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REVIEW

A review of carbon dots in biological applications

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Abstract Luminescent carbon-based nanomaterials have inspired tremendous research interests due to their tunable optical properties as well as superior biocompatibility. In this review, distinct light emission properties of carbon dots (CDs) derived from different synthesis methods are summarized. The optical properties of as-synthesized CDs can be further controlled by element doping and surface functionalization of CDs for tunable band gap. Due to their low cytotoxicity and tunable optical behaviors, luminescent CDs have been extensively studied for their potential biomedical applications, such as analytical sensors, and bioimaging devices. This review presents a comprehensive overview of the emerging luminescent CDs and their applications as biosensors and bioimaging agents. The challenges and perspectives in the near future are also discussed.

Introduction

Diverse carbon allotropes have attracted extensive interests for their potential applications from electronic devices to biosensors and bioimaging agents. Graphitic structural materials like zero-dimensional spherical fullerene [1–5], diamond nanocrystals (DNs) [6–9] and carbon dots (CDs) [10–14], one-dimensional cylindrical carbon nanotubes (CNTs) [15–18] and two-dimensional graphene quantum dots (GQDs) [19, 20], and graphene [21–23] have been widely investigated in the last decade.

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Although CDs, DNs, and GQDs are three similar quantum-confined fluorescent carbon materials that have been widely employed as biosensors and bioimaging agents [24], the different spatial arrangements of carbon atoms result in distinctive physical and chemical properties [25]. Generally, DNs are mainly composed of a sp^3-hybridized core and a graphitic carbon shell. In comparison, both CDs and GQDs are mainly composed of sp^2 carbon, oxygen, and nitrogen elements and other doped heteroatoms [26, 27]. Actually, the CDs do not have perfect crystal structures as GQDs [28–31]. In addition, the size of luminescent CDs is usually below 10 nm [32–34], while luminescent GQDs obtain a lateral dimension up to 100 nm [34, 35]. Due to their low cost, high quantum yield, abundant source, low cytotoxicity, superior chemical and photo stability, CDs are generally regarded as a potential candidate in biosensing, bioimaging, and other biologically related applications [27, 36–39].

CDs were firstly discovered in the process of purifying single-walled carbon nanotubes fabricated by arc discharge methods by Scrivens in 2004 [40]. In the past few years, various starting materials and synthetic methods have been developed to obtain CDs, including electrochemical synthesis [29, 41–43], supported routes [44–46], combustion/heating [36, 47, 48], hydrothermal [49–56], acidic oxidation [57–59], microwave/ultrasonic [60–67], arc discharge [40], laser ablation [26, 68], and plasma treatment [13, 69]. These above approaches can be classified into two categories: top-down and bottom-up methods. The top-down method refers to cleavage of larger carbonaceous materials, such as CNTs [40, 70, 71], graphite [29, 41, 70, 72], NDs [73], and commercial activated carbon [74]. The bottom-up method generally involves supported route and carbonization [32]. The supported route means employing supports to localize the growth of CDs by blocking nanoparticle

agglomeration during high-temperature treatment [44]. Another bottom-up synthetic strategy is precursor carbonization, which is a facile and common way to achieve CDs via various treatment methods like heating [36], hydrothermal [49], and microwave [65]. Candle was often used as a precursor to fabricate fluorescent CDs [30, 57]. After combustion, the collected candle soot is refluxed with HNO_3 to oxide particles. After cooling, as-synthesized CDs are collected after centrifugation or dialysis. Similarly, the natural gas is also used as a carbon source to achieve CDs via combustion method [28]. Subsequently, different precursors including molecular compounds and natural sources have been widely explored to synthesize CDs. Giannelis and co-workers employed the ammonium citrate [75], octadecylammonium citrate [75], and sodium 11-aminoundecanoate [76] as molecular precursors to achieve luminescent CDs. Small organic compounds, such as citric acid [36, 49, 77], boric acid [78, 79], amino acid [78], glycerol [45, 80], glucose [58, 81–83], ethylene glycol [84], N-acetylcysteine [85], diamine [86], and benzene [69] have also been extensively used as precursors. In addition, natural sources, such as orange juice [87], banana juice [88], soy milk [89], meat [90], beverage [91], coffee [92], beer [93], egg [94], potato [54], punica granatum fruit [48], bombyxmori silk [50], sugar [95], bread [95], jiggery [95], lysozyme [96], sucrose [58, 97], starch [58], and grass [98] have been studied to fabricate CDs. Generally, these CDs are rich in surface functional groups including carboxyl, amino, hydroxyl, and other groups, which facilitate further modifications to tune optical properties. The synthetic methods of CDs have been systematically discussed in several published reviews [32, 99, 100].

