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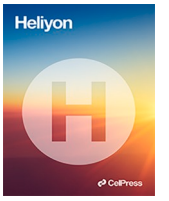

Review article

Luminescence of carbon quantum dots and their application in biochemistry

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ABSTRACT

Similar to fullerenes, carbon nanotubes and graphene, carbon dots (CDs) are causing a lot of research work in their own right. CDs are a type of surface-passivated quantum dot that contain carbon atoms. Their distinctive characteristics, such as luminescent emission that varies with size and wavelength, resistance to photobleaching, easy biological binding, lack of toxicity, and economical production without the need for intricate synthetic processes, have led to a noteworthy surge in attention within the research community. Different techniques can be utilized to create these CDs, spanning from basic candle burning to laser ablation. This review article delves into the principles of fluorescence technology, providing insights into how different synthesis methods of quantum dots impact their luminescent properties. Additionally, it highlights the latest applications of quantum dots in catalysis and biomedical fields, with special emphasis on the current status of luminescent properties in biology and chemistry. Towards the end, the article discusses the limitations of quantum dots in current practical applications, pointing out that CDs hold promising potential for future applications.

1. Introduction

Carbon-based materials are crucial to the advancement of materials science and biomedicine. Extensive research has been dedicated to exploring and developing novel carbon nanomaterials, including carbon fibers, graphene, and carbon nanotubes (CNTs). These materials have gained significant popularity within the domains of chemistry and materials science due to their unique properties and promising potential for diverse applications in fields such as aviation, machinery, and other interdisciplinary areas [1–3] , due to their small specific gravity [4], high strength [5], excellent properties and environmental friendliness [6]. Common carbon nanomaterials encompass carbon nanotubes, graphene, fullerene, and carbon quantum dots. Among these, carbon nanotubes, also known as bucky tubes, are seamless and hollow nanotubes formed by single-walled or multi-walled graphite sheets curling around the center at specific angles. They can be further classified into single-walled, double-walled, and multi-walled carbon nanotubes [7,8]. Graphene is a hexagonal two-dimensional carbon nanomaterial, and layers of graphene can be stacked into graphite. Fullerenes are a general term for a range of clusters, among which C60 is a typical isolated fullerene that may have a spheroid-like structure [9].

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However, the development of nanotechnology has brought along a disadvantage; carbon materials are challenging to use effectively as fluorescent materials due to the absence of a suitable band gap [10]. Carbon QDs (CDs) are another common carbon nanomaterial consisting of nanoparticles with dimensions smaller than 10 nm [11–13]. Because of their size-dependent photoluminescence and dependence on excitation wavelength, CDs are attracting considerable attention, especially in terms of scale, cost and biocompatibility [14]. CDs stand as innovative carbon materials, distinguished by their exceptional fluorescence traits. In contrast to prevalent carbon nanomaterials, they offer numerous benefits, such as excellent water solubility, remarkable stability, absence of toxicity, effortless functionalization, cost efficiency, and a straightforward synthesis process. Carbon material that possess unique fluorescence properties. Compared to other common carbon nanomaterials, they offer numerous benefits, such as excellent water solubility, remarkable stability, absence of toxicity, effortless functionalization, cost efficiency, and a straightforward synthesis process [15]. These attributes position them as a promising contender across a spectrum of applications. Because CDs have luminescent properties similar to semiconductor quantum dots [16–18], resulting in their application across diverse domains, encompassing biomedicine [19], catalysis, optoelectronic devices and anti-counterfeiting [20,21].

In 2004, Xu et al. at the University of South Carolina, USA, in an experiment to purify single-walled carbon nanotubes, a unique carbon nano was accidently found using arc discharge, which they first called CDs [22]. Through the analysis, we found the great prospect of this carbon material. Along with the discovery of CDs, nanodiamonds were also discovered, which were carbon-based nanomaterial have similar properties to carbon dots in surface function and size [23,24]. The distinctions are that the amount of graphite carbon on the nanodiamond surface is small, its CDs also have larger sp2 characteristics, and contain small amounts of carbon with higher oxygen content [25–27]. Whereas CDs show a broad spectrum in photoluminescence analysis, fluorescent nanodiamonds emit from point defects, particularly negatively charged nitrogen vacuoles, which absorb at 569 nm with emission wavelengths close to 700 nm [28]. Researchers have discovered that CDs possess size-dependent fluorescence properties, which make them a promising material for various applications. While the initial discovery of their potential was unexpected, numerous researchers have since successfully synthesized CDs using various synthesis methods. These endeavors aim to gain a deeper understanding of their optical properties and elucidate their luminescence principles. Consequently, these efforts have led to the emergence of new research ideas and protocols, further showcasing the potential of CDs as a highly promising material for future investigations across diverse fields.

The first section of this article discusses the classification of fluorescence and factors influencing its intensity. Section 2.2 examines various synthesis routes used in the preparation of CDs, while section 2.3 delves into the process of doping these CDs with other chemical elements. Section 2.4 analyzes the luminescence principle behind CDs fluorescence, including different factors that affect its properties. Finally, section 2.5 introduces some of the most promising applications of CDs in various fields such as biochemistry detection, cellular imaging, chemical catalysis, cancer treatment, and drug delivery.

This review aims to provide valuable insights for future research in the field of emerging nanocarbons and stimulate further investigation into the origin of their unique properties. By fostering exploration across various disciplines, this knowledge has the potential to drive new discoveries and unveil novel applications for CDs.

1.1. Fluorescence technology

1.1.1. Fluorescence principle

Luminescence encompasses various forms, including photoluminescence, chemiluminescence, and other distinct types of luminescence [29–31]. Photoluminescence can be further categorized into fluorescence and phosphorescence, while chemiluminescence also encompasses bioluminescence [32]. Additionally, other forms of luminescence, such as acoustoluminescence and electroluminescence, exist. The underlying principle of fluorescence involves the excitation of certain atoms by light, causing the electrons

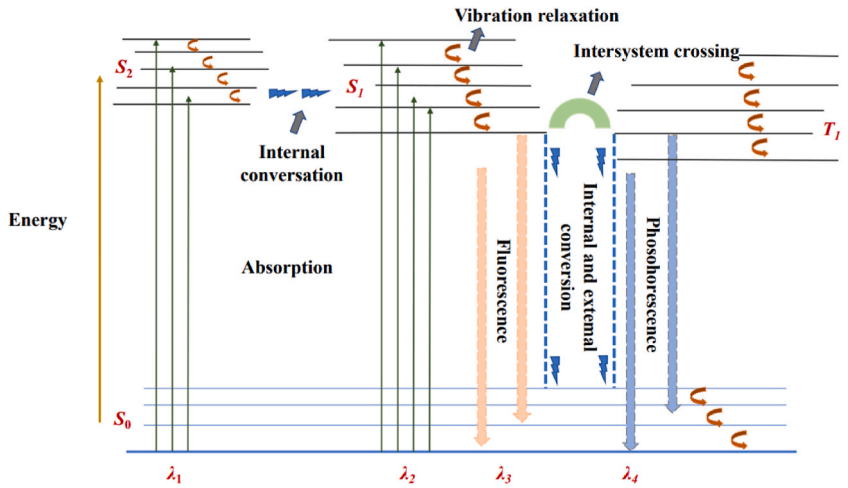

Fig. 1. Jablonski energy level diagram.

orbiting their nuclei to transition from the ground state to either the unstable first excited singlet state or the second excited singlet state. Subsequently, as the electrons return to the ground state from the first excited singlet state [33,34], the energy is released in the form of light, thus producing fluorescence. Fluorescent sticks, phosphors and glow-in-the-dark paints are all common fluorescent phenomena in life [35,36].

The absorption and emission processes of molecules are generally represented by Jablonski energy level diagrams (Fig. 1). At room temperature, a molecule typically remains in its ground state. However, upon absorption of external energy, the molecule can undergo a transition to a higher energy state through an interlevel jump [37]. The multiplicity of the determined electron energy levels is denoted by the symbol M, and the total number of orbital spins is denoted by S; $M = 2S + 1$. The energy state of the electron is denoted by the symbol S when the electron is in the singlet state, where $S = 0$ and $M = 1$. The energy state of the electron is denoted by the symbol T when the electron is in the triplet state, where $S = 1$ and $M = 3$.

1.1.2. Fluorescence intensity and lifetime

The substance that causes the fluorescence intensity to decrease is called a fluorescent bursting agent. The fluorescence burst is divided into dynamic burst and static burst [38]. Static quenching involves the formation of non-fluorescent complexes between the quenching agent and the fluorophore, preventing its excitation and subsequent fluorescence emission [39,40]. A dynamic burst refers to the fluorescent molecules and the burster collision, so that the fluorescent molecules do not release radiation but leap back to the ground state and cause the fluorescence to burst [41].

Photobleaching refers to the photochemical degradation of a dye or fluorophore molecule. It occurs due to covalent bond breakage or non-specific reactions between the fluorophore and surrounding molecules [42]. Additionally, environmental factors such as temperature can also affect the fluorescence intensity of the dye or fluorophore [43,44]. Therefore, choosing low temperature conditions for fluorescence detection will be beneficial to improving the sensitivity of the analysis [45].

Upon removal of the excitation light source, the fluorescence intensity of a molecule gradually diminishes, and the time taken to reach 1/e of the maximum fluorescence intensity is defined as the fluorescence lifetime [46]. Presently, two primary approaches are employed for fluorescence lifetime measurements: the time domain method (Fig. 2A) and the frequency domain method (Fig. 2B). The schematic diagram illustrating fluorescence lifetime measurement using the time domain method and frequency domain method are depicted in Fig. 2.

1.1.3. Fluorescence applications

Förster resonance energy transfer (FRET) is a widely utilized technique in the field of biochemistry for studying fluorescence interactions. Resonance occurs when there is a partial overlap between the emission spectrum of a fluorescent group and the absorption spectrum of other groups, and when the two fluorescent groups are maintained at an optimal distance. This enables the observation of fluorescence emitted by the acceptor group upon excitation of the donor group. This phenomenon, known as resonance, is employed as a powerful analytical tool [47]. Resonance energy transfer is the technique used for observing fluorescence emitted by the acceptor group when the donor group is excited. The donor group provides the emission spectrum and is called the fluorescence donor, while the acceptor group provides the absorption spectrum and is called the fluorescence acceptor. The selection of the donor and acceptor groups in this technique is generally based on three principles. (1) The donor should first have a high fluorescence quantum yield. (2) There is a certain effective overlap. (3) There should be a certain suitable distance between the two groups, generally within (1 ± 0.5) R_0 [48].

