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Review article

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Carbon dots as versatile nanomaterials in sensing and imaging: Efficiency and beyond

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ABSTRACT

Carbon dots (CDs) have emerged as a versatile and promising carbon-based nanomaterial with exceptional optical properties, including tunable emission wavelengths, high quantum yield, and photostability. CDs are appropriate for various applications with many benefits, such as biocompatibility, low toxicity, and simplicity of surface modification. Thanks to their tunable optical properties and great sensitivity, CDs have been used in sensing as fluorescent probes for detecting pH, heavy metal ions, and other analytes. In addition, CDs have demonstrated potential as luminescence converters for white organic light-emitting diodes and light emitters in optoelectronic devices due to their superior optical qualities and exciton-independent emission. CDs have been used for drug administration and bioimaging in the biomedical field due to their biocompatibility, low cytotoxicity, and ease of functionalization. Additionally, due to their stability, efficient charge separation, and low recombination rate, CDs have shown interesting uses in energy systems, such as photocatalysis and energy conversion. This article highlights the growing possibilities and potential of CDs as adaptable nanomaterials in a variety of interdisciplinary areas related to sensing and imaging, at the same time addressing the major challenges involved in the current research and proposing scientific solutions to apply CDs in the development of a super smart society.

1. Introduction

The advent of carbon-based materials heralds a transformative era in material science, delineated by their remarkable versatility and environmentally benign nature. Historically, the utilization of carbon materials has traversed a diverse spectrum, from the conventional industrial variants such as activated carbon and carbon black to the pioneering realms of carbon fibers and graphite. This evolution reflects not only the material advancements but also the shifting paradigms of industrial and scientific endeavors. In recent decades, the exploration of carbon's potential has taken a quantum leap with the advent of carbon nanomaterials, notably graphene and carbon nanotubes (CNTs), which have opened new vistas in a multitude of interdisciplinary fields [1]. These materials, characterized by their unique properties, have paved the way for innovations across chemistry, materials science, and beyond. However, despite their impressive attributes, traditional and macroscopic carbon forms often grapple with inherent limitations, such as the

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absence of a necessary band gap, which precludes their efficacy as luminous materials. This constraint underscores a pivotal challenge in harnessing carbon's full potential, paving the way for the emergence of Carbon Dots (CDs) as a groundbreaking solution.

CDs, a novel entrant in the carbon family, have emerged as a focal point of research interest, attributed to their exceptional photoluminescence (PL), high quantum yield (QY), and a plethora of desirable properties that render them apt for a wide range of applications. Tracing back to their serendipitous discovery during the purification of single-walled carbon nanotubes, CDs have evolved from mere scientific curiosities to central figures in nanomaterials research. CDs flaunt a suite of properties that make them particularly enticing for sensing and imaging. Their quantum confinement and edge effect endow them with photoluminescence, which can be tuned from the ultraviolet to the near-infrared spectrum by manipulating size, surface state, and doping [2]. Moreover, the high surface area to volume ratio and abundant functional groups on their surface facilitate the adsorption or conjugation of various biomolecules or analytes, enhancing their sensing capabilities [3]. The allure of CDs lies not just in their optical properties but also in their high biocompatibility. These characteristics, coupled with their abundance and cost-effectiveness, have positioned CDs as captivating subjects for scientific research and application, bridging the gaps observed with their carbon predecessors. The synthesis and functionalization of CDs have been subjects of intense study, aimed at optimizing their properties and expanding their application landscape.

The field of CDs is a testament to the dynamic nature of material science, where the confluence of creativity, innovation, and interdisciplinary collaboration shapes the future of technological advancements. As researchers delve deeper into the nuances of CDs, they unravel the complexities that govern their behavior, unlocking new potentials and challenging existing paradigms. This journey from macroscopic forms of carbon to the nano-scale marvels of CDs encapsulates the essence of scientific exploration, driven by curiosity and the quest for solutions to real-world problems. CDs stand at the forefront of this quest, embodying the promise of carbon materials and the unexplored territories they hold. Their rise from a niche area of research to a field of significant interest underscores the evolving landscape of material science, where the infusion of nanotechnology and environmental consciousness paves the way for innovative solutions.

