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CARBON DOTS - AN EMERGING NANOPARTICLE FOR NANOMEDICINE

Jeya Jeyamani [*1], Suguna Rajendran [2] and Renuka Radhakrishnan [3]

Department of Microbiology [1], Department of Biochemistry [3], V. V. Vanniaperumal College for Women, Virudhunagar - 626001, Tamil Nadu, India.
Department of Biotechnology [2], Madura College, Madurai - 625011, Tamil Nadu, India.

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Correspondence to Author:
Jeya Jeyamani

Assistant Professor,
Department of Microbiology,
V. V. Vanniaperumal College for Women, Virudhunagar - 626001,
Tamil Nadu, India.

E-mail: jeyasivakumar4@gmail.com

ABSTRACT: Carbon dots are small nanoparticles having a size of less than 10nm. They have distinctive properties such as chemical stability, water solubility, fluorescence emission, easy synthesis, and low toxicity. Because of their special properties, they have been applied in various scientific disciplines. The fluorescence property of Carbon dots enables them for various biomedical applications such as bioimaging, drug delivery, gene delivery, and cancer therapy. Green synthesized fluorescent carbon dots have biomedical applications, especially for imaging and treatment of *in-vitro* cancer cells. Carbon dots have visible/natural light-activated antibacterial activities. This review discusses the recent advances of carbon dots in the medical field and their applications.

INTRODUCTION: Nano-crystals of carbon materials with dimensions smaller than 10 nm are understood as Carbon quantum Dots (CDs) [1]. Carbon dots (C-dots) have gathered wide attention and considerable potential in biological applications. C-dots mainly contain abundant and nontoxic carbon, and they are different from other Nano Particle (NPs) families. Due to their biocompatibility, low toxicity, strong photoluminescence (PL), and synthetic and photograph steadiness, C-dots became a desirable material for bioimaging and, therefore the detection of various analytes [2]. C-dots ordinarily contain discrete, quasispherical NPs with sizes of about 10 nm.

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Sp2- characterized C-dots contain different functional groups like carbonyl, ether, epoxy, amino, carboxylic acid, and hydroxyl on their surface. The presence of such groups on C-dots results in their high hydrophilicity. The surface groups might impart fluorescence properties of the C-dots [3]. They exhibit optical properties like photoluminescence, chemiluminescence, electrochemical luminescence and photoinduced electron transfer [4]. Besides, the high aqueous dispersibility, biocompatibility, good elasticity in modification, high resistance to photo bleaching and chemical inertness make it well applicable in bio-imaging [5], bio-sensing [6], chemical-sensing, and biomedical applications.

Being a replacement quite fluorescent nanomaterial and having excellent biocompatibility, CDs are widely utilized in the world of bio-imaging both *in-vitro* and *in-vivo* and in diagnosis purposes, Photothermal also as photodynamic therapy and drug/gene delivery carriers [7, 8].

CDs could also be applied for the determination of cellular levels of biomolecules and ions (bio-sensor), like Cu^{2+}, Hg^{2+}, NO_3-[9], $C_6H_{12}O_6$, pH [10], H_2O_2, *etc*. In the current scenario, natural sources like ayurvedic plant leaves are highly attractive for producing various materials for agriculture and biomedical applications due to their biocompatibility [11]. In particular, fabricating carbon dots from ayurvedic plant leaves may be a new and upcoming development with huge potential within the field of biomedical diagnosis. Moreover, absorbance in broad near-infrared range and emission property of carbon dots makes them attractive and suitable for cell imaging and phototherapy [12]. C-dots are generally referred to as benign and nontoxic *in-vitro* and *in-vivo*. However, with their effective light-harvesting over a broad spectral range from UV to near-IR, C-dots have exhibited strong photodynamic effects with relevant uses in cancer therapy reported [13]. Similarly, photoexcited C-dots can synthesize Reactive Oxygen Species (ROS), which are known to kill/inhibit microorganisms.

Discovery of CDs: CDs were first synthesized in 2004 during the purification of single-wall carbon nanotubes (SWCNTs) [14]. In 2006, Carbon quantum dots were synthesized as stable photoluminescent carbon nanoparticles of different sizes [15]. Water-soluble CDs passivated with poly-propionyl ethylenimine-co-ethylenimine showed two photon-induced luminescence spectra and were used to detect human carcinoma MCF-7 cells.