In this review, tunable optical properties and cytotoxicity of CDs are addressed. Recent advances in CDs including biosensors and bioimaging agents are presented. Current challenges and perspectives for the future development of CDs are also discussed.

Advances in bioapplications

Absorbance and photoluminescence

Although tremendous efforts have been devoted to investigate the origin of photoluminescence (PL), the mechanism is still unclear. Quantum effect and defect emission are currently regarded as the main emissive mechanism of CDs [101].

In general, CDs display strong optical absorption in the UV region, with a tail extending out into the visible range (as shown in Fig. 1a). Most of the one-step prepared CDs exhibit absorbance in the range 280–360 nm [26, 49, 102, 103]. The absorption band could be regulated via various surface passivation or modification (as shown in Fig. 1a) [25, 58].

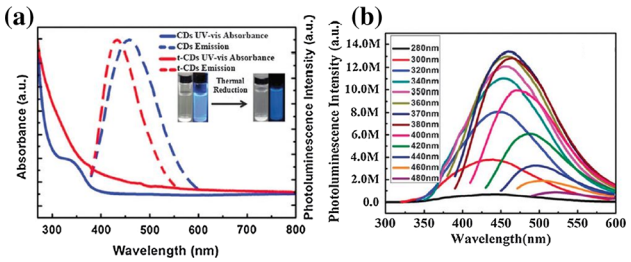

Fig. 1 **a** UV–Vis absorption and normalized photoluminescence spectra of the CDs and t-CDs. Inset: photograph of the CD (*left*) and t-CD (*right*) aqueous solutions under visible light and 365 nm UV light, respectively. **b** Emission spectra of the CDs with excitation at different wavelengths. Reproduced from Ref. [54] with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry

CDs usually show the excitation-dependent emission properties in wavelength and intensity (as shown in Fig. 1b) [104]. Liu et al. reported that the polyethyleneimine (PEI) passivated CDs exhibited colorful luminescence. The blue, green, or red luminescence were excited under ultraviolet (330–385 nm), blue (460–495 nm), or green (530–550 nm), respectively [38]. The PL intensity decreased extraordinarily under longer wavelength excitation. This phenomenon may result from the size and surface state non-uniformity of PEI passivated CDs. In contrast, the excitation-independent CDs were also reported [29, 49]. The CDs co-doped with nitrogen and sulfur (N, S-CDs) indicated excitation-independent emission, since the PL properties of the N, S-CDs depended on surface states rather than morphology, and the surface states of the N, S-CDs were uniform [49]. In our previous study, the similar results of N, S-CDs were also achieved [77]. Recently, Hu and Trinchi reported a new type of CDs whose peak fluorescence emission wavelengths were tunable across the entire visible spectrum via adjustment of the reagents and synthesis conditions [47]. In this report, various synthesized types of CDs achieved an optimal emission peak, however, in some cases (at longer wavelengths) a small shift in emission with varying excitation was observed, which indicated that the excitation-dependent or -independent behavior mainly result from surface state of CDs.

Although CDs are generally regarded as zero-dimensional (0D) nanoparticles with a small size range below 10 nm, the size effect is a key factor that can attribute to the PL properties of CDs. Kang and Lee reported that UV light emission, bright visible light emission, and near-infrared emission were illuminated from small-sized (1.2 nm), medium-sized (1.5–3 nm), and large-sized (3.8 nm) CDs under UV light, respectively [105]. Pang et al. reported that the CDs prepared by electrochemical oxidation method exhibited obvious size-dependent PL in

two fractions (19 ± 0.3 and 3.2 ± 0.5 nm) [29]. In our previous research, the charge-dependent emission was discovered in CDs derived from stacked graphene nanofibers [53]. Figure 2 shows different emissions from various surface-charged CDs under 365 nm UV lamp.