CDs have attracted a lot of attention in biomedicine for their use in medication delivery, theranostics, and bioimaging. CDs have demonstrated significant potential as contrast agents for high-resolution bioimaging, enabling real-time monitoring of biological processes [4,5] as well due to their special optical properties. They are the perfect candidates for cellular imaging because of their small size, which enables effective cellular uptake [6]. Furthermore, tailored drug delivery systems have been made possible by their biocompatibility and surface functionalization, which offer controlled release profiles for better therapeutic results [7,8]. To improve the selectivity, efficacy, and safety of various therapeutic agents and medication administration, scientists are intensively investigating the possibilities of CDs.

Fluorescent CDs are a new type of fluorescent nanomaterial and are gaining popularity as benign alternatives for quantum dots (QDs) in bioimaging, biosensing, and disease detection due to their small particle size (<10 nm), excitation wavelength-dependent photoluminescence and great biocompatibility. CDs were used as fluorescent agents in sensing applications to detect various analytes, such as heavy metal ions, pH, and mercury ions [9–11]. They allow effective and targeted detection due to their tunable optical characteristics, high quantum yield, and sensitivity. Multi-emission fluorescence sensor arrays have also been coupled with CDs to improve their detection capabilities [12]. In various applications, including bioimaging, biosensing, and disease detection, fluorescent CDs have become popular safe substitutes for QDs [13]. C-dots are appropriate for biomedical applications due to their tiny particle size (<10 nm), excitation wavelength-dependent photoluminescence, and high biocompatibility [12]. Additionally, fluorescent CDs are useful fluorescent agents for detecting diverse analytes in sensing applications due to their tunable optical characteristics, high quantum yield, and sensitivity [12]. Besides these disciplines, CDs have found use in optoelectronics, sensing, catalysis and in many industries [9,10,13–16]. Innovative materials and technologies have been created by their tunable emission wavelengths, simple synthesis processes, and compatibility with various matrices.

CDs can catalyze various reactions, including the photocatalytic degradation of organic pollutants, the reduction of pollutants, and the synthesis of useful compounds [10]. In the field of optoelectronics, CDs have demonstrated potential as light emitters for



Fig. 1. a) Scholarly output focusing on Carbon Dots, b) Research disciplines of carbon dots.

optoelectronic devices such as light-emitting diodes and photovoltaic devices, as well as luminescence converters for white organic light-emitting diodes [17]. High PL quantum yield, color tunability, and exciton-independent emission are just a few of the excellent optical characteristics that make them desirable candidates for creating effective and high-performance devices. CDs have been made in the biomedical industry, particularly in medication delivery and bioimaging. They are suitable for cellular imaging and tracking because of their biocompatibility and low cytotoxicity [18]. Additionally, drug loading and functionalization of CDs are simple, allowing for regulated drug release and targeted drug administration [7]. CDs have been used in many ways in the energy sector. For instance, according to Chu et al. (2019) and Liu et al. (2021), they have been used as effective photocatalysts for energy conversion and environmental remediation [19,20]. Remediation of the environment is another crucial area where CDs have demonstrated tremendous potential. Thanks to their photocatalytic and photoluminescent characteristics, they have been used to detect and degrade contaminants in water and the air [10]. These nanoparticles can effectively eliminate harmful chemicals, providing a viable approach to solving environmental problems. Fig. 1a, b shows the impact of CDs in scientific research and the various applications these nanomaterials cover.

CDs become more effective and selective when functionalized with ligands, increasing their affinity for contaminants [6]. Additionally, they are desirable candidates for extensive environmental cleanup applications due to their low cost and simplicity of synthesis. With CDs, there have been notable breakthroughs in energy storage and conversion. CDs have improved light absorption and charge transfer capabilities when used as sensitizers in solar cells, increasing photovoltaic efficiency [20]. Next-generation solar cells have benefited from their capacity to capture solar energy efficiently. Additionally, they have great charge-discharge rates and high energy storage capacities thanks to their use in supercapacitors, which has aided in the creation of effective energy storage devices [1, 21]. With better power densities and quicker response times, CDs have demonstrated potential as electrode materials, helping to meet the growing demand for sophisticated energy storage systems.