Classification of CDs: CDs are classified based on their precise carbon core structure, surface groups, and properties into graphene quantum dots (GQDs), carbon quantum dots (CQDs), carbon nanodots (CNDs), and carbonized polymer dots (CPDs) [16]. The GQDs have one or few graphene sheets with lattices or chemical groups on the sting that give distinct properties, like the quantum confinement effect and edge effect [17, 18]. The CQDs are spherical and possess notable crystal lattices and chemical groups on the surface, which show intrinsic state luminescence and the quantum confinement effect of the CQDs' size. The CNDs do not possess lattice structure and polymer features but have a high carbonization degree with some chemical groups on the surface. Photoluminescence mainly originates from the surface state and subdomain state within the graphitic carbon core. The CPDs possess a polymer/carbon hybrid structure comprising of abundant functional groups/polymer chains on the surface and a carbon core.

Architecture of CDs: CDs are recently discovered nanoparticles. They are spherical in shape, having an average diameter but 10 nm. They have several functional groups, such as hydroxyl, carboxyl, carbonyl, amino, and epoxy groups, over their surfaces so that inorganic and organic moieties can bind. The functional groups on the surface of CDs provide hydrophilic or hydrophobic and thermodynamic stabilities in several solvents, especially in water [19]. Surface modifications of CDs with functional groups provide them with strong photoluminescent signals [20]. Surface modification of CDs by different functionalities, passivating agents, and solvents, reflects a sensible variation in their properties [21].

Synthesis of Carbon Dots: The C-dots are synthesized by carbonizing carbon sources. They are synthesized by two techniques, "bottom-up" and "top-down" methods. These can be achieved by means of chemical, electrochemical or physical systems [22]. Top–down strategies involve the fragmentation of carbon matter into carbon nanoparticles, including arc discharge, laser ablation, electrochemical approach, etc. However, drawbacks of this approach include the requirement of expensive materials, harsh reaction conditions, and long reaction times. On the other hand, bottom-up strategies incorporate template strategy, thermal routes, pyrolytic process, hydrothermal and aqueous method, supported synthetic technique, reverse micelle technique, microwave-assisted strategy, and substance oxidation.

Top-Down approach: "Top-down" methodology of synthesis of CDs includes laser ablation, electrochemical oxidation, and arc discharge method.

Electrochemical Method: The electrochemical method is used to synthesize ultrapure CDs from larger molecular matter like nanotube, graphite, and carbon fiber by an electrolytic process where larger organic molecules are used as an electrode within the presence of proper electrolytes. CDs were

synthesized from multiwalled carbon nanotubes within the presence of tetrabutylammonium perchlorate as the electrolyte by electrochemical method [23]. Then water-soluble pure CDs were prepared using graphite as an electrode within the presence of phosphate buffer at neutral pH and used in biosensor. Crystalline CDs synthesized from graphite exhibit photoluminescence (PL) properties and are employed in photocatalysis.

Laser Ablation Method: The laser ablation technique has been widely used to create CDs of various sizes. In the laser ablation route, complex organic macromolecules are exposed under laser radiation operated in CW or pulsed mode, and nanosized carbon particles are detached from the larger molecular structures. CDs are synthesized by graphite powder's laser excitation (1064nm, 10Hz) at 900° C and 75 kPa. CDs synthesized by using laser light wavelength 1064 nm from bulk graphite within the presence of ethanol have a broad spectrum peaked at 325nm. A laser irradiation technique synthesizes photoluminescent CDs with ~3 nm size from glassy carbon particles within the presence of polyethylene glycol 200are used in bioimaging for cancer epithelial human cells [24].

ARC Discharge Method: Arc discharge method synthesizes carbon nanotubes where a direct-current arc voltage is applied across two graphite electrodes immersed in an inert gas such as Helium. When pure graphite rods are used, MWCNTs are deposited on the cathode. When a graphite anode containing a metal catalyst iron or cobalt is used with a pure graphite cathode SWCNTs are generated.