In addition, pH value also has a significant impact on the PL intensity of CDs. Pan et al. found that the PL intensity of CDs gradually enhanced with pH value increasing from 1 to 13 while reduced with pH value decreasing from 13 to 1 [106].

Up-conversion PL is a strong benefit for luminescent nanomaterials so that their applications can be expended to biosensor and bioimaging agents. Recently, Kang and his co-workers developed ultrasonic synthesized CDs that exhibited strong emission upon two-photon excitation in the near-infrared region [105]. Recently, a novel and facile approach was reported to fabricate three types of CDs that could emit bright and stable red, green, or blue (RGB) colors under a single ultraviolet-light excitation. Interestingly, up-conversion PL was also found in these CDs [107].

Quantum yield (QY)

Brightness and photostability are two major points that should be taken into account in the application of luminescent CDs. Generally, the theory that CDs achieve high resistance to photobleaching is widely acknowledged [100]. In our previous study, excellent photostability was observed in pristine CDs, thermally reduced CDs, and N, S-CDs [54, 77]. This superior optical property attracts researchers to concentrate on developing CDs with higher QY. QY is an essential parameter for luminescent nanomaterial. In early studies, CDs prepared from candle soot, citric acid, stacked graphene nanofiber, or graphite possessed low QY, which was usually below 10 % [26, 53, 54, 57]. To enhance the QY of CDs, diverse designs have been developed including surface passivation or modification, element doping, and inorganic salt doping.

Surface passivation or modification is the common way to improve the QY of CDs due to its facile processibility and high efficiency. Sun et al. reported the PEG_{1500N} (diamine-terminated poly(ethylene glycol)) could endow the non-luminescent CDs with bright luminescence. The most luminescent CDs with a high QY of 60 % were achieved via rigorous separation by an aqueous gel column [108]. Chi and his co-workers synthesized polyamine-functionalized CDs with high QY (42.5 %) via a one-step method combining low-temperature carbonizated CDs and branched polyethylenimine (BPEI) [102]. In our previous study, the QY of CDs was improved via thermal reduction with less carboxyl groups on its surface, the scheme is shown in Fig. 3 [54].

Recently, element doping has been utilized to increase the QY of CDs [59, 67, 109]. Nitrogen is widely used as a doping element to synthesize nitrogen-doped CDs with a high QY using various nitrogenous compounds [77, 110–112]. Jiang et al. [113] employed amino acids as the raw materials to prepare CDs with high QY (44.9 %) via a microwave method. Since some nitrogenous compounds can serve as both surface passivator and nitrogen source, it should be noted that the element doping and surface passivation of nitrogenous reagents are hard to discriminate. For instance, Li and Yu et al. prepared nitrogen and sulfur co-doped CDs via hydrothermal method with extremely high QY (73 %) [49]. In this method, L-cysteine was used as both surface passivator and element-doped source. To our knowledge, the QY of N, S-CDs is highest (>70 %) among all the luminescent CDs by introducing both nitrogen and sulfur elements. In addition, Xu et al. synthesized sulfur-doped CDs with significant fluorescence QY (67 %) via a hydrothermal method, which indicated that the sulfur doping achieved an efficient QY improvement [53]. In addition to nitrogen and sulfur, boron is another doping element to enhance the QY of CDs. Bourlinos and Zboril et al. reported a green and simple route to improve QY of CDs via boron doping, and the boron-doped CDs achieved significantly enhanced non-linear optical properties [67]. Phosphorus was also doped into CDs to improve the QY via microwave pyrolysis of polyol in the presence of inorganic ions [65]. Recently, Yang et al. reported a facile and economical one-step approach for synthesizing CDs with a high QY (around 80.3 %). In this approach, citric acid and glutathione were employed as precursors [114].