In the dynamic realm of nanotechnology, Carbon Dots (CDs) emerge as a revolutionary class of nanomaterials heralded for their versatility and groundbreaking applications across numerous interdisciplinary domains. This review paper aims to encapsulate the



Fig. 2. Classification of Carbon dots (CDs) including Graphene Quantum Dots (GQDs), Carbon Nitride Quantum Dots (CNQDs), Carbon Quantum Dots (CQDs), Carbon Nano Dots (CNDs) and Carbonized Polymer Dots (CPDs). Images reproduced with permission from refs. ([1,22]).

Z.A. Qureshi et al.

comprehensive advancements and multifaceted uses of CDs, with a particular emphasis on sensing and imaging—delving deep into their transformative role in these specific areas, while also acknowledging their broader implications in biomedicine, optoelectronics, catalysis, energy conversion, and environmental sustainability.

As we unfold the narrative of CDs, our discourse is dedicated to exploring the core attributes that set these nanomaterials apart: their exceptional photostability, tunable fluorescent emissions, remarkable biocompatibility, and facile surface modification. These physicochemical properties position CDs not just as an innovation within nanotechnology but as a seminal breakthrough that challenges and expands upon traditional material limitations. Through a detailed examination of cutting-edge synthesis methods, surface functionalization techniques, and doping strategies, this paper provides insights into the boundless possibilities that CDs offer. Herein, we elucidate how CDs can be ingeniously tailored to meet the exacting demands of wide-ranging applications—chief among them, their pivotal role in enhancing the precision and efficacy of sensing and imaging technologies.

Focusing intently on the domain of biomedicine, we highlight how CDs, benefiting from their biocompatibility and targeted delivery capabilities, are revolutionizing drug delivery systems. In the realm of sensing, CDs are lauded for their unmatched sensitivity and selectivity, reshaping our approach to monitoring and detection applications.

Despite the optimistic outlook, the path from laboratory innovation to market application for CDs is beset with challenges—ranging from the scalability and reproducibility of synthesis methods to the intricate understanding required of their behavior within complex biological and environmental matrices. This review delves into the ongoing endeavors within the scientific community aimed at overcoming these obstacles, with the ultimate goal of fully harnessing CDs' capabilities. By meticulously examining the current hurdles faced in CD research, this article posits a roadmap of scientific strategies not only to address these challenges but also to pave the way for groundbreaking applications of CDs.

As we traverse the complex landscape of CDs' applications, especially in sensing and imaging, this discussion is poised to serve as a guiding light for future explorations, endeavoring to unlock the full potential of CDs. In doing so, it accentuates the transformative impact of CDs on propelling technology and healthcare forward, envisioning a future enriched by the profound capabilities of nanotechnology. This review asserts the critical role of CDs in driving the nanotechnological frontier towards realizing a 'super smart' society, where sensing and imaging are but facets of their extensive utility in advancing human progress and environmental stewardship.

2. Classification of carbon dots

Classifying Carbon Dots (CDs) is a challenging endeavor due to their diverse nanostructure characteristics. However, a more structured classification has emerged, distinguishing CDs into five principal types based on the variance in their carbon core compositions (Fig. 2). This classification greatly aids in understanding the distinct functionalities and applications of each CD type.

Graphene Quantum Dots (GQDs): GQDs are essentially single-layer graphene fragments with diameters less than 10 nm. However, most GQDs commonly possess a few atomic layers and may include edge or internal layer defects due to suboptimal synthesis conditions [23]. The physical thickness of GQDs directly influences their physicochemical properties, such as absorbance [24]. Despite this, GQDs retain certain graphitic characteristics, such as the in-plane lattice spacing similar to that of bulk graphite [25]. The presence of heteroatoms through doping can modify these properties to tailor GQDs for specific applications. Moreover, the edge sites of GQDs, characterized by their "edge effect," significantly contribute to their predominant properties and performance [26].

Graphitic Carbon Nitride Quantum Dots (g-CNQDs): Often analogized with GQDs owing to their graphene-like two-dimensional structure, g-CNQDs differ markedly in their elemental composition and nanostructure. Comprising tri-s-triazine units linked by amino groups and characterized by periodic vacancies, g-CNQDs exhibit a 'poly(tri-s-triazine)' framework [27]. This structure endows them with remarkable catalytic properties, distinct from other carbon dot variants [28]. High crystallinity g-CNQDs with a unique lattice parameter have been observed, demonstrating the profound influence of the structural design on their properties [29,30].