CDs are accidentally synthesized by an arc discharge method during SWCNTs synthesis. SWCNTs are discharged across two graphite electrodes, forming small carbon fragments or CDs. Photoluminescent CDs prepared from pristine and SWCNTs using an arc discharge method show PL within the violet-blue and blue-green regions, respectively [60]. Recently, Boron- and nitrogen-doped QDs were synthesized using B2H6 and NH3 from graphite [25].

Bottom-up Approach: In the "bottom-up" methodology, CDs are synthesized from small molecules by microwave irradiation, hydrothermal, and pyrolysis method.

Microwave Irradiation Method: The microwave irradiation method of CDs synthesis is the carbonization of small organic molecules such as carbohydrates, citrate, and polymer silica nanocomposites. This method can provide uniform heat for the formation of CDs. The advantages of this method of CDs synthesis are that it is less time-consuming, cost-effective, and eco-friendly to the environment. CDs synthesized from carbohydrates using the Mw technique have outstanding photophysical characteristics and a rapid reaction time [26]. Using an Mw irradiation approach, Liu *et al.* created photoluminescent CDs from glycerol and 4, 7, 10-trioxo-1,13-tridecylenediamine as a surface-passivating agent.

Hydrothermal Method: Hydrothermal method is the popular method for synthesizing CDs. This method is a nontoxic, environmentally friendly, low-cost, and simple. In this process, an organic precursor solution is sealed in a synthetic reactor, where the reaction takes place at high temperature and pressure. The first one-pot hydrothermal technique for producing CD worked out from ascorbic acid in the presence of ethanol as a solvent in 2010. Pang *et al.* used a hydrothermal technique to synthesize co-doped nitrogen and sulphur in CDs (NS-CDs) generated from methionine [27].

Pyrolysis Method: Pyrolysis is a simple method to synthesize CDs from organic compounds through a simple chemical reaction carried out at very high temperatures in the presence of strong acids or bases. A one-step pyrolysis process is used to synthesize stable CD Guo *et al.* from hair (keratin) at 200°C with a reaction time of 24 hours. They successfully produced the CD and used it to detect Hg2+ with higher sensitivity and selectivity [28].

Highly photoluminescent nitrogen-doped CDs (NCDs), which are derived from guanidine chloride and citric acid by pyrolysis, and fluorescence quenching is observed in the presence of Fe_3+[29]. The NCDs obtained through its synthesis have been widely used in metal ion detection and biological imaging.

Green approach Forsynthesis of Carbon Dots: The green synthesis of nanoparticles is nowadays very popular than chemical methods. It is a very simple and economical process. They were using

plant and plant parts as bio-reductants of metal ions into their elemental form in size range 1- 100nm. Green synthesis of nanoparticles has several advantages, including lower failure probability, lower cost, and easy characterization [30]. The synthesis of nanoparticles from plant-based extracts at room temperature is simple and completed in a few minutes to a few hours. Metal salts are synthesized with plant extracts, silver (Ag), and gold (Au) NPs synthesized by using plant-based materials are safer than other metal nanoparticles. Chemical methods of nanoparticle synthesis release toxic chemicals that repeatedly pollute the environment and cause health hazards to humans [31]. Using plant extract and green synthesis methods reduces environmental pollution and toxicity.

In addition, carbon precursors such as coffee powder used, tea, grass, and light sediments are also used to develop carbon dots. The carbon dots produced by these precursors are abundant and economical. CDs synthesized from plant sources are simple, reproducible, eco-friendly, and low-cost [32]. Therefore, considerable efforts have been made to synthesize C-dots from abundant natural resources. For example, C-dots obtained from apple juice emit a wavelength of 428 nm, and when excited with 340 nm ultraviolet light, the quantum yield is 6.4% [33].

The quantum yield of C-dots made from strawberries is 6.3%. When they are excited by a wavelength of 340 nm, the maximum wavelength is 427 nm. The quantum yield of C-dots obtained from orange juice is 26%. Recently, C-dots from Acacia seeds have also been reported, which show strong emission at 468 nm when excited at 390 nm, with a quantum yield of 10.2% [34].

In addition to juice, plant wastes such as grapefruit peel, willow bark, and watermelon peel can also be used to synthesize C-dots. Most natural C-dots emit short wavelengths when excited by short excitation wavelengths. However, short, high-energy excitation wavelengths can cause tissues and cells to be destroyed, which limits their applications in bioimaging and biomedicine. The synthesized carbon dots are characterized by numerous techniques such as Nuclear magnetic resonance (NMR), X-Ray diffraction (XRD), transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FTIR), UV spectroscopy and photoluminescence.