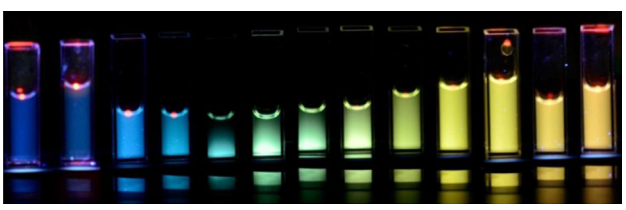

Fig. 2 The photograph under 365 nm UV lamp of charge-separated CD fractions. Reprinted with permission from [53]. Copyright © 1999–2015 John Wiley & Sons, Inc. All Rights Reserved

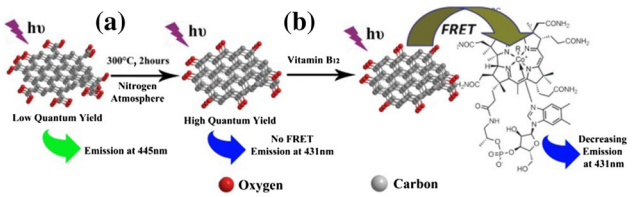

Fig. 3 **a** Thermal reduction process from CDs to t-CDs and **b** fluorescence resonance energy transfer process from t-CDs to vitamin B_{12}. Reproduced from Ref. [54] with permission from the Centre National de la Recherche Scientifique (CNRS) and The Royal Society of Chemistry

Moreover, some other methods have been developed to improve the QY of CDs. For example, Sun and his co-workers developed CDs doped with inorganic salt to achieve high QY [115]. The QY of the CDs doped with ZnS was around 45 % while that of the CDs doped with ZnO was enhanced to more than 50 %. Interestingly, the brighter CDs (QY = 78 %) were achieved via a similar method with little modification [116]. In addition, highly luminescent oil-soluble CDs were synthesized by Liu et al. [117]. In their study, the 1-hexadecylamine (HDA) was used as the surface passivation agent. After that, the N-(b-aminoethyl)-c-aminopropyl methyldimethoxy silane (AEAPMS) was also used as the surface passivation agent to fabricate oil-soluble CDs with a high QY [118].

Recently, Tong and Liu et al. synthesized amorphous CDs with high two-photon fluorescence via a hydrothermal approach. In this method, citric acid and hyperbranched poly(amino amine) were used as the carbon source and surface passivation agent, respectively. Although the QY of CDs in aqueous solution was only 17.1 %, the CDs emitted bright fluorescence in the solid state with a QY of 16.3 %, which is the highest solid-state fluorescence value obtained for carbon-based nanomaterials [55].

Cytotoxicity

In order to be considered in bioapplications, the prerequisite condition for luminescent nanomaterials is low cytotoxicity. So far, the cytotoxicity of luminescent CDs in vitro has been comprehensively investigated [37, 38, 73, 107, 119–122], while the studies of the cytotoxicity of CDs in vivo are still rare. Therefore, more and more studies have been done to explore the properties and potential applications of CDs in vivo [27, 70, 121, 123–125]. The previous studies reported that CDs are generally non-toxic in in vitro experiments [32, 104].

Sun's group devoted lots of efforts to study the biocompatibility and cytotoxicity of surface-passivated CDs and the surface passivation agents in vitro and in vivo [27, 121, 123]. They first investigated the cytotoxicity of the PEG_{1500N}-passivated CDs and PEG_{1500N} both in vitro and in vivo. The human breast cancer MCF-7 and human colorectal adenocarcinoma HT-29 cells were incubated by PEG_{1500N}-passivated CDs and PEG_{1500N}. Figure 4 clearly shows that the effects of CDs on the proliferation, mortality, and viability of the cells were as weak as PEG_{1500N}. In addition, in vivo biodistribution and cytotoxicity of CDs were investigated via intravenous injection in mice. No obvious toxic effects of CDs were observed in mice at the concentration required for PL bioimaging. Although some CDs were found in some essential organs including liver, spleen, and kidney, the levels of accumulation were extremely low that no significant toxicity was observed. In addition, CDs were cleared via renal excretion within about 24 h in mice and no clinical symptoms were observed after 28 days. Furthermore, four different polymers were used as surface passivation agents to prepare CDs and it was found that the cytotoxicity of CDs was derived from the passivation molecules. Therefore, surface passivation agents with low cytotoxicity are more suitable to develop CDs with high compatibility in order to be applied in bioapplications as biosensors and bioimaging agents.