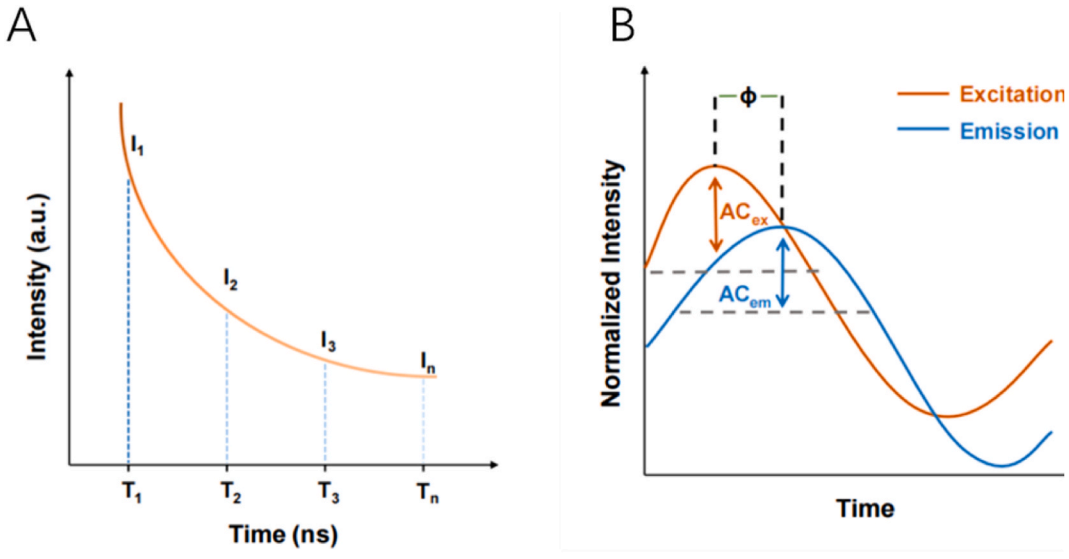

Fig. 2. Schematic diagram of fluorescence lifetime measurement by time domain method(A) and Frequency domain method(B).

Nowadays, FRET has emerged as a widely utilized technique in various applications within the field of biochemistry. For instance, FRET can be utilized to detect changes in protein kinase activity within living cells, providing insight into protein-protein interactions and cellular signaling pathways [49]. The use of FRET technology to design fusion reporter proteins in response to changes in enzyme activity for studies on apoptosis [50]. Moreover, FRET can be applied to observe the lateral spread of receptor activation effects on cell membranes following local stimulation, by employing the proximity of green fluorescent protein and Cy3 dye to induce FRET [51]. For example, using FRET to detect intracellular molecular interactions, such as the interaction between Rac and PDB, GFP and Alexa will be close enough for FRET to occur, thus allowing real-time detection of the relationship between changes in Rac localization and Rac activation in a living cell.

Fluorescent nanomaterials can be broadly classified into two categories: organic and inorganic [52]. Organic fluorescent materials are composed of small organic molecules that possess certain conjugated heterocycles and various color-generating groups (Fig. 3). These molecules can be used as organic fluorescent dyes, with some common markers being fluorescein-based and rhodamine-based [53]. Fluorescein-based dyes generally have higher quantum yields, while rhodamine-based dyes have longer emission wavelengths. In contrast, inorganic fluorescent materials are exemplified by rare-earth-based fluorescent materials, which include semiconductor quantum dots and rare-earth-doped upconversion materials [54].

Fluorescence technology has numerous applications, including bioimaging and sensing. In fluorescence imaging, fluorescent substances are added to the sample being analyzed [55]. When these substances are excited, they emit a fluorescent signal, and the intensity of this signal is linearly related to the content of fluorescein within a certain range [56]. This characteristic enables the quantitative detection of corresponding substances [11,22,25,26,57].

1.2. Synthesis methods

Two general approaches are often employed to synthesize CDs: top-down and bottom-up. The top-down strategy involves stripping large carbon sources of smaller carbon spots. This approach is often accomplished through methods such as arc discharge, in which high-voltage electrical discharges are used to break down carbon-containing materials into smaller carbon structures [22], laser ablation [57–64] and electrochemical oxidation [65–68]. The advantages and disadvantages for the different synthesis methods are shown in Table 1.

Arc discharge is an early “top-down” method for preparing CDs (Table 2). Xu et al. unexpectedly obtain three distinct CDs, each displaying varying fluorescence attributes when purifying single-walled carbon nanotubes by the arc discharge method [22]. These three CDs emit green blue, yellow, and orange in order of elution and increasing size under excitation at 366 nm. Although this method enables the synthesis of CDs with favorable water dispersibility, the size of the CDs formed during the discharge process exhibits variability.

Electrochemical method is an irregular “top-down” chemical cutting process for carbon materials such as graphite, carbon

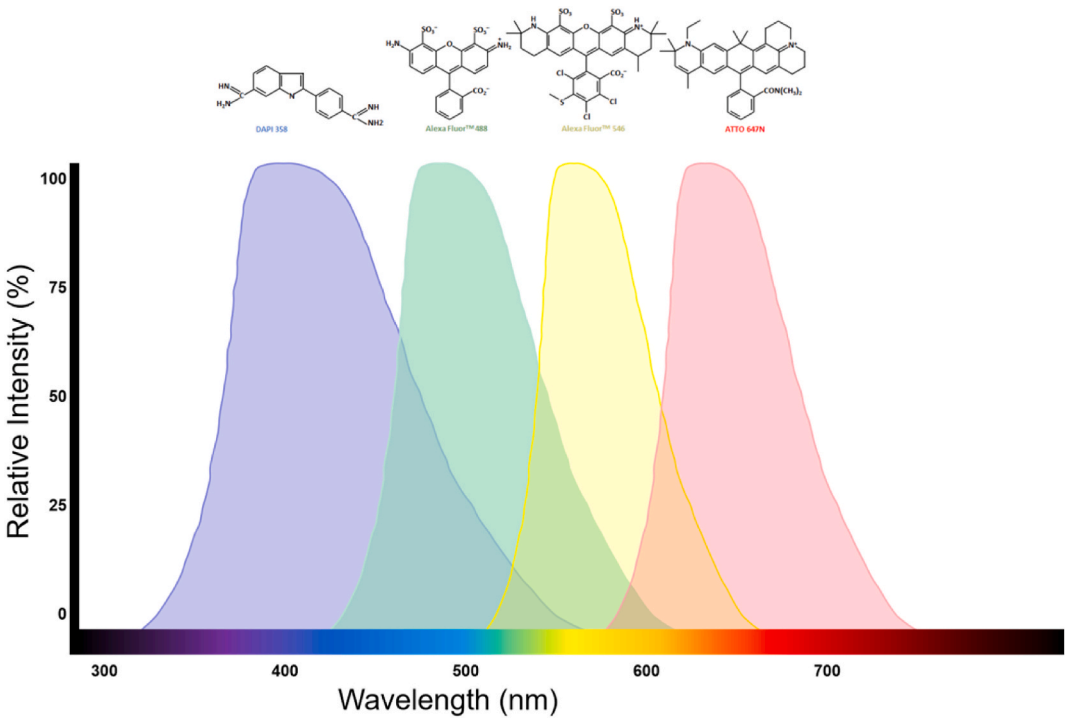

Fig. 3. Molecular structures of common fluorophores.

Table 1
The advantages and disadvantages for the different synthesis methods.

Method	Classification	Advantages	Disadvantages	Reference
Arc discharge	"Top-down" method	Excellent water dispersibility	Particle size with large different	[22]
Electrochemical method	"Top-down" method	Easy to adjust the particle size and fluorescence properties, high yield	Special equipment required	[69]
Laser ablation	"Top-down" method	High fabrication rates, fine particle size, and high monodispersity	Low yield, inhomogeneous particle size and complex operation	[70]
Chemical exfoliation	"Top-down" method	Mass production of high-quality CDs without complex equipment	Complex removal of chemical reagents	[71]
Microwave radiation	"Bottom-up" method	More prominent size and focusing applications.	Time-consuming, complex, and economically prohibitive	[72]
Hydrothermal/ solvothermal method	"Bottom-up" method	Homogeneous particle size	The reaction requires a high temperature	[73]
Combustion	"Bottom-up" method	Simple operation	Low yield and inhomogeneous particle size	[74]

Table 2
Examples of CDs in oncology treatment.

Theory	Materials	Model	Function	Reference
FRET	Carbon powders	OVCAR3 cells	Monitor the presence of tumour cells	[75]
FRET	Glucose, PAAS	HeLa/HepG2 cells	Determining prognosis of cancer and investigation of cancer metastasis	[76]
ECL	Zinc acetate, PEI	MCF-7 and MDA-MB-231	Monitor the presence of tumour cells	[77]
Character dependent fluorescence properties	Citric acid, ethylenediamine	–	Diagnosis of early lung cancer	[78]
DOX	1,4,5,8-tetraminoanthraquinone, citric acid	Brain glioma mice beared U87 cells, cervical carcinoma mice beared Hela cells	Mimicking large amino acids, cross the BBB	[79]
DOX	Glycerol, PEI	Liver cancer mice beared MHCC-97L cells	Treatment synergetic monitoring	[80]
Pt(IV)	Citric acid, polyene polyamine	Hepatoma mice beared H22 cells	Multicolor imaging	[81]
Amygdalin	Golden acid	Hep3B cells	Natural targeting tumor cells	[82]
Dextran	Acrylic acid and ethylenediamine	SKOV3 cells	Synergistic nanogels release drug in controlled manner	[83]
PTT	Purchased from Xianfeng Nano (China)	Breast cancer mice beared 4T1 cells	Synergetic immunotherapy	[84]
PTT	TNP, BPEI1800	Breast cancer mice beared 4T1 cells	Enhancement of photothermal conversion efficiency by doping black phosphorus	[85]
PDT	L-cysteine, *m*-phenylenediamine	Cervical carcinoma mice beared U14 cells	Targeting tumour nuclei	[86]
PTT&PDT	FAC, polylysine acid	Cervical carcinoma mice beared Hela cells	Synergetic MR imaging	[87]
PTT&PDT	MnO_2 and Cu(II)	Breast cancer mice beared 4T1 cells	Four combination therapy of PTT/PDT/ starving-like/immunotherapy	[88]
PTT&PDT	–	B16–F0 tumor-bearing nude mice	Synergetic FL and PA imaging	[89]

nanotubes, or carbon fiber electrodes [90]. The CDs obtained by electrochemical methods have different sizes, so further purification is necessary through filtration or chromatography to obtain monodisperse CDs. Deng et al. published work on the improved synthesis of CDs through a one-pot electrochemical method [91]. In their study, CDs were synthesized using alcohol in a three-electrode system. Platinum (Pt) sheets served as working and counter electrodes, while reference electrodes were calomel electrodes fixed to a lugging capillary. The distance between the electrodes could be adjusted using rubber plugs [69]. Although specialized equipment is required, the particle size and fluorescence properties can be easily adjusted in this method.

The laser ablation method is used to obtain the colloidal solution of nanoparticles in various solvents. Laser ablation has the advantages of high manufacturing rate, fine particle size, and high monodispersity [92]. The two parameters for controlling material properties through the laser ablation method are the laser tuning and multi-target precursors. A laser ablation synthesis method for synthesizing CDs using boron nitrite as a precursor has been proposed. This method was the first to tune the photoluminescence of boron nitride quantum dots (BNQDs) from UV to the green region through the femtosecond laser ablation [93]. N. Ramila Devi et al. [69]. used hexagonal boron nitride (hBN) powder as a nitrogen source to react in ethanol, diethylamine, and ethylenediamine to prepare CDs that emit three different types of luminescence, including ultraviolet, blue, and green luminescence. These CDs can be used as fluorescent probes for biological imaging. However, it is important to note that this method has some limitations, such as low yield, inhomogeneous particle size, and complex operational procedures. Chemical exfoliation is a facile and convenient method for

mass production of high-quality CDs without complicated devices. The precursor carbon materials (carbon fibers, graphene oxide, carbon nanotubes) are cleaved by strong acids or oxidizing agents. Mao and coworkers fabricated for the first time fluorescent GDs with different sizes from candle soot by using HNO_3 under a relatively high temperature in 2007 [74]. In fact, except for strong acids, various powerful oxidants were also used in the preparation of CDs. For example, Kailasa and coworkers utilized H_2SO_4 as an oxidant to prepare blue-, green- and yellow-color fluorescent CDs by chemically oxidizing tomato. The obtained CDs were evaluated for assaying Fe^{3+} ions and exhibited low limit of detection up to 0.016 μM. Fe^{3+} ion was quantified in iron supplements and biofluids [94]. Although one pot acid-free route is highly efficient for synthesis of CDs, the purification process of the final product was tedious and complex for the removal of oxidant.

In contrast, bottom-up synthesis is the opposite of large-to-small synthesis, where carbon quantum dots are synthesized from smaller sized carbon sources, and small-to-large methods include combustion/thermal pathways [25,27,74,95,96], supported synthesis [25,97] and microwave methods [98].

Combustion/thermal pathways involves obtaining CDs from the products of combustion organic matter. Nitric acid reflux candle soot to prepare water-soluble multicolor fluorescent carbon nanoparticles with readily available and inexpensive starting materials [74]. However, it has limitations such as low yield of CDs and variations in particle size. A cost-effective and clean microwave-assisted strategy is commonly employed for the synthesis of CDs in a shorter time period ，which can offer the advantage of providing uniform heat for the formation of CDs. As mentioned in the literature review, some possible methods for improving reactions using microwave-assisted strategy are as follows: (i) thermal effects [99] (ii)specific microwave effects [100], and (iii)non-thermal effects [72]. The common lemons and onion biomass on the market were first used as raw materials to synthesize CDs by microwave method. The analysis method is used to determine commercial vitamin and mineral supplements [101].

Hydrothermal synthesis is a bottom-up method for preparing CDs. This method is a solution reaction approach. In hydrothermal synthesis, it is usually necessary to apply a very high-temperature range to the reaction materials in the high-pressure vessel. The CDs synthesized from carrot juice by hydrothermal method exhibited maximum PL excitation at a wavelength of 360 nm and emission at 442 nm, which reveal a general stroke shift of 82 nm [102]. Due to the wide size distribution of CDs, the emission peak wavelength depends on the excitation wavelength [103]. Xie et al. synthesized green CDs by hydrothermal method using highland barley as carbon source and ethylenediamine as nitrogen source. These synthesized CDs have been applied for Hg^{2+} detection based on their sensitivity and selective detection quenching effect. The CDs synthesized by this method exhibits excitation wavelength dependent characteristics at 4.5–7 nm size and strong blue fluorescence [104].

To enhance the functionality of CDs, researchers commonly synthesize composite CDs that are doped with other elements. These composite CDs are typically prepared using methods such as sonication, mixing, hydrothermal synthesis, or micro-blogging assisted techniques [105]. During these processes, additional elements can be incorporated into the CDs, thereby further enhancing their properties and expanding their versatility for a wide range of applications.

1.3. Elemental doped CDs

The conventional synthesis method for CDs typically results in the introduction of numerous oxygen-containing functional groups on their surface. However, CDs consisting solely of carbon and oxygen elements exhibit low quantum yields. To overcome this limitation, surface modifications are required to enhance their quantum yields CDs [61,106,107]. This presents challenges for the wider application and further development of CDs. In 2012, some researchers proposed that N-doped CDs [108], which provide a better way to regulate the performance of CDs [109]. At present, there are only two main types of methods to synthesize such heteroatom-doped CDs, which are multi-step synthesis and one-step synthesis. The latter is more convenient [110].

Currently, the most commonly studied doped CDs are N-doped CDs. However, besides nitrogen, other elements such as sulfur and phosphorus have also been used for doping CDs [111]. Furthermore, there are various heteroatoms that can be co-doped with CDs [112]. The incorporation of different dopants in CDs leads to diverse effects on their fluorescence properties, including enhancing the quantum yield, altering the emission wavelength, and adjusting the luminescent color [113]. After various chemical modifications, quantum dots can exhibit good biocompatibility and reduced harm to organisms, making them suitable for biological labeling and detection experiments. In contrast, fluorescent dyes generally have higher toxicity and poorer biocompatibility [114]. It is precisely due to these unique optical properties that quantum dots have become an ideal fluorescence probe. By replacing organic fluorescent dyes with quantum dots, they will play an important role in research on cell localization, signal transduction, and the movement and migration of molecules within cells.

1.3.1. N-doped CDs

N-doped CDs currently represent the most prevalent type of doped CDs. These doped CDs exhibit significant potential, effectively enhancing their own fluorescence quantum yield and inducing a red shift in the wavelength of fluorescence emission [19]. Lin et al. [115] synthesized N-doped CDs with red, green, and blue fluorescent colors, suggesting the possibility of creating carbon quantum dots that emit various fluorescence colors through this approach. However, there is limited evidence to conclusively attribute the redshift solely to the presence of nitrogen. Nevertheless, some studies have demonstrated that the incorporation of nitrogen in N-doped CDs can induce a negative electric induction effect, leading to a phenomenon known as blueshift [116].

The nitrogen doping process in CDs introduces various nitrogen forms into their structure, resulting in a diverse range of properties that depend on the configuration of nitrogen atoms. This complexity arises due to the significant impact that different nitrogen configurations can have on the properties of N-doped CDs [117,118]. Therefore, comprehending the nature of nitrogen doping in CDs is crucial for enhancing their properties and expanding their potential applications [119]. The complexity of nitrogen doping in CDs

stems from the existence of four main nitrogen forms that can be present in their structure [120]. One commonly doped form is graphitic nitrogen, which is frequently incorporated into the graphene skeleton [121,122]. However, there is often confusion regarding the diverse roles played by nitrogen in N-doped CDs. To address this issue, Guo et al. [123] conducted research aimed at gaining a deeper understanding of the nature of nitrogen doping in CDs. Their study emphasized that only aromatic nitrogen-doped CDs have an impact on the luminescence of the CDs themselves [124]. This finding is significant as it provides crucial clarification on the relationship between nitrogen doping and luminescence in CDs, shedding light on the distinctive properties of these compounds and offering new insights for future investigations.

1.3.2. Non-metallic elements doped with CDs

While nitrogen is currently the most commonly utilized element for doping CDs, other non-metallic elements such as sulfur and phosphorus also exhibit potential for enhancing their properties. Although research on sulfur and phosphorus doping in CDs is not as extensive as that on nitrogen, there are nevertheless reports exploring the application of these elements [125]. This indicates that there are additional opportunities to develop new and intriguing composite CDs in the future. Among the alternative dopant CDs, sulfur-doped CDs represent the second most prevalent type [126], with sulfur potentially present in the form of thioether groups, sulfhydryl groups, or sulfonic acid groups within the CDs [127].

Sulfur is usually used as a co-dopant in N-doped CDs [128,129]. Yu et al. have introduced a novel type of co-doped CDs incorporating nitrogen and sulfur elements [130]. In these doped CDs, the fluorescence is primarily influenced by the functional group containing nitrogen. It is observed that the sulfur atom likely plays a role in stabilizing and enhancing the surface state of the nitrogen atom while inhibiting the surface state of the oxygen atom.