Biomedical Applications of CDs: C-dots play an important role in biomedical applications. Because of their small size and good biocompatibility, they are widely used in biomedicine. Special physicochemical and catalytic properties, low toxicity, high hydrophilicity, water solubility, fluorescence emission, and chemical stability make it suitable for some biomedical applications. Here, we discussed some important biomedical uses of c-dots and the latest developments in each field.

Bioimaging: Biological imaging generates images of biological bodies using technologies such as magnetic resonance imaging (MRI), X-rays, and ultrasound. It is also used to determine three-dimensional structural information. Lei et al. produced water-soluble, blue and yellow fluorescent CDs from carbon black and nitric acid, which are introduced into HepG2 cells and used for biological imaging [35]. TTDD (4, 7, 10 trioxa 1, 13 tridecanediamine) inactivated CD can use to image COS7 cells by Joe *et al.* Highly fluorescent graphene dots (GD) synthesized from graphene oxide by a one-step solvothermal method by Zhu *et al.* (2011) He used this CD for excitation-dependent fluorescence bioimaging of MG63 cells [36].

Biocompatible CD/silica (silica-encapsulated CD) nanoparticles with a size of 12 nm were produced using the decomposition pyrolysis method, and Wang *et al.* used this CD for bioimaging of BGC823 cells [37]. Yang *et al.* used a glucose-derived green fluorescent CD as a bioimaging agent to label HepG2 cells. They also checked the intracellular localization of CD by counterstaining with 40.6 diamidino-2-phenylindole (DAPI). They showed the cytoplasmic localization of CD around the nucleus [38]. The water-soluble fluorescent CD produced from carbon fiber was used by Peng *et al.* for the biological imaging of breast cancer T47D cancer cells [39]. Yellow fluorescent CD derived from carbon nanotubes and graphite for *in-vivo* bioimaging and labelling purposes have reported by Tao *et al.* Zhang *et al.* used an electrochemical process was used to synthesize a yellow fluorescent CD with a diameter of 5 to 10 nm from a graphite rod, then reduced with hydrazine, and used for

bioimaging of human lung A549 and breast MCF7 cancer cells [40]. The CD (15 nm) was obtained by chemical oxidation of XC72 black by Dong *et al.*, and he used this CD to label MCF7 breast cancer cells [41].

For bioimaging of MC3T3 cells, Zhu *et al.* used CD derived from citric acid and ethylenediamine [42]. Chen *et al.* used synthetic CD from sucrose to image 16HBE cells at 488 nm [43]. Hu *et al.* synthesized the blue luminescent N-doped CD by hydrothermal treatment for imaging HeLa cells. N-doped CD based on folic acid synthesized by Wang *et al.* is used to image U87 glioma cells [44]. Ge *et al.* synthesized a re-emission CD derived from polythiophene-phenyl propionic acid [45]. and used for *in-vitro* and *in-vivo* imaging. Krishna *et al.* used citric acid, PEG-diamine and glycerol to synthesize CD and functionalize it with digitonin for cholesterol detection and imaging [46]. Parvin *et al.* used the hydrothermal synthesis of N and P co-doped CD for biological imaging of RAW 264.7 cells [47]. Boss *et al.* used CDs derived from urea and polyethylene glycol (PEG) for bioimaging in L929 cells.

Multimodal biological imaging can be defined as a combination of optical imaging and MRI modalities. MRI shows high spatial resolution and the potential to obtain anatomical and physiological information. On the other hand, rapid screening is determined by optical imaging. For example, for multimodal bioimaging, CD doped with iron oxide can be used for bioimaging of spleen cells. Due to the biocompatibility of CD, the combination of different imaging techniques and CD fluorescence imaging also has advantages [48]. Loading CDs with enzyme-responsive mesoporous silica nanocarriers, with a pH-adjustable zwitterionic surface, can be used for targeted imaging and drug delivery to tumors [49].

Drug Delivery: Drug delivery systems require the design of systems capable of delivering a drug to a specific target in the body and the appropriate drug interaction with that target. Drug-conjugated nanostructured materials can improve drug delivery through drug absorption, distribution, and elimination. CDs can be used as a means of drug delivery due to their synthesis from inexpensive sources, easy surface functionalization, small size and increased biocompatibility. Furthermore, the assay route is also followed due to the intrinsic nature of CDs fluorescence.

Zhou *et al.* used fluorescent CD-gated mesoporous silica nanoparticles (MSPs) as a pH-responsive drug carrier and bioimaging system to deliver the antineoplastic antibiotic (DOX) into cancer cells. These CD-gated mesoporous oxide nanoparticles (MSPs) were biocompatible and showed robust glow each *in-vitro* and *in-vivo*. DOX-loaded CD-gated mesoporous oxide nanoparticles (MSPs) entered cancer cells via endocytosis, showed a pH-responsive drug unleash behaviour in gently acidic conditions, and increased toxicity in Hela cells [50].

CDs synthesized with HA (hyaluronic acid) were targeted onto tumor cells by Yang *et al.* For a drug delivery system, the β-cyclodextrin (βCD), oligoethylenimine (OEI), and Phosphoric acid-based green luminescent CDs. These CDs were noncytotoxic and possessed green fluorescence in H1299 cells. The DOX-loaded CD nano complexes exhibit more cytotoxicity towards H1299 cells than that of free DOX [51].

The CDs/DOX nano complexes synthesized by Wang *et al.* used for an image-guided drug delivery system. They produced the CDs from citric acid and o-phenylenediamine, and the positively charged DOX was loaded on the surface of negatively charged CDs through electrostatic interactions. These CDs have less fluorescence after loading with DOX, and strong fluorescence after the release of DOX from the surface of the CDs; hence it was used as an image-guided drug delivery system [52]. These CDs have high cytotoxicity towards cancerous cells (HeLa).

Recently, citric acid and ethylenediamine-based CDs synthesized by Kong *et al.* were used for drug delivery systems. They show higher cellular uptake and antitumor efficiency on the breast cancer MCF-7 cells than free DOX [53]. Zheng *et al.* synthesized biocompatible fluorescent CDs with an anticancer drug, cytotoxic oxaliplatin (CD-Oxa) *via* condensation reaction between the carboxyl groups of oxaliplatin derivative and the amino groups on the CD surface to increase its anticancer efficacy. These CDs can be used as image-guided drug delivery systems and have both *in-vitro* and *in-vivo*

a-plications. By using citric acid and diethylenetriamine as the precursor, Feng *et al.* synthesized cisplatin (IV) pro-drug-loaded charge convertible CDs. These CDs were used for image-guided drug delivery and had a higher therapeutic capability in the tumour extracellular environment. The charge convertible CDs loaded with ciprofloxacin (a broad-spectrum antibiotic) acted as an efficient nanocarrier for controlled drug release.

Gene Delivery: Nowadays, Gene therapy is used to correct the origin of diseases. It is based on the delivery and expression of exogenous DNA encoding for the missing or defective gene product. Gene vector is an important factor for gene therapy [54].

The CDs with unique properties such as biocompatibility, low toxicity, strong fluorescence emission, broad excitation spectra, and stable PL is used as a vector for gene delivery. The CDs can condense with plasmid DNA and form CDs/pDNA complexes. This complex can enter the cell by caveolae-and clathrin-mediated endocytosis pathways [55, 56].

The CDs with the positive surface can condense with plasmid DNA. These CDs can form a complex with negatively charged siRNAs. The folate-conjugated reducible polyethyleneimine passivated CDs delivered siRNA in a reducing environment [104]. The CDs conjugated with plasmid SOX9 have unique properties such as high solubility, low cytotoxicity, and fluorescence emission [54]. It is used as a gene vector for chondrogenesis from fibroblasts. Wu *et al.* used folate-conjugated reduction-sensitive polyethyleneimine passivated CDs for targeted EGFR and cyclinB1 siRNA delivery and targeting in H460 lung cancer cells. The combined siRNAs were released in reducing intracellular conditions and increased the anti-cancer activity in H460 cells [57]. Recently Cao *et al.* fabricated dual functional cationic CDs from glucose and arginine for imaging and SOX9 plasmid delivery in mouse embryonic fibroblasts (MEFs). They observed that CDs/pSOX9 nanoparticles possessed low cytotoxicity towards MEFs, helped in the intracellular tracking, and SOX9-dependent chondrogenic differentiation [58]. Several other researchers have also worked with CDs, as non-viral gene delivery vectors [59].

Photo-Dynamic Therapy (PDT) and Photo-Thermal (PTT):

Therapy: Photodynamic therapy (PDT) and Photothermal therapy (PTT) are applied for the treatment of cancer using laser light (most often by near IR radiations). PTT refers to using NIR, which is absorbed by a photo absorber to generate local heat and destroy diseased tissue. PTT offers several advantages over conventional chemotherapy, radiotherapy, and surgery. Therefore, attracting much interest from researchers in the field of cancer treatment. However, the effectiveness of nanomaterials as photothermal agents (gold nanostructures, graphene, and graphene oxide) in PTT is still under consideration due to the difficulties in their synthesis and high production cost. Unlike PTT, PDT requires O_2 to produce ROS and destroy targeted cells. In PDT, a photosensitizer is irradiated with laser light that produces ROS, ultimately destroying cancer cells/tissue. Upon excitation with a suitable wavelength, photosensitizer gets excited from its ground singlet state to a higher energy singlet state and suffers intersystem crossing to form a long-lived triplet state. The triplet state reacts with oxygen molecules, producing ROS that can effectively destroy cancer cells [60].

Recently CDs have been intensively used by researchers in PTT and PDT due to their smooth and low-cost synthesis, facile surface functionalization and superior, caring capacity, excellent biocompatibility and ability to convert absorbed light into heat due to a large number of pi electrons. PDT and PTT involve the use of light having less energy and hence is less injurious to healthy cells/tissue [61]. Ge *et al.* fabricated red-fluorescent CDs from polythiophene phenyl propionic acid for photoacoustic/FL (NIR) imaging and *in-vitro* and *in-vivo* photothermal therapy. They demonstrated that the as-synthesized CDs showed extensive cytotoxicity towards HeLa cells upon NIR laser irradiation. The CDs were also found to show *in-vivo* PTT efficacy in HeLa tumor-bearing mice without any sign of systemic toxicity [62]. Beack *et al.* synthesized CDs-Ce6-HA, hyaluronic acid-modified CDs conjugated with chlorine Ce6 (Ce6, a photosensitizer) for targeted therapy of melanoma skin cancer.

They observed that CDs conjugation to Ce6 increased the photodynamic reaction of Ce6, and produce more singlet oxygen than free Ce6. Transdermal administration of CDs-Ce6-HA resulted in suppression of B16F10 melanoma cells in tumor mice upon laser irradiation [63]. Zheng *et al.* synthesized carbon nitride (C3N4) doped CDs and functionalized it with a targeting agent and a photosensitizer to increase the targeted PDT efficiency in solid tumor under hypoxic condition. They demonstrated that the nanocomposite produces oxygen inside the hypoxic tumor region via water splitting upon light exposure, producing ROS and showing enhanced PDT efficiency under both *in-vitro* and *in-vivo* conditions [64].

Furthermore, cetuximab-conjugated CDs exhibit superior photodynamic effects in HCC827 and MDA-MB-231 cancer cells and MDA-MB-231 tumor-bearing mice [65]. Recently Guo *et al.* synthesized Cu, N co-doped CDs via one-step hydrothermal treatment using EDTA. 2Na and $CuCl_2$. They demonstrated that the Cu, N co-doped CDs signifcantly inhibited cancer (B16 melanoma) cell growth via photodynamic and photothermal therapy.

Crossing Blood-Brain Barrier: An important and major parameter for using any nanostructures in living biomedical applications is the size of the nanoparticles. The smallest human capillaries are less than 4 mm. Therefore, it is essential to keep the size of the nanoparticles below this size for use in a living body. Smaller sizes of the nanoparticles would prevent blood vessel blockage and their elimination by the reticuloendothelial system [66]. Delivering imaging probes to brain tumors is a complex technique because of the Blood–Brain Barrier (BBB). Crossing the BBB depends on the size of the probes and their surface properties [67]. Furthermore, delivering the drugs to the brain is difficult because of the BBB properties, which prevent drugs such as foreign proteins, chemicals, and peptides from crossing. Nanoparticles and QDs can be used for drug delivery by crossing BBB [68–71]. The CQDs may also be used for drug delivery across BBB as well. Li *et al.* reported that synthesized CQDs had been observed in all organs, including the brain, after 6 h post-injection. The CQDs without functional decorating could pass across the BBB. Polymer-coated nitrogen-doped CQDs (with sizes in the range of 5–15 nm), which were synthesized by a solvothermal reaction, could enter glioma cells *in-vitro* and be used for *in-vivo* glioma fluorescence imaging. The physiological pore size upper limit for the blood–tumor barrier (BTB) of malignant glioma microvasculature is around 12 nm, implying that the synthesized CQDs can cross the barrier. Furthermore, the hydrophilic polymer coating on the CQDs prolongs blood circulation time and improves the probability of proper targeting at the tumor site [72].

Carbon Dots as Potent Antimicrobial Agents: The adhesion of CDs to the bacterial surface, the photoinduced production of ROS, the disruption and penetration of the bacterial cell wall/membrane, the induction of oxidative stress with damages to DNA/RNA, leading to the alterations or inhibitions of important gene expressions, and the induction of oxidative damages to proteins and other intracellular biomolecules [73-75]. Under visible/natural light illumination, CDs in contact with the bacteria cell can efficiently generate ROS by activating the oxygen in air or water, leading to the production of hydroxyl free radicals (OH•) and/or singlet oxygen (1O2), which can destroy some of the critical biomolecules in cell and lead to cell death [125, 126].

Li, *et al.* synthesized CDs by a one-step electrochemical method with vitamin C as a precursor, and found their broad-spectrum antibacterial activities against *S. aureus*, *B. subtilis*, *Bacillus* sp. WL-6, *E. coli*, and the ampicillin-resistant *E. coli.* There have been a few reported studies on the anti-fungi activities of CDs. Li, *et al*. found that the CDs prepared to inactivate bacteria also exhibited broad-spectrum antifungal activities against *R. solani* and *P. grisea.* Jhonsi, *et al.* also found antifungal effects of CDs against *C. albicans* [76]. Similarly, CDs conjugated with ciprofloxacin were tested on *Saccharomyces cerevisiae* yeast cells, with the observed bright green fluorescence emissions attributed to the CDs inside the cells [77]. Priyadarshini, *et al*. explored the potential of CDs and their derived conjugates for anti-fungi activities against the fungal pathogen *C. albicans* [78]. Studies have been scarce on the use of CDs to inactivate viruses and/or reduce infection rates. Du, *et al.* reported that procine kidney (PK-15) cells and monkey kidney (MARC-145) cells treated with

CDs could significantly inhibit the multiplication of pseudorabies virus (PRV) and porcine reproductive and respiratory syndrome virus (PRRSV). The inhibitory effect of CDs was rationalized as being due to the activation of IFN-α and the production of ISGs, which in turn inhibited viruses' replications [79]. Type I interferons (IFNs), including IFN-α and IFN-β, are the best-known antiviral innate immune molecules with a powerful antiviral response to viral infection in the human body. More recently, Huang, *et al.* found that CDs synthesized from benzoxazine monomer could block the infection of life-threatening flaviviruses (Japanese encephalitis, Zika, and dengue viruses) and non-enveloped viruses (porcine parvovirus and adenovirus-associated virus) *in-vitro*, probably *via* directly binding to the surface of the virion and eventually impeding the first step of virus-cell interaction [80].

CONCLUSION: In this review, we have elaborated on the recent advancement in CQDs, emphasizing their synthesis methods and characterization, followed by their biomedical applications. The unique properties of CQDs are beneficial for potential biomedical science and research applications. C-dots have extensive adequacy for *in-vivo* and *in-vitro* bioimaging and drug delivery studies. Because of their biocompatibility, low toxicity, strong PL, and synthetic and photograph steadiness, C-dots have become a fascinating material for bioimaging and cancer therapy. This review summarized the research progress on CDs regarding their synthesis from small organic molecules and biomedical applications. However, more research is needed to evaluate their blood circulation, toxicity and conjugate them in multifunctional platforms for simultaneous bioimaging and drug/gene delivery.

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