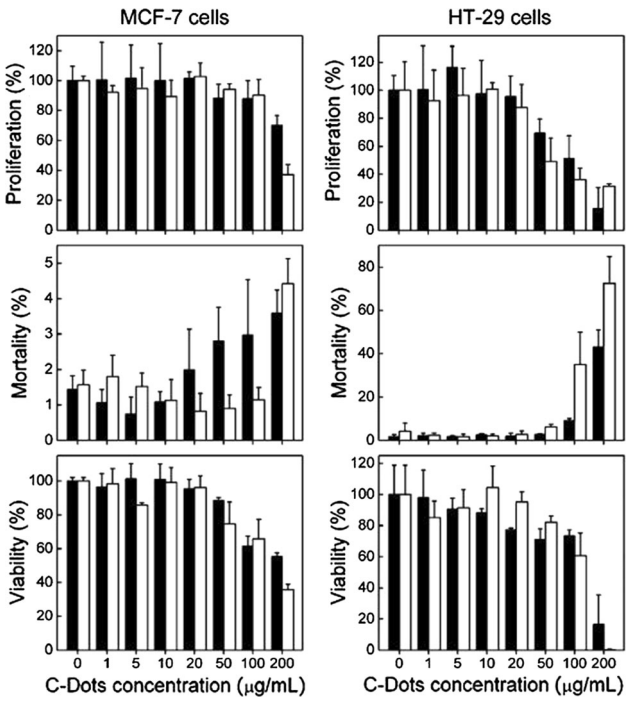

Fig. 4 Results from cytotoxicity evaluations of C-Dots (*black*) and PEG_{1500N} (*white*). Data presented as mean ± SD ($n = 4$). Reprinted with permission from [121]. Copyright © 2009, American Chemical Society

Similar conclusions were achieved by other researchers' studies. Tao et al. performed an in vivo cytotoxicity investigation in mice for over three months, which exhibited no death and even a significant body weight drop in treated mice [70]. In addition, no apparent toxic effects were observed in treated mice with the injection dosage of CDs (20 mg/kg). Subsequently, blood tests and histological analyses of treated mice showed no obvious toxic effects of CDs in in vivo biodistribution.

Bioapplications

Biosensors

In addition to their superior biocompatibility, CDs possess the ability to serve as either excellent electron donors or electron acceptor. Therefore, CDs can be used for

intracellular detection of ions, biological pH value, protein and enzymes, vitamins, and nucleic acid, etc.

Although CDs derived from various raw materials were used to detect different ions, including Cu^{2+}, Hg^{2+}, Ag^{2+}, Cr^{3+}, Fe^{3+}, K^{+}, Cl^{-}, and H^{+} [14, 43, 53, 67, 98, 102, 126–133], the ion detection mechanisms are similar. The surface functional groups on CDs indicate distinctive affinities to different target ions, which results in the quench of PL intensity through an electron or energy transfer process and high selectivity to other ions. For instance, Qu et al. used dopamine as the raw material to develop CDs, which could be used as a Fe^{3+} sensor with an excellent detection limit of 0.32 μM [127]. The fundamental mechanism is simply depicted in Fig. 5. The interaction between CDs and Hg^{2+} or other ions results in the quench of PL intensity. The PL is recovered when the interaction is broken via external force. Recently, the fluorescent turn-on system was also designed. Yang et al. fabricated a novel oligodeoxyribonucleotide (ODN)-CDs and graphene oxide optical sensor to detect Hg^{2+} [134]. In detail, fluorescence of ODN-CDs was efficiently quenched by graphene oxide via fluorescence resonance energy transfer (FRET). With the addition of Hg^{2+}, the luminescence was recovered by releasing ODN-CDs from GO due to the formation of T-Hg^{2+}-T duplex. An ultrasensitive CDs-based sensor was achieved via a one-step hydrothermal treatment of potatoes to detect phosphate [54].

In addition, CDs are also used to determine the physiological pH value in living cells and tissues. Tian's group synthesized aminomethylphenylterpyridine (AE-TPY) CDs to probe the pH value changes in physiological conditions

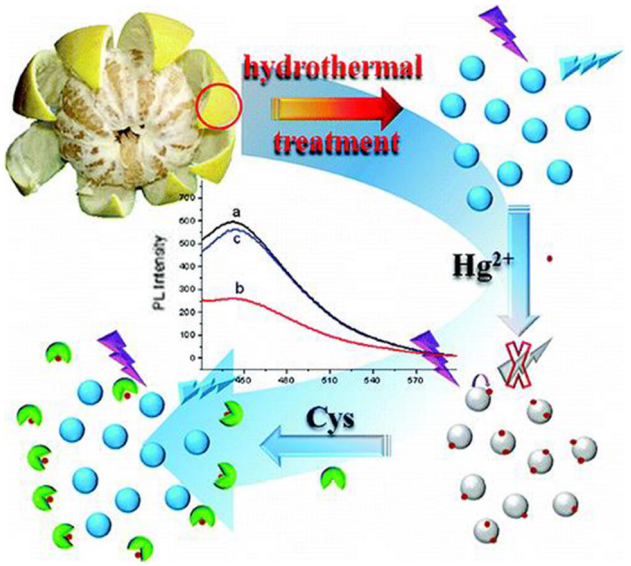

Fig. 5 Scheme of CDs to detect Hg^{2+} in aqueous solution. Reprinted with permission from [128]. Copyright © 2012 American Chemical Society

[135]. This PL sensor could be employed to monitor pH value gradients in a range of 6.0–8.5 with high sensitivity and selectivity. In addition, this sensitive sensor was also successfully applied in living cells and tumor tissues of mice, which demonstrated that the CDs-based pH sensors could be further used in vitro and in vivo. Recently, the CDs were employed inside living pathogenic fungal cells to detect the intracellular pH value [134].

Most recently, CDs-based sensors have been developed to detect nucleic acid. For instance, Li et al. designed a CDs-based strategy for DNA sensing that CDs were labeled with single-stranded DNA (ssDNA) which quenched the PL of CDs [136]. Until the combination of the target DNA and the labeled ssDNA to form double-stranded DNA (dsDNA), the PL was recovered when the labeled ssDNA disconnected from CDs.

On the other side, the DNA-labeled CDs have also been developed to detect protein and enzymes [137]. For instance, a novel CDs-dsDNA sensor was synthesized to detect histone [138]. In this sensor, the PL was quenched in the presence of dsDNA, while with the addition of histone, the luminescence was turned on through unwinding dsDNA from CDs due to the strong affinity between histone and dsDNA. With the binding affinity between DNA and proteins, the quantitative detection of protein could be calculated from fluorescence restoration.

CDs-based sensors have received extensive interests to detect bio-molecules including vitamins and amino acids. In our previous studies, riboflavin and vitamin B_{12} were detected by surface-functionalized CDs to target molecules [54, 77]. The ratio-metric sensing protocol is widely accepted due to the influence of temperature variation and local probe concentration variation could be eliminated successfully. It is mainly because the ratio of two interconnected PL signals is considered as a detection index [139–142]. In our previous study, the riboflavin was effectively detected by CDs-based ratio-metric sensor with high sensitivity (1.9 nM) [77]. Recently, a novel turn-on CDs-based sensor was fabricated to detect cysteine with excellent selectivity and sensitivity [143]. In addition, Liu and Zhang et al. designed a nanosensor composed of CDs and gold nanoparticles to detect cysteine with multiple signals [144].

Furthermore, Yu's group developed naphthalimide azide derivatives anchored CDs to detect H_2S with a detection limit of 10 nM, which is the lowest among the fluorescent H_2S sensors [139]. In this method, the highly sensitive sensor could be employed to detect H_2S not only in aqueous media and serum, but even inside living cells, which further broadened the applications of CDs in biomedical area.

Recently, Dong et al. reported a type of nitrogen-rich CDs via a microwave-assisted pyrolysis approach, which

could be applied as a dual sensing platform for both the fluorescent and electrochemical detection of 2,4,6-trinitrotoluene (TNT). In this report, the fluorescence sensor was developed based on the TNT-amino interaction, which could efficiently quench the luminescence of amino-functionalized CDs via charge transfer [63]. Algarra and coworkers investigated potential applications of CDs as an analyzer of four heterocyclic aromatic amines [66]. In addition, CDs-based fluorescent sensor was used to determine the concentration of hydroquinone in waste water. [145] Furthermore, CDs were also employed as a fluorophotometric sensor to determinate the critical micelle concentration (CMC) of ionic and non-ionic surfactants via Stokes shift [146].