Given that silicon belongs to the same family as carbon, it possesses the potential for being doped into the graphene skeleton of CDs. Consequently, silicon doping has emerged as a promising area of research in the field of CDs, offering opportunities to enhance their functionality and broaden their applications. Feng et al. successfully synthesized silicon-doped CDs with a fluorescence quantum yield of 19.2% by utilizing hydroquinone as a carbon source and silicon tetrachloride as a silicon source [131]. Such silicon-doped CDs have prominent applications in biological imaging and fluorescence sensing [132]. However, it should be noted that silicon doping leads to an approximate 80 nm blue shift in the main fluorescence emission peak of CDs, causing it to enter the ultraviolet region, thereby deviating from the characteristics of traditional CDs. Wang et al. employed the thermal decomposition method to synthesize co-doped CDs containing boron and phosphorus. In brief, CDs were dispersed in 100 ml of water through sonication [133]. Subsequently, triphenylphosphine and boric acid were added to the carbon dot suspension and stirred until noticeable agglomeration occurred. The resulting mixture was heated at 80 °C to evaporate the water, followed by overnight drying in a vacuum oven at 60 °C. The resulting solid complex was ground to a powder using a mortar. Finally, the complex was placed in a quartz boat within a tube furnace and pyrolyzed under argon for 45 min at 750 °C. The resulting powder was immersed in nitric acid for an additional 8 h, followed by centrifugation, evaporation, and dialysis. These CDs induce a redshift in the emission wavelength from 513.8 nm to 526.8 nm. Additionally, chlorine-doped CDs synthesized by Zhao et al. have been shown to effectively enhance the photoelectric performance of CDs [134], while the CDs doped with fluorine synthesized by Gong et al. can enhance the magnetic properties of them [135]. Researchers employed a graded fluorine sacrifice strategy to synthesize fluorine-doped CDs with controllable size and tunable fluorine content, using bulk fluorine fossil ink as a starting material. Experimental results revealed that the introduction of fluorine-induced defects significantly improved the paramagnetic properties of the fluorine-doped CDs, potentially leading to a five-fold increase compared to fluorine-doped CDs synthesized without fluorine. This advancement opens up new opportunities for tailoring the properties of CDs and enhancing their functionality, particularly in applications where magnetic properties are desired. More particularly, Xie et al. prepared doped CDs with high selenium content, and took sulfur and selenium as examples to believe that the emission wavelength of doped CDs may depend on the electronegativity of the heteroelements [136]. A possible reason for the effectiveness of sulfur and selenium doping in CDs may be their lower electronegativity relative to carbon, making them more likely to act as electron acceptors. In addition, researchers have also successfully implemented a “fluorescence on and off” technique using selenium-doped CDs for the detection of oxidative hydroxyl radical (• OH) and reductive glutathione (GSH). This innovative approach demonstrates the potential applications of selenium-doped CDs in the field of biological sensing and disease detection [137]. The authors proposed that the yellow fluorescence exhibited by selenium-doped CDs is attributed to a special energy level formed by the C–Se bond on the surface, while fluorescence quenching occurs when the C–Se bond is converted to a C–Se–Se bond form by hydroxyl radicals. However, carbon selenide dots possess distinctive chemical properties, including REDOX responsiveness, owing to their unique electronegativity, although few studies have been conducted on this subject. Li et al. used mild hydrothermal treatment to prepare selenium doped carbon quantum dots with green fluorescence [138]. The incorporation of selenium heteroatoms in CDs can introduce redox-dependent reversible fluorescence, adding a unique functionality to the materials. Furthermore, selenium doping of CDs has been shown to effectively scavenge hydroxyl radicals and other harmful free radicals, reducing the levels of reactive oxygen species in cells upon internalization of the Se-doped CDs [139,140]. This property positions Se-doped CDs as a promising candidate for protecting biological systems from oxidative stress and suggests potential applications in biomedical research and therapeutic interventions.

1.3.3. CDs associated with metal elements

Influenced by doped semiconductor quantum dot materials, Sun et al. [60] developed a kind of zinc salt doped CDs, which is the earliest metal salt doped CDs. This kind of CDs has green fluorescence characteristics. Subsequently, other researchers also saw the development prospect of this aspect, and tried to synthesize the doped CDs of this metal element [141,142]. Their objective was to explore the unique functionalities that could be achieved through doping with these specific metal elements [112].

Feng et al. synthesized doped CDs with manganese elements, which emit yellow fluorescence [143]. They believe that manganese is distributed in the interior of the CDs. Furthermore, whether the complex formation occurs through chelation of manganese prior to CDs synthesis or by doping manganese after CDs synthesis, the final result is the generation of "functionalized" manganese-doped CDs. Subsequently, the doping of CDs with divalent cobalt ions was proposed to induce a charge-transfer effect, ultimately leading to the production of doped CDs with orange fluorescence [144].

Tian et al. synthesized N-doped CDs with magnesium elements, which were synthesized by hydrothermal method using citric acid as a carbon source [145]. They observed a strong fluorescence enhancement property in these CDs; however, they concluded that the presence of magnesium atoms did not contribute to the fluorescence enhancement of the N-doped CDs. On the other hand, Wu et al. synthesized N-doped CDs with copper elements [146]. Researchers utilized a direct pyrolysis method to synthesize copper-doped CDs using Na_2[Cu(EDTA)] as the raw material, resulting in a copper content of 2.1%. Although the copper-doped CDs did not exhibit distinctive fluorescence properties, they demonstrated significantly enhanced electron donor and acceptor capacities, 1.5 and 2.5 times higher than those of regular CDs, respectively. These findings suggest that copper-doped CDs may possess excellent photocatalytic performance and hold potential for applications in photocatalysis. In a study by Ding et al. doped CDs containing iron were synthesized, where the iron element was found to exist on the surface of the CDs in the form of trivalent iron ions [147]. Interestingly, the presence of iron exerted a pronounced negative influence on the properties of the CDs, leading to substantial shifts in their spectral behavior. This observation emphasizes the importance of cautious selection and consideration of doping elements during the synthesis of CDs to prevent any adverse effects on their properties and potential applications. CDs.

1.4. Luminescence mechanism and influencing factors

CDs have garnered significant attention due to their unique and exceptional fluorescence properties. However, the mechanism underlying carbon dot fluorescence remains a subject of debate among researchers. Currently, there are three prevailing perspectives on the fluorescence luminescence mechanism of CDs [148]. The first viewpoint attributes the optical properties of carbon nuclei to the quantum size effect, which is primarily associated with the size-dependent behavior of carbon nuclei. The second perspective links the fluorescent luminescence to the presence of various functional groups on the surface of CDs, specifically the optical properties of the surface defect state. Lastly, the third viewpoint proposes that the fluorescence is induced by the extensive π bond conjugated structure formed by CDs [149,150]. As our understanding of the fluorescence luminescence mechanism of CDs advances, it will aid in the optimization of carbon dot properties for a range of applications, including sensing and nanomedicine.

1.4.1. Crystallization of carbon nuclei and quantum size effects

1.4.1.1. Quantum size effects. Bao et al. found that the π electron system possessed by carbon nuclei can have an important impact on the energy band of CDs by comparing the differences in the luminescence characteristics caused by CDs of different sizes and molecular weights [151]. This impact will have a red shift as the carbon nuclei grow. Miao et al. found that the fluorescence color emitted by CDs continuously changes as the size of CDs increases, which may be caused by different graphitization degrees of carbon nuclei [152]. Peng et al. described the impact of different reaction temperatures on the luminescent properties of CDs [153]. By changing the reaction temperature to produce different types of CDs, the size of graphitized carbon nuclei of these CDs will also vary, resulting in different fluorescent luminescence colors [154]. In addition to experimental studies, researchers have also conducted theoretical studies to help explain the various optical phenomena observed in CDs. These studies aim to provide a more complete understanding of the factors that contribute to the optical properties of CDs, which will help guide the design and synthesis of improved carbon dot materials for various applications. By combining experimental and theoretical research, it is hoped that a deeper understanding of carbon dot properties and behavior can be achieved, leading to more innovative and effective future applications. Sk et al. have calculated theoretically that original CDs with different sizes will obtain different emission wavelengths [155]. The carbon dot size increases from 1 nm to 7.9 nm, the fluorescence wavelength of the carbon dot changes from 460 nm to 580 nm (Fig. 4). Kwon et al. have developed fluorescent CDs with full chromatographic emission, which are synthesized by solvothermal methods [156]. This method can change the content of amines to change the size of the CDs, thereby changing the color change of the fluorescent emission

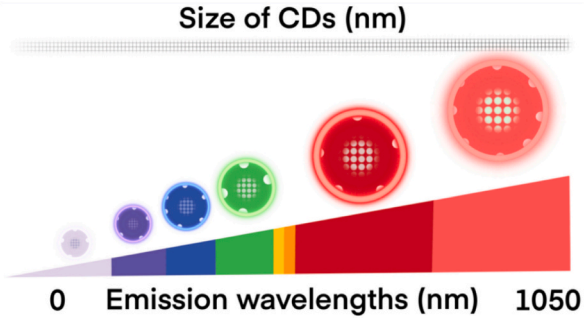

Fig. 4. Fluorescence wavelength as carbon dot size increases.

of the CDs. At the same time, as the size of the CDs continues to increase, the bandwidth of the corresponding CDs will continue to decline.

1.4.1.2. Precursor ratio. The synthesis of CDs commonly involves the use of various precursors in different ratios. The ratio of these precursors has been shown to significantly impact the degree of carbonization of CDs, which in turn can lead to differences in carbon nuclei and affect the fluorescence and luminescence characteristics of the materials [73]. Therefore, careful monitoring and control of precursor ratios during carbon dot synthesis are crucial for producing tailored carbon dot materials with specific optical properties, and for ensuring consistent and reproducible results. Liu et al. confirmed that the use of different precursors during carbon dot synthesis can result in significant differences in the materials' fluorescence luminescence characteristics [157]. To illustrate this point, the scientists employed various precursors, including urea, hexamethylenetetramine, ethylenediamine, and formamide. Although the CDs produced from the hydrothermal method exhibited fluorescence of the same color, these dots underwent solvothermal reaction using toluene as a solvent, and as a result exhibited fluorescence emission of different colors [158,159]. This finding highlights the importance of careful precursor selection and synthesis protocol optimization for the production of high-quality CDs with controlled fluorescence properties for various applications, including bioimaging and sensing. Zhang et al. also have significant differences in the luminescent properties of the CDs obtained by changing the subtle proportions of the same precursor [160]. In a recent experiment, ethylenediamine tetra acetic acid and ammonium citrate were used as precursors to synthesize CDs. By varying the proportions between the two precursors, the types of surface functional groups could be altered, resulting in CDs with differing fluorescence emission colors. This approach highlights the versatility of carbon dot synthesis, as well as the importance of careful selection and control of precursor ratios for producing CDs with desirable optical properties. Further research into the impact of precursor selection on carbon dot properties may aid in the development of improved carbon dot materials for a range of applications.

1.4.1.3. Effect of reaction temperature on carbon nucleus luminescence. The reaction temperature will have a significant impact on the degree of ordering of CDs formed in the carbon nucleus region, thereby affecting the luminescent properties of CDs. Krysmann et al. explored the mechanism of forming CDs at different reaction temperatures through experiments, thereby analyzing how different reaction temperatures have different effects on the luminescence of CDs [161]. In a recent study, researchers examined the factors contributing to the fluorescence of CDs at different temperatures [162]. The results indicated that the fluorescence observed at lower temperatures was not solely attributable to the CDs themselves, but was rather the result of a combined reaction between the CDs and the small molecules of precursor that remained unreacted. Conversely, at higher temperatures, the fluorescence was predominantly generated by the carbon nuclei, demonstrating a clear temperature-dependent shift in fluorescence behavior. This finding provides valuable insights into the mechanisms underlying carbon dot fluorescence and may have important implications for optimizing carbon dot synthesis and performance across a range of different applications. Based on previous researchers, Zhu et al. elaborated in more detail the effects of different reaction temperatures on the synthesis of carbon nuclei [163]. The fluorescence emitted by CDs is influenced by temperature, with different effects observed at lower and higher temperatures. At lower temperatures, the fluorescence may arise from the surface state of CDs, while at higher temperatures, the fluorescence luminescence effect of carbon nuclei becomes more significant. This phenomenon can be attributed to changes in the energy states and reactivity of CDs at different temperatures. These results underscore the complex nature of carbon dot fluorescence and suggest that careful control of synthesis conditions and temperature may be necessary for obtaining CDs with desired fluorescence properties for various applications.

1.4.2. Surface state and surface modification

Large specific surface area is one of the defining characteristics of nanomaterials, including carbon quantum dots. These materials also possess high surface area, making surface modification a common approach to altering their luminescent properties. Surface modification of CDs can improve water solubility, biocompatibility, and reactivity, among other important properties. In previous studies on fluorescence luminescence mechanisms, the optical properties of carbon dot surface defect states were found to play a key role in regulating their luminescent properties [164,165]. As the field of carbon dot research continues to evolve, further investigation into the underlying mechanisms driving carbon dot luminescence may shed new light on the potential applications of these versatile materials.

Bao et al. found that there are a large number of different surface functional groups connected to the surface of the carbon dot outside the carbon dot [136], and these different surface functional groups have different effects on the properties of the carbon dot, and some of the oxygen-containing functional groups can cause dynamic fluorescence quenching of the carbon dot.

1.4.2.1. Surface passivation treatment. Surface passivation treatment is a common method for improving the surface defect state of CDs by enhancing the functionality of surface functional groups. Specifically, this treatment aims to modify the types of functional groups present on the carbon dot surface to enable more efficient recombination of electron-hole pairs on the surface state [166]. By enhancing surface passivation through surface modification, the luminescent properties of CDs can be significantly improved, resulting in enhanced fluorescence and other optical properties. This approach holds great promise for the continued development of high-performance carbon dot materials with tailored optical and functional properties for a wide range of applications in fields such as bioimaging, sensing, and electronics [167]. In the early days of carbon dot research, Sun et al. used polyethylene glycol to passivate the surface of CDs, resulting in fluorescent CDs with high quantum efficiency [61]. CDs with improved surface passivation also exhibit enhanced resistance to photobleaching. In some cases, acid treatment can be used to passivate the surface of CDs that have been peeled off by laser light, resulting in CDs with diminished fluorescence. This approach offers a versatile method for modifying carbon dot

properties with respect to fluorescence, and may have potential applications in fields such as imaging and sensing. By carefully controlling surface passivation treatments, advanced carbon dot materials with tailored fluorescence and other properties may be developed, paving the way for continued innovation and discovery in this exciting area of nanophotonics research [168,169].

There are currently two main types of surface passivating agents used in the production of CDs: organic molecules such as ethylenediamine and thiourea, and polymeric materials [170]. However, since many current carbon dot synthesis methods rely on single-step processes, incorporating organic molecules into the reaction can complicate attempts to assess their exact role in the passivation of carbon dot surfaces [171]. Therefore, utilizing polymeric materials as surface passivating agents has emerged as a promising approach for the further development and application of CDs. Such agents have been found to effectively enhance the emission intensity of CDs, as well as improve their overall biocompatibility. By utilizing advanced surface passivation techniques such as these, researchers can continue to explore new and innovative applications for CDs in fields ranging from medicine to electronics [172].

1.4.2.2. Reaction solvent action. Due to the involvement of solvent molecules in the reaction of carbon point synthesis to modify the surface functional groups of carbon points, different reaction solvents can also affect the fluorescence properties of carbon points, which is called reaction solvent action. Tian et al. prepared fluorescent CDs in the full spectrum of visible light by changing the solvent used in the synthesis process of CDs [173]. The size of CDs can be adjusted by changing the ratio of the reaction solvent and altering the degree of dehydration and carbonization of the carbon source material. By regulating carbon dot size through this approach, it is possible to exert fine control over the luminescence properties of the material. By modulating important factors such as energy states and surface chemistry, researchers can continue to refine the properties of CDs for use in a wide range of cutting-edge applications, spanning fields as diverse as optoelectronics, biomedicine, and sensing [174].

During the synthesis process, CDs also react with ions in solution. Many documents have reported that the fluorescence luminescence changes of CDs respond to external acidic environments [175,176]. Wang et al. have prepared a special carbon dot that is extremely sensitive to pH changes and can therefore be used as a probe for testing pH changes in enzymatic reactions [177].

1.4.2.3. Atomic doping. As mentioned above, atom doping is an effective method to change the fluorescence characteristics of CDs, and it is also another way to modify the surface of CDs. The doping process of atoms can occur both on the carbon nucleus and on the surface of CDs [178]. Different types of doped CDs can have different effects on the different fluorescence properties of CDs, such as improving the quantum yield of CDs, changing the emission wavelength of CDs, and adjusting the luminescent color of CDs [179].

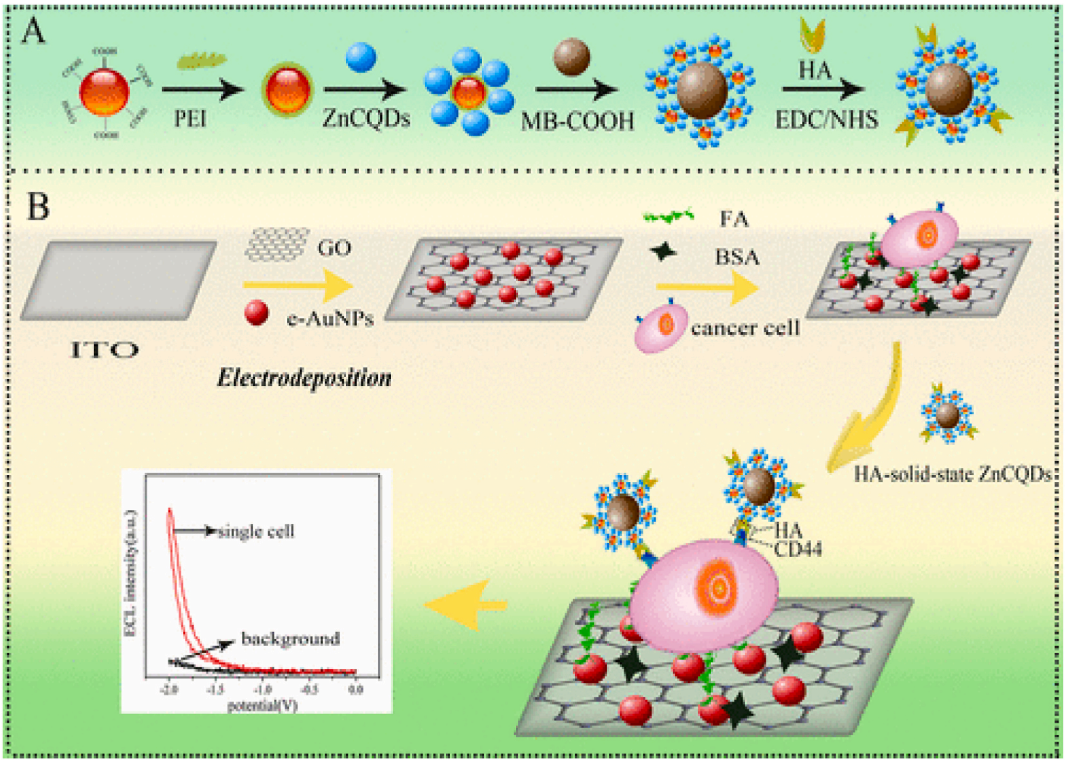

Fig. 5. Preparation process of ZnCQDs nanocomposite ECL Probe(A) and Fabrication of the proposed single-cell analysis Platform(B) [77].

1.5. Applications of CDs in biological and chemical fields

Owing to their remarkable fluorescence properties and other favorable chemical characteristics, CDs find extensive utility in various domains, such as biological imaging, chemical sensing, photoelectric devices, photocatalysis, and more. The subsequent section provides an overview of recent studies focusing on the application of CDs in these areas, encompassing ion and molecular detection, bioimaging, catalytic activity, drug delivery, and disease treatment CDs [180,181].

1.5.1. Ion and molecular detection

CDs have gained significant traction in the field of ion and molecular detection. With their distinctive fluorescence properties and excellent biocompatibility, CDs have been extensively utilized for various detection purposes [132,182–186]. Qin et al. prepared a unique CDs: PL C-dots that can specifically detect divalent mercury ions [132]. The detection principle relies on the selective quenching of fluorescence emission by divalent mercury ions, which can be attributed to electron or energy transfer resulting in the fluorescence quenching of CDs. PL C-dots can be effectively employed for the detection of heavy metal ions in the environment, thereby mitigating the environmental pollution caused by these heavy metal ions and reducing potential harm to human health. Zhu et al. conducted the synthesis of a novel carbon quantum dot capable of detecting ferric ions [182]. This new type of double-emission carbon dot was synthesized using a solvothermal method and doped with copper, resulting in specific wavelength changes corresponding to the concentration of ferric ions. In a similar vein, Yan et al. synthesized a carbon quantum dot that effectively detects bivalent copper ions, inducing specific changes in bivalent copper ion concentrations that can be accurately detected [183]. The carbon quantum dots (ZnCQDs) synthesized in the study by Youyi Qiu et al. [77] were utilized as electrochemiluminescence (ECL) probes. These probes were functionalized with hyaluronic acid (HA) in a solid-state format, enabling specific recognition of HA carrying CD44 on the cell surface. This approach facilitated the labeling of individual breast cancer cells, serving the purpose of breast cancer detection and evaluation of CD44 expression levels (Fig. 5).