Carbon Quantum Dots (CQDs): Representing a broader category, CQDs are quasi-spherical nanoparticles characterized by a crystalline core composed of both sp2 and sp3 hybridized carbons [31]. CQDs' distinctive lattice structures and high carbon content, as evidenced by specific Raman spectral features, highlight their core composition's complexity [32]. Notably, the lattice spacing of CQDs plays a pivotal role in their functionality, offering potential for a range of applications from sensing to catalysis [33,34].

Carbon Nanodots (CNDs): Although CNDs are occasionally conflated with CQDs, they are primarily characterized by an amorphous core, differentiating them from the crystalline core of CQDs [35,36]. Their luminescent properties, coupled with relatively lower costs, position CNDs as promising candidates for future applications across various fields.

Carbonized Polymer Dots (CPDs): CPDs emerge from the aggregation or cross-linking of polymers or monomers, significantly distinguishing themselves from the other CD types [37]. The carbonization degree within CPDs depends on reaction conditions and precursor materials, contributing to the enhanced fluorescence and stability of these particles. Such properties make CPDs particularly suitable for various applications, especially in biomedical fields for drug, gene, or protein delivery systems.

This classification not only sheds light on the distinct types of CDs available but also underscores the importance of understanding their unique structural and functional properties. As research in this area continues to evolve, the nuanced understanding of each CD type's characteristics facilitates their application in diverse fields, ranging from optoelectronics to biomedicine [38,39].

3. Synthesis & functionalization of carbon dots

Methods for producing Carbon Quantum Dots (CQD) are divided into two primary categories: (i) top-down approaches and (ii) bottom-up strategies, as illustrated in Fig. 3 [40]. In top-down strategies, either physical or chemical techniques are applied to

fragment or disintegrate larger carbon sources into finely divided CQD particles. This category includes techniques such as arc-discharge, laser ablation, and acidic oxidation [41]. Conversely, bottom-up strategies consist of a sequence of chemical reactions designed to build up CQDs from smaller molecular precursors through polymerization and carbonization processes. Specific methods within this strategy encompass microwave pyrolysis, various combustion methods, electrochemical synthesis, and hydrothermal or solvothermal processes.

3.1. Top-down approaches

3.1.1. Laser ablation

The laser ablation method represents a sophisticated approach for synthesizing Carbon Quantum Dots (CQDs), focusing on the use of high-energy laser pulses to engineer these nanomaterials from carbonaceous sources. This method illuminates the precursors, inducing a thermodynamic state characterized by extreme pressure and temperature, resulting in the rapid formation of a plasma state where vapor crystallizes into carbon nanomaterials. The method begins by directing high-energy laser pulses onto a carbon precursor's surface. This interaction prompts the system to reach extremely high pressures (approximately 75 KPa) and temperatures (exceeding 900 °C), facilitating the transition into a plasma state where vapor crystallizes to form various carbon nanomaterials, including CODs [46]. Following the irradiation with laser, carbon nanoparticles aggregate. Initially, these particles may not exhibit observable photoluminescence. However, post-synthesis treatments, such as interaction with a dilute HNO3 solution and refluxing followed by passivation with substances like polyethylene glycol (PEG), are employed. These processes introduce functional groups to the CQDs' surfaces and refine their photophysical properties [47,48]. Through the judicious selection of organic solvents and surface passivating agents, the surface condition of CQDs can be tailored. This modification enables variation in the photoluminescence (PL) properties of the synthesized CQDs. Particularly, passivated CQDs exhibit high PL with diameters around 5 nm. The method is lauded for its ability to produce CQDs with limited size distribution, high water solubility, and enhanced fluorescence [49,50]. The initiation of carbon dots (C-Dots) was pioneered through a laser ablation technique [51]. Following this, a novel approach was developed to fabricate carbon dots leveraging a liquid-phase laser ablation, incorporating a solution of ammonia, ethylenediamine, and pyridine to enrich the dots with amine and pyridine nitrogen groups [52]. This innovation allows for the tuning of the carbon dots' fluorescