as eye drops to treat bacterial keratitis in 2017 (Figure 12I). The CDs show good biocompatibility and can induce the opening of the tight junction corneal epithelial cells. It has a strong therapeutic effect on rabbit bacterial keratitis induced by S. aureus (Figure 12J). Thus, CDs show great potential as viable alternatives to conventional eye drops.
for treating ocular diseases caused by bacterial infections.\textsuperscript{104}

4.2 Antimicrobial coatings

Matrix and carrier are necessary for CDs in biosensing, drug delivery, and bioimaging applications. Due to the polymer’s unique properties, it has become the favorite material to combine with CDs. And CDs-polymers composites have gradually permeated many aspects of CDs. Therefore, CDs nanocomposites can also be used as antimicrobial agents.\textsuperscript{105} Stanković et al.\textsuperscript{106} deposited uniform and homogeneous Langmuir Blodgett (LB) hydrophobic CDs thin films on different substrates in 2018 (Figure 13A,C). This kind of CDs generated singlet oxygen under blue light irradiation. The antibacterial and anti-biofouling tests on the films showed that the growth of bacteria (\textit{E. coli} and \textit{B. cereus}) was inhibited by several orders of magnitude on surfaces coated with LB hCQDs (Figure 13B). Moreover, the hydrophobic CDs thin film showed good biocompatibility. These properties enabled the potential usage of hydrophobic CDs thin films in antibacterial and anti-biofouling coatings. Kováčová et al.\textsuperscript{107} used the swell-encapsulation-shrink method to produce a hydrophobic CQDs/polyurethane nanocomposite in 2018. Under the low-power blue light irradiation, it can effectively generate singlet oxygen (Figure 13D). After 60 min irradiation, the growth of \textit{S. aureus} and \textit{E. coli} was effectively inhibited (Figure 13F). The nanocomposites have smooth and uniform surface without any streaks, it could be produced directly and coated on various surfaces such as wood, metal, glass, or plastics (Figure 13E). And an impermeable antibacterial barrier formed on the coated surface. It can be used to design catheters, hospital bedding, and wound dressings.\textsuperscript{108} Ye et al.\textsuperscript{109} immersed sterile medical gauze in 30 \textmu g/ml C-dots solution synthesized by p-phenylenediamine and prepared antibacterial gauze in 2020 (Figure 13G). The antibacterial tests indicated that antibacterial gauze can effectively inhibit the bacterial growth, no colonies grew in the area covered by the antibacterial gauze (Figure 13H). Marković et al.\textsuperscript{110} encapsulated hydrophobic CDs in medical-grade silicone-polydimethylsiloxane using a swelling-encapsulation-shrink method in 2019. The nanocomposites generated singlet oxygen initiated by 470 nm blue light irradiation and displayed excellent antibacterial properties towards \textit{S. aureus}, \textit{E. coli}, and \textit{Klebsiella pneumoniae}. The rough surface of the nanocomposites significantly contributed to the better surface adhesion of bacteria, which improved the antibacterial activity. It is a promising visible-light-triggered antibacterial material in sterile areas, such as hospitals, pharmaceuticals, and food industries. Sobhi and Sunish\textsuperscript{111} synthesized CDs composites by dispersing green N and S-doped CDs in biocompatible polymeric matrices in 2021. The CDs composites enhanced the stability and photoluminescence properties and displayed antibacterial activity. These composites have potential applications for targeted drug delivery, the development of drug delivery devices, and bioimaging studies.

5 SUMMARY AND OUTLOOK/PROSPECT

Compared with traditional antibacterial drugs, CDs possess good biocompatibility and solubility, excellent optical properties, and rich and adjustable surface functional groups. Their photodynamic antibacterial mode is an effective strategy to combat bacterial resistance. CDs can affect bacteria metabolism and destroy the cell membrane by adsorbing onto bacterial cell membranes. The photogenerated ROS can destroy the bacterial membranes to kill bacteria. During the antimicrobial process of CDs, the CDs particle size, surface functional groups, surface charges, light intensity, and precursors of the synthetic CDs affect their antimicrobial activity. CDs can be made into various dressing materials and compounded with different polymer composite materials as wound dressings to prevent bacterial infections and accelerate wound healing. Their unique properties can also be exploited to design multifunctional antimicrobial agents.

The current issues for the development of CDs antimicrobial mainly include: (a) structure of CDs is ambiguous, lack of methods for exact preparation. (b) The MIC value is higher than that of antimicrobial drugs. (c) Many CDs only kill Gram-positive bacteria. (d) The ROS produced by CDs also damage normal tissue cells during photodynamic antibacterial therapy. (e) Some CDs are not easily degradable and have poor water dispersibility. To make further breakthroughs in the future research of antimicrobial CDs, and promote the application, some challenges still need to be addressed:

(1) For the synthesis, defining the structure–activity relationship of the antimicrobial CDs is primary. Low-cost and large-scale preparation methods should be developed for consistent and exact structure CDs.

(2) Improve the antimicrobial activity of CDs. First, high-performing precursors should be selected as carbon sources of CDs, such as quaternary ammonium salts, polyamines, silver ions, and antibiotics. The new high-activity precursors should be developed. Second, the CDs’ particle size should be tuned
as small as possible to easily pass through the bacteria cell membrane. And try to make the surface charge of CDs positive, which is the benefit of binding between the CDs and bacteria cells. Finally, the shell of CDs should be functionalized by spermine, putrescine, or other molecules to make it easy to pass through and damage the membrane and efficiently kill bacteria.

(3) APDT mechanism of CDs should be used to achieve broad-spectrum antibacterial. The sterilization process should be under illumination. The CDs produce many ROS under the light excitation that induces the oxidative damage of cell membrane and DNA/RNA, and this process is effective for all microorganisms. This kind of CDs should possess good absorbing properties, and the PL emission must be weak.

(4) Excellent biocompatibility is one of the advantages of CDs, but producing ROS could be toxic for all cells. Thus, the photoexcited CDs should be enriched or exposed to pathogenic microorganisms. On the one hand, CDs should be functionalized to target the bacteria. On the other hand, CDs can be composited with polymer materials. The polymer can interact with bacteria, leading to the CDs being released in the infection site. These all can prevent the tissue cell from the effect of ROS.

(5) In the vivo applications of antimicrobial CDs, higher water solubility and dispersibility are essential for penetration and delivery. Thus, the precursors of CDs should be chosen as water-soluble substances. The CDs were prepared in high polarity solvent environment and/or modified by PEI or other aqueous polymers.

Based on the outstanding properties, after understanding the existing scientific problems and supplementing the performance deficiencies, CDs will become the high-activity antimicrobial agent to treat emerging infectious diseases and a new strategy to fight against antimicrobial-resistant infections.

ACKNOWLEDGMENTS
This study was financially supported by the Beijing Municipal High-Level Innovative Team Building Program (No. IDHT20180504), the Beijing Outstanding Young Scientists Program (No. BJWZYJH01201910005017), the National Natural Science Foundation of China (Nos. 21872001, 51801006, and 21805004), the Key Project of the National Natural Science Foundation of China (Nos. 21936001 and 21801092), the Beijing Natural Science Foundation (No. 2192005), and the Beijing Municipal Science and Natural Science Fund Project (No. KM201910005016).

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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How to cite this article: Li P, Sun L, Xue S, et al. Recent advances of carbon dots as new antimicrobial agents. *SmartMat.* 2022;3:226-248. doi:10.1002/smm2.1131