He et al. developed a novel fluorescent probe capable of detecting dopamine molecules using FRET technology [184]. In this probe, CDs were used as donor probes and gold nanoclusters were used as acceptor probes. The principle of this probe detection is that dopamine will selectively quench the fluorescence of the probe. In addition, Wang et al. synthesized a carbon quantum dot composed of manganese dioxide mixed with CDs [185], which can be used to test glutathione because manganese dioxide can oxidize 3,3,5, 5-tetramethylbenzidine to produce a blue product whose absorption at 655 nm is weakened in the presence of glutathione. Huang et al. synthesized a carbon dot for detecting hemoglobin, which used sucrose as carbon source and phosphoric acid as dopant to synthesize a nitrogen and phosphorus double doped carbon point [186]. Nitrogen and phosphorus-doped CDs have been found to exhibit high

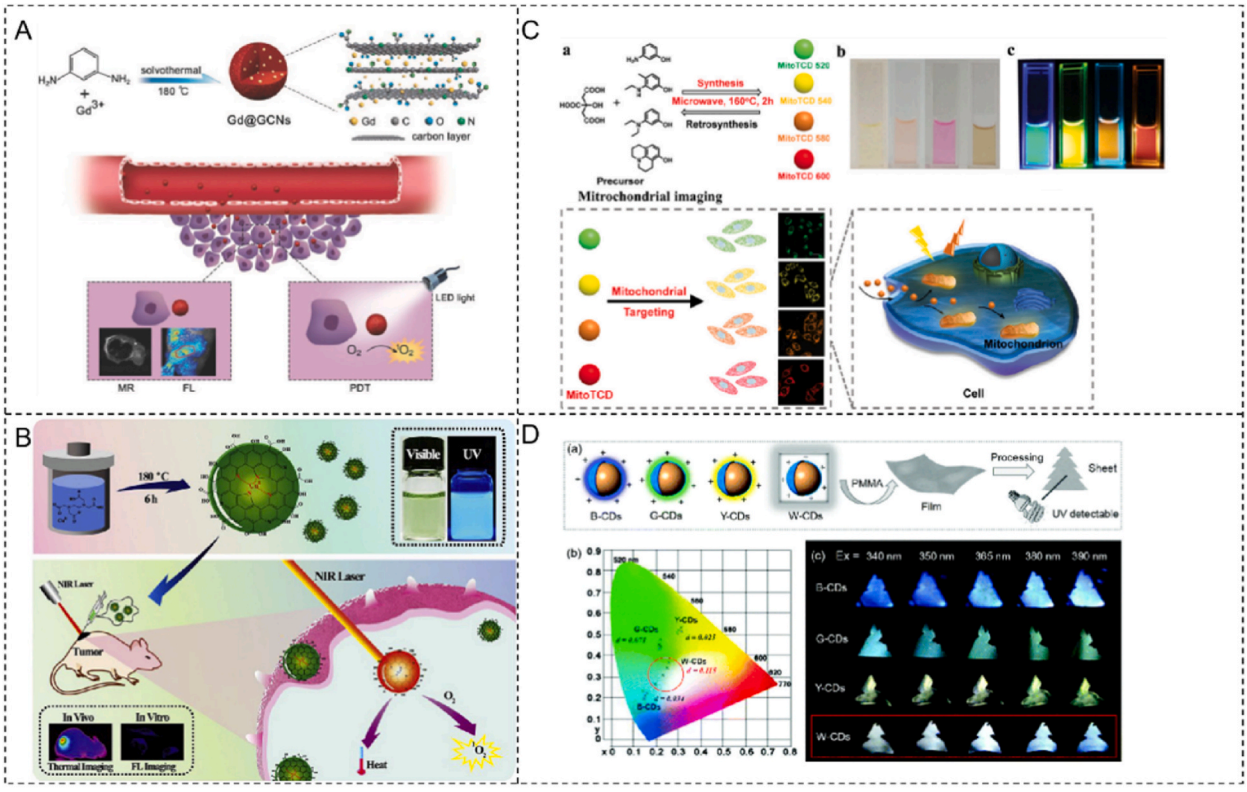

Fig. 6. Examples of CDs in bioimaging applications [123,192,194,195].

sensitivity to hemoglobin, making them ideal candidates for use as ultra-selective fluorescence probes for hemoglobin detection. The surface chemical groups on these CDs, including doped nitrogen and phosphorus atoms, promote strong binding with hemoglobin, while the four ferro globin units in hemoglobin ensure a strong accumulation effect between the protein and the CDs. Importantly, these CDs also exhibit excellent biocompatibility, which enables their use in the detection of hemoglobin inside the human body. By exploiting the unique optical and chemical properties of nitrogen and phosphorus-doped CDs, researchers may be able to develop advanced diagnostic and therapeutic tools for a wide range of medical applications, including disease detection and treatment monitoring [187,188].

1.5.2. Biological imaging

In biological imaging, because of the advantages of CDs, CDs become the first choice for fluorescent probes in biological imaging. First advantage is that carbon dot has low toxicity, little side effects, and good biocompatibility; Secondly, the fluorescence signal generated by the CDs is relatively stable and has a high intensity; Thirdly, the carbon dot has a good water solubility without complex surface processing modification; The fourth advantage is that carbon dot has the property of near infrared emission. The CDs synthesized by Li et al. can emit yellow fluorescence [189]. This yellow CDs not only have excellent optical performance, but also has very good biocompatibility and low cytotoxicity, so it is very suitable to be used as a probe for fluorescence cell imaging. The CDs can also be used to detect the hydrogen peroxide generated in real time during biocatalysis, but in this case, they need to be combined with silver nanoparticles. Gong et al. synthesized the CDs has achieved a new application in tumor treatment by integrating it with fluorescence imaging [190]. This CDs have a high fluorescence intensity, which can improve the accuracy of the CDs in the optical monitoring process.

Magnetic resonance imaging (MRI) is the most landmark application of CDs in clinical transformation [191]. In previous studies, quantum dots usually doped paramagnetic Gd (III) ions in the process of synthesis to achieve MRI effect. In recent years, the rapid development of this field has endowed CDs with more functions and realized the integrated mode of diagnosis and treatment. Hongmin Chen et al. found that after implantation, cells can be clearly differentiated from labeled cells, allowing them to be visualized in deep tissue. Gadolinium-encapsulated graphene carbon nanoparticles (Gd@GCNs) loaded with CDs, made it integrated with fluorescence, MRI, and photodynamic therapy (PDT) properties [192]. In addition, hydrophilic CDs exhibited significant chemical exchange saturation transfer (CEST) contrast ratio due to the abundant hydroxyl, amide and guanidine protons on their surfaces [193] (Fig. 6A).

Cu-doped carbon dots (Cu-CD) prepared via a one-pot hydrothermal method by incorporating transition metals, such as EDTA·2Na and $CuCl_2$, have been employed in near-infrared dual-modal therapy and diagnostic applications. The Cu doping enhances the NIR absorption of the CDs and promotes NIR-mediated photothermal therapy (PTT) and PDT in tumor treatment. They serve as fluorescent cell imaging agents and infrared thermal imaging agents for visualizing both in vitro and in vivo therapeutic processes (Fig. 6B) [123]. Furthermore, Xin Geng et al. [194]. synthesized mitochondria-targeted carbon dots (MitoTCD) with intramitochondrial imaging capability and tunable long-wavelength fluorescence from green to red. MitoTCD enables long-term cell imaging for up to six generations, utilizing rhodamine-based lipophilic cations as the luminescent center of the CDs. They exhibit excellent biocompatibility and superior photostability (Fig. 6C). Dongxiao Zheng et al. [195]proposed the preparation of W-CDs, which are cationic surfactant-based CDs possessing multicolor and dual-emission fluorescence. W-CDs enable visual detection of specific wavelengths in the UV region. They achieve unique multi-channel cell imaging visualization based on the electrostatic interaction with ct-DNA (Fig. 6D).

A. Schematic illustration of the Gd@GCNs as cancer nanotheranostic tools for imaging-guided phototherapy in solid tumors [192]. B. Illustration of Cu,N-CDs preparation and their PTT/PDT of mouse melanoma (B16) cells [123]. C. a) The synthesis produces for four kinds of MitoTCD (MitoTCD 520, MitoTCD 540, MitoTCD 580, and MitoTCD 600). The images of them b) under daylight and c) different excitation and the application of MitoTCD in mitochondria imaging [194]. Dual-emission strategy for the large-scale enhancement of fluorescent tint control. a) Description of the preparation of the CD doped PMMA film. b) The fluorescent color coordinates of the CD samples. c) Photographs of the CD doped film sheets under UV light at different excitation wavelengths [195].

1.5.3. Catalytic activity

Due to the excellent upconversion luminescence properties of CDs, CDs and their compounds have high photocatalytic activity and have been widely used in the field of photocatalysis, such as photocatalytic chemical reactions and photocatalytic degradation of organic pollutants [196]. In 2017, Huang et al. synthesized a new type of nanocomposite material [197], which was composed of $ZnFe_2O_4$ and CDs. This composite catalyst has a good catalytic effect in photocatalytic degradation of nitric oxide. Compared with the single catalytic system with only $ZnFe_2O_4$ nanoparticles, the composite catalytic system with CDs has a higher spectral utilization range. Yu et al. prepared another composite catalytic system, which combines CDs with α-Fe_2O_3 to form a composite catalyst [198], which can effectively photocatalyzed methylene blue degradation and has the ability to be recycled. Guo et al. [199] developed a carbon-coated $CuFe_2O_4$ composite catalyst with excellent catalytic activity in catalytic reduction. At present, CDs and its composite catalytic system have been used in various kinds of catalysis, especially in terms of hydrogen production [200].