Bioimaging

Although semiconducting quantum dots (QDs) such as CdSe and other related core–shell nanoparticles have been investigated in bioimaging in vitro and in vivo, serious health problems and environmental concerns limit their bioapplications due to the existence of heavy metals. CDs with superior photostability and low cytotoxicity have been widely studied in optical imaging applications as an alternative to QDs. Both in vitro and in vivo evaluations indicated that CDs are excellent candidates in bioapplications due to their visible excitation and emission wavelengths, high brightness at the individual dot level.

A lot of studies were performed via fluorescence from diverse CDs in cell imaging of various cell lines, including Caco-2 cells [147], Ehrlich ascites carcinoma cells [30], HEK293 [148], pig kidney cell line [149], B16F11 [148], murine P19 progenitor cells [44], BGC823 cells [118], HeLa cells [54], Escherichia coli cells [150], etc. [38, 50, 77, 87, 114, 151, 152]. For instance, Sun et al. reported the PEG_{1500N}-passivated and PPEI-EI-passivated CDs were used to label cells for bioimaging [26, 150]. Liu et al. developed CDs via one-step microwave-assisted pyrolysis of glycerol in the presence of TTDDA, which exhibited strong PL and multicolor emissions. These CDs existed in cells could be observed via multicolor PL under a confocal microscope [153]. Hahn et al. developed PEG diamine-capped CDs to label B16F1 and HEK293 cells. The confocal laser scanning microscopic images of B16F1 and HEK293 cells after incubation with CDs are depicted in Fig. 6 [148]. Recently, Wang and Leung et al. widely investigated the effect of nitrogen doping ratios in CDs for in vitro and in vivo bioimaging [154].

In addition, the bioimaging application of CDs was further developed by Hahn [148]. In this report, the CDs could be employed for real-time bioimaging of target-specific delivery of hyaluronic acid (HA) derivatives. HA/CDs hybrids were fabricated by amidation reaction

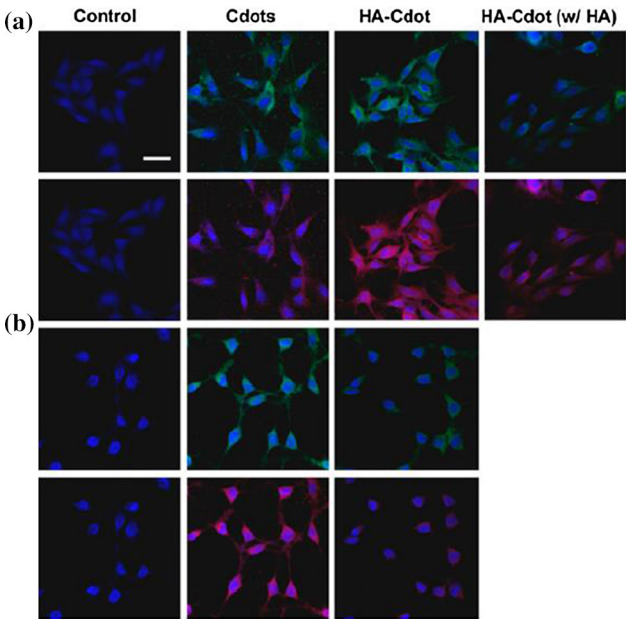

Fig. 6 Confocal laser scanning microscopic images of **a** B16F1 and **b** HEK293 cells after incubation at 37 °C for 24 h with C-dots and HA–C-dot conjugates in the absence and presence of 100-fold molar excess HA. Scale bar indicates 30 μm. Reprinted with permission from [148]. Copyright © 2012 American Chemical Society

between amino groups on the surface of CDs and carboxylic groups of HA. According to in vivo real-time bioimaging, the HA/CDs hybrids revealed the target-specific delivery to liver. Sun's group comprehensively investigated the effect of PEG_{1500N}-C_{ZNS}-dots in the mice body with various injection methods [27]. The results demonstrated that CDs injected in various ways into mice maintained strong fluorescence in vivo (as shown in Fig. 7). Due to their high biocompatibility and low cytotoxicity, CDs are very promising for bioimaging and other related bioapplications.