1.5.4. Antitumor effects

As is known to all, the recurrence, heterogeneity and metastasis of tumors are the main reasons for the decreased survival rate of tumor patients after surgery. However, currently, chemotherapy and radiotherapy are still used as the main treatment strategies [201]. The low specificity of chemotherapeutic drugs makes them damage the division of normal tissue cells when they work. Radiotherapy, on the other hand, exposes patients to radiation areas, which also affect normal tissue cells to kill tumor cells [202,203]. Therefore, the early diagnosis of tumor and the inhibition or killing of tumor cells have become the current direction of antitumor research. Carbon dot CDs, with its small size and enhanced permeability and retention effect EPR, can be highly enriched and retained in tumor tissues

with abundant blood supply. At the same time, CDs has strong modification, fluorescence and thermal sensitivity [204,205], which makes it applied in the research of tumor therapy [206,207]. These include tumor drug delivery, photothermal therapy and collaborative photodynamic therapy, tumor diagnosis and antitumor mechanism.

Tumor tissue provides a suitable pH environment for the release of anticancer drugs DOX. A pH-responsive hydrogel system combined with nitrogen doped carbon quantum dots NCQDs loaded with hydroxyapatite and DOX is applied to the tumor site [208, 209]. The Schiff base broke away and DOX was released to play an anti-cancer role. In addition, using the fluorescence properties of CQDs, Hang He et al. s study encapsulated CDs and DOX into mesoporous silica nanoparticles (MSN) [210]. Due to the acidic conditions of tumors, the MSN surface structure was destroyed, and DOX and CQDs were released.

Doxorubicin (DOX) can inhibit the synthesis of RNA and DNA, inducing the death of tumour cells in various growth cycles, which is widely used in oncology treatment [211,212]. Fluorescence changes of CQD were monitored to verify the release of antitumor drugs. Photothermal therapy and photodynamic therapy PDT is an emerging strategy of tumor treatment mechanism at present. PTT or PDT alone cannot completely eliminate tumors, and the combination of these two methods can improve the efficacy of scientific research (Fig. 7) [213,214]. In the study of combined materials application, it was found that CQDs is a heat-sensitive agent and ZnPc is a photosensitizer. CQDs can produce obvious thermal effect under 980 nm laser irradiation, ablation of tumor cells in focal areas and enhance DOX release. Based on the upconversion luminescence (UCL), the combined material transfers energy to conjugated PDT reagent (ZnPc) to produce ROS to kill tumor cells [215,216]. In addition, a nanoparticle system loaded with Cu2+ and CDs-Ce6 (Cu/CC NPs) using the synergistic principle [217], partly due to FRET, the assembled Cu/CC NPs hide the fluorescence of CQD, which decomposing and releasing CQD when entering tumor cells as a means of monitoring, the other part of PDT function was effectively restored under the stimulation of tumor microenvironment TME (Fig. 7A). Ding Qu et al. designed a multi-component heat-sensitive lipid nanocomposite based on GNR modifications to enhance anti-breast cancer treatment. Hypothermia (LT-PTT) can temporarily maintain tumors at fever-like temperatures and increase the recruitment of immune cells [218] (Fig. 7B).

Copper-dependent Redox reactions can lead to a depletion of GSH in tumors, which in turn increases intracellular oxidative stress and amplifies the therapeutic efficacy of reactive oxygen species (ROS) in cancer treatment [221]. CDs have played an important role in the discovery, diagnosis, and treatment of tumors. Despite this, there are still several challenges that need to be addressed, and the combination of CDs with other therapies such as immunotherapy, gene therapy, and radiotherapy requires further investigation. By exploring the synergistic effects of CDs and other treatments, researchers may be able to unlock new approaches for fighting cancer and other diseases. Ge et al. used Polythiophene phenyl propionic acid polymer (PAA) as the carbon source and prepared near-infrared CDs by hydrothermal kettle reaction [222]. Under 671 nm excitation light, CDs produced a lot of heat energy in mice. After 10 min of irradiation, the temperature of the tumor site reached 57 °C, which could kill the tumor. Photothermal therapy can achieve local heating, but the temperature should not be too high, otherwise it may cause damage to normal cells or tissues. The participation of carbon quantum dots in photothermal therapy significantly improves this defect. Carbon quantum dots can specifically accumulate at

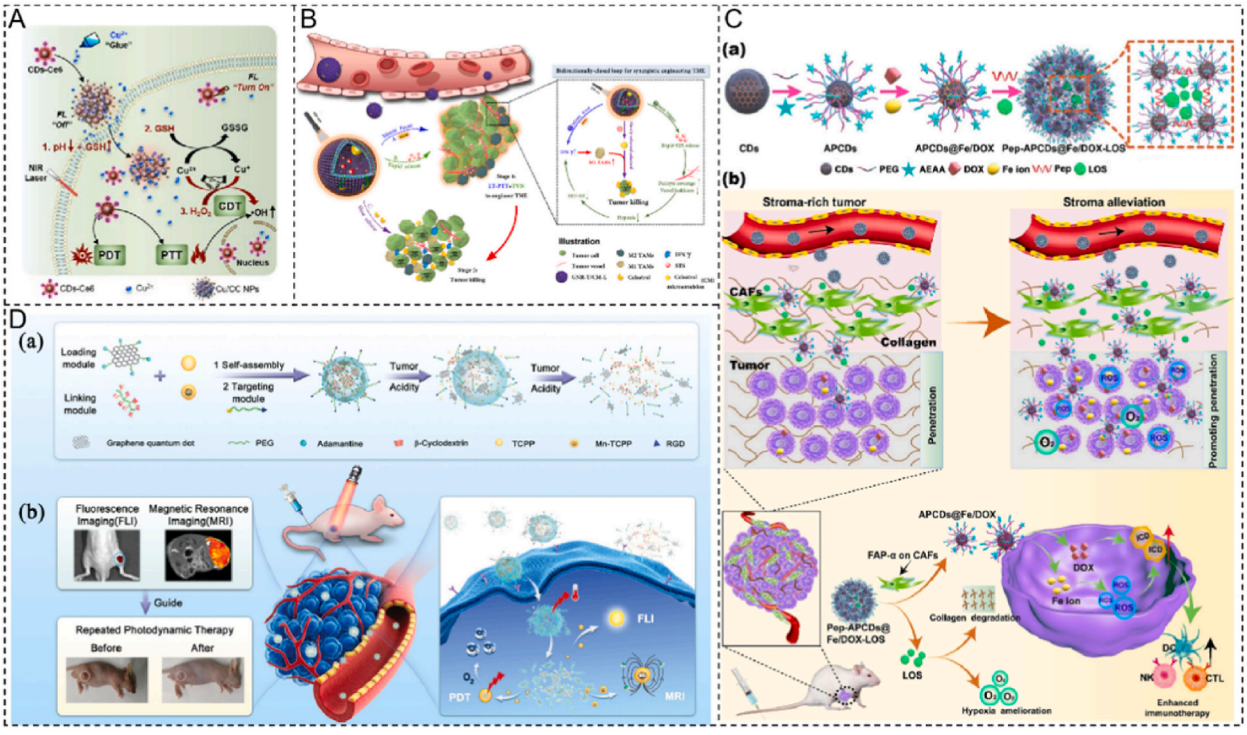

Fig. 7. Examples of CDs in oncology treatment [217,218,219,220].

the tumor site and do not generate excessive temperature under near-infrared irradiation, which not only kills cancer cells but also improves the safety and effectiveness of the treatment process. In addition, the carbon quantum dots can also generate singlet oxygen through multi state sensitization process, and the quantum yield exceeds [223,224]. Through in vitro and in vivo research, they have demonstrated that this carbon quantum dot can serve as a photodynamic therapy drug, while achieving imaging and efficient cancer treatment. CDs have excellent photothermal conversion performance, including a large absorption cross-section, excellent photothermal conversion efficiency, and excellent photothermal stability; CDs not only significantly improve the water solubility of photosensitizers, but also transfer energy to photosensitizers through FRET, greatly improving the fluorescence performance of photosensitizers. The composite system has a lethal effect on tumor cells, and the photodynamic therapy effect is significant. Carbon quantum dots have efficient TPACS, so the carbon quantum dot photosensitizer system under two-photon excitation has great application value. Meanwhile, efficient TPACS significantly enhances the singlet oxygen yield of photosensitizers and the efficiency of tumor treatment [225]. Continued research into the properties and potential applications of CDs holds great promise for the future of medical science and healthcare. Lin Hou et al. [219] integrated immunogenic cell death (ICD)-inducing chemotherapy drug (DOX), ICD-enhancer (Fe ions), and tumor microenvironment (TME) modulator (LOS) within a mesoporous nanostructure (Pep-APCD). Pep-APCDs@Fe/DOX-LOS reduces the production of extracellular matrix collagen and improves tumor oxygenation to regulate TME, thereby enhancing anti-tumor immunity for eradicating tumor cells and combating tumor metastasis (Fig. 7C). Xin Zhou et al. have developed an acidity-activated graphene quantum dots-based nanotransformers (GQD NT) by continuously “deforming” in the tumor, prolonging the residence time of nanodrugs in the tumor, and enhancing the penetration of drugs in the tumor, long-term MRI detection and efficient treatment of cancer tumors are realized at very low drug doses (Fig. 7D) [220].

A. Schematic illustration of a tumor microenvironment (TME) stimuli-responsive fluorescence imaging and trimodal synergistic cancer treatment nanoplatform is facilely constructed via assembling carbon dots and Cu2+ [217]. B. Schematic illustration of synergistic engineering of a tumor immune microenvironment after the treatment with GNR-T/CM-L upon NIR illumination [218]. C. Preparation and immune activation mechanism of Pep-APCDs@Fe/DOX-LOS. a) Schematic illustration for the preparation of drugs-loaded nanoassemblies. b) The transformation and enhanced antitumor immunity mechanism of Pep-APCDs@Fe/DOX-LOS [219]. D. A modularized self-assembly method to fabricate graphene quantum dots-based nanotransformers (GQD NT) for long period tumor imaging and repeated PDT. a) Synthesis of the GQD NT. b) In Vivo Fluorescence and Magnetic Resonance Duplex Imaging of TCPP@GQD NT [220].

1.5.5. Drug delivery

Quantum dots have emerged as promising biological systems for drug delivery, owing to their small size, high surface activity, excellent biocompatibility, and versatile modifiable properties. Furthermore, CDs offer the added benefit of fluorescence, which enables real-time imaging during drug delivery procedures. These unique properties make CDs ideal candidates for targeted drug delivery systems with minimal side effects [226]. By exploiting the potential of CDs for drug delivery and imaging, medical researchers can develop more effective and efficient therapeutic regimens for a wide range of diseases and conditions. Continued exploration of carbon dot properties and techniques for drug delivery has the potential to revolutionize the field of medicine and improve outcomes and quality of care for patients [227]. Devi et al. found that NCDs of aloe vera extract showed a bright blue glow under ultraviolet light. They found that the Aloe vera extract NCDs demonstrated bright blue luminescence under UV light. And staphylococcus aureus and Escherichia coli have very good bactericidal effect [228]. In the treatment of neurodegenerative diseases, the main obstacle is that drug delivery is difficult to cross the blood-brain barrier, resulting in the inability to exert its efficacy. Dopamine, a large molecule of high polarity, plays an indispensable role in neurodegenerative diseases and cannot cross the blood-brain barrier. Sheril Ann Mathew’s study encapsulated dopamine in CDs carbonized from chitosan to form a 144 nm dopamine@CS/CD nanocomposite that stably

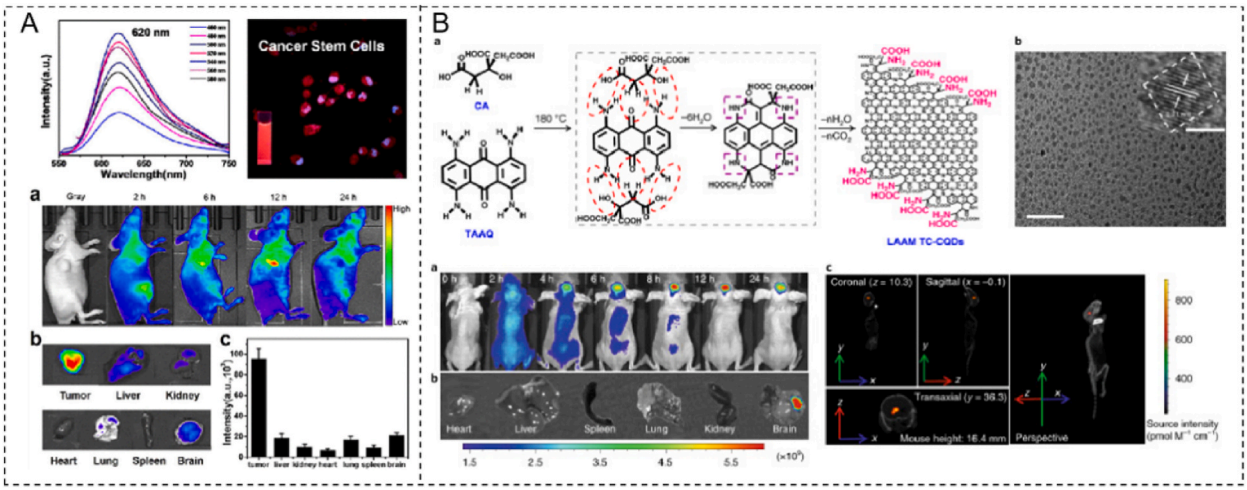

Fig. 8. Examples of CDs in Drug delivery [79,232].

transmembrane for drug release in neurodegenerative diseases [229]. Zilin Wang can induce the epithelial-mesenchymal transition (EMT) process by microwave-assisted heating of CDots synthesized from ascorbic acid and polyethylenimine (PEI) for the treatment of skin wounds activating transforming TGF-β)/p38/Snail signaling pathway [230]. Xiaoting Ji et al. [231] designed dual-functional composite nanospheres for cancer embryonic antigen (CEA) sensing and targeted drug delivery. These nanospheres exhibit high sensitivity for CEA detection in vitro while exerting effective anti-cancer effects. Wen Su et al. [232]synthesized CSCNP-R-CQDs as anti-cancer drug carriers, efficiently delivering DOX to the nucleus of cancer cells, especially cancer stem cells, and demonstrating excellent cytotoxicity against tumor cells (Fig. 8A). Shuhua Li et al. [79] designed and prepared a LAAM CQD that selectively targets tumors by binding to large neutral amino acid transporter 1. This CQD can penetrate the blood-brain barrier and deliver chemotherapy drugs, while also enabling near-infrared fluorescence and photoacoustic imaging of tumors. LAAM CQDs have the potential for clinical applications in imaging and drug delivery for various types of tumors and central nervous system diseases (Fig. 8B).

A. Schematic representation for the fluorescence emission spectra of CSCNP-R-CQD aqueous solution and LCSM images of breast CSCs incubated with CSCNP-R-CQDs. (a-c) In vivo imaging of tumor-bearing mice after intravenous injection of CSCNP-R-CQD/DOX at different time points [232]. B. Schematic diagram of LAAM TC-CQD synthesis (a) and TEM and HRTEM characterization (b). Scale bars, 10 nm and 1 nm (inset). The NIR fluorescence images of representative U87-tumour-bearing mouse (a) and the indicated organs and tumour (b) that received intravenous injection of LAAM TC-CQDs. c) 3D reconstruction of the distribution of LAAM TC-CQDs in the mouse [79].

2. Summary and outlook

This comprehensive review offers a survey of the latest advancements in the synthesis, luminescence mechanism elucidation, and the application of CDs within the realms of biochemistry and medicine. CDs are significant popularity within the domains of optics, energy, and biomedicine, which attributed to their straightforward synthesis, eco-friendliness, diverse synthesis methodologies, impressive optical and electrical traits, cost-effectiveness, and exceptional biocompatibility [233,234]. Extensive research efforts have been dedicated to exploring synthesis strategies, elucidating the structure-property relationships, investigating the underlying mechanisms, and advancing the applications of CDs. Various factors, such as precursor ratios, solvent variations, reaction modes, and size effects during synthesis, as well as extrinsic modifications like doping with other elements and surface functionalization [235], have been used to adjust the physicochemical properties and luminescence characteristics of CDs [236].

The unique properties of quantum dots are based on their inherent quantum effects. When the particle size enters the nanoscale range, size confinement leads to coulomb blockade effects, size effects, quantum confinement effects, macroscopic quantum tunneling effects, and surface effects. These give rise to nanosystems with different low-dimensional properties compared to macroscopic materials, exhibiting many distinct physical and chemical properties. Therefore, quantum dots demonstrate significant advantages in applications such as catalysts, biosensors, drug delivery systems, and bioimaging agents. They also play a crucial role in disease diagnosis and treatment by providing the characteristics of quantum dot photothermal and photodynamic therapies.

CDs possess a broad excitation wavelength range and a narrow emission wavelength range. The fluorescence peak position can be controlled by altering the physical size of the CDs. This allows for the simultaneous excitation of multiple types of quantum dots using the same excitation light, resulting in the emission of fluorescence at different wavelengths for multiplex fluorescence detection. Additionally, CDs can display different colors without overlap, enabling the simultaneous labeling of different components in experiments. Quantum dots exhibit strong resistance to photobleaching and have fluorescence lifetimes 10–50 times higher than those of ordinary organic fluorescent dyes, demonstrating high photostability.

Despite encouraging findings and wide-ranging applications of CDs in the advancement of biology, physics, chemistry, and medicine, there are still numerous critical issues to be addressed. In comparison to quantum dots and other carbon materials, research concerning CDs is still in its infancy [237]. Among the foremost challenges lies the absence of systematic and scalable synthetic strategies to generate high-quality CDs with the required structure, encompassing dimensions, morphology, crystallinity, count of functional groups, defect type and location. In addition, the non-standard synthesis pathways and impurities, the exact reaction mechanism, nuclear mechanism and the formation process of CDs are unknown [238–240]. Furthermore, due to their high surface activity, quantum dots are prone to aggregation, leading to the formation of larger aggregates with weak interfacial connections. The surface of the particles is not smooth and contains numerous defects, which can affect the luminescence efficiency of the nanoparticles. Therefore, surface modification of quantum dots with appropriate functional groups or their multifunctionalization has become the mainstream direction in current quantum dot research. However, the preparation conditions for quantum dots are stringent, involving complex reaction steps and high costs, which pose significant obstacles to the development of quantum dots.

In order to attain the goal of producing high-performance carbon dots on a significant scale, an efficient approach is necessary to systematically explore the influence of precursors and parameters (including temperature, duration, and pH) on CDs performance, and develop purification schemes based on size or polarity [241,242]. Notably, the development of in situ techniques plays a decisive role in the characterization of the mechanism of CDs formation., which facilitates the controlled synthesis of CDs with defined nanostructures [243]. Addressing these challenges will lead to the realization of the full potential of CDs in various applications and propel the field forward.

Tumor is a major disease threatening human life and health, especially brain tumor. The imaging diagnosis of brain tumor has extremely important clinical significance. At present, the research on CDs as optical imaging probes internationally is still in its early stages, and the key issues are mainly as follows: the emission wavelength of most CDs is in the shorter wavelength visible light range of 400 nm–500 nm, which has poor penetrability to body tissues and limits their optical imaging of deep tissues in the body [244,245]. Additionally, interference from the spontaneous fluorescence of organisms is a concern. The distribution of CDs in the body lacks

selectivity and fails to concentrate on lesion sites such as tumor sites, resulting in weak contrast between tumor tissue and normal tissue boundaries, leading to suboptimal imaging outcomes [246]. The particle size scale and surface properties of CDs result in a shorter biological half-life in vivo, further weakening the imaging effect [247]. Therefore, future research directions may focus on CDs that emit light in the red or near-infrared region and have tumor targeting properties to improve their tumor signal-to-noise ratio. Thus, achieving precise and targeted rapid labeling and imaging analysis of relevant lesion locations, achieving non-invasive real-time, dynamic, highly sensitive, and rapid tracing and monitoring, truly achieving integration of diagnosis and treatment.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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