Compared to down-conversion fluorescence, up-conversion fluorescence has a lot of advantages including noninvasive and deep penetration of NIR radiation in bioapplications. Various CDs can achieve obvious up-conversion PL properties, which have been widely investigated and reported [81, 105, 155, 156]. For instance, Salinas-Castillo et al. reported that PEI-CDs exhibited excellent up-conversion PL properties with the emissions located in the range of 308–550 nm by long-wavelength light excitation [130]. These up-conversion CDs have been effectively employed for in vitro bioimaging with two-photon excitation [26, 55, 115, 150]. As depicted in Fig. 8, CDs show a strong emission with either the 458 nm excitation or 800 nm two-photon excitation. In addition, in vivo NIR fluorescence imaging was also investigated by Zhang, Kang, and Liu et al. [70].

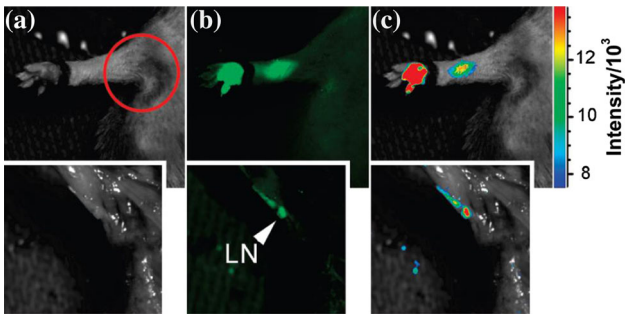

Fig. 7 Interdermal injection of C_{ZnS}-Dots: **a** bright field, **b** as-detected fluorescence, and **c** *color-coded* images. *Insets* dissected (in the *circled area*) axillary lymph node (LN). Reprinted with permission from [27]. Copyright © 2009 American Chemical Society (Color figure online)

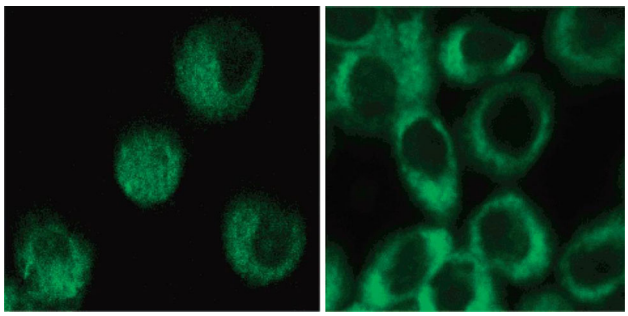

Fig. 8 Representative two-photon luminescence image (800 nm excitation) of human breast cancer MCF-7 cells with internalized C-Dots. Reprinted with permission from [26. Copyright © 2007 American Chemical Society

Recently, Kailasa et al. [48] employed CDs synthesized from punica granatum fruit as an imaging agent for bacterial and fungal cells, which demonstrated that CDs were non-toxic and did not inhibit the growth of *B. subtilis*. Therefore, the CDs can be applied as fluorescent probes for imaging of both animal and plant cells.

Summary and perspectives

In this review, an overview of CDs has been summarized to introduce the recent advances in bioapplications. Firstly, the physical and chemical properties including photoluminescence and cytotoxicity that attributes to bioapplications have been reviewed. Then, the bioapplications, such as biosensors and bioimaging agents, have been systematically introduced.

As an emerging luminescent material, tremendous efforts have been devoted to synthesis, performance, mechanism, and applications of CDs. With the high photostability and chemical stability, low cytotoxicity, and high quantum yield, CDs are employed as non-toxic alternatives to replace traditional heavy metal-based QDs. However, two major problems still exist and impede the further bioapplications of CDs: (a) CDs prepared from diverse methods exhibit large size distribution and photoluminescence non-uniformity, and the complex and time-consuming separation and purification severely limit their further applications; (b) The mechanism of photoluminescence is still unclear. Therefore, more efforts on CDs might be concentrated on the following fields. (1) Effective synthesis with a high yield should be developed to fabricate CDs with a high quantum yield in a small size distribution; (2) A physic understanding of CDs' photoluminescence phenomenon, especially their bright multiphoton emission, should be explored, which can facilitate their in vivo applications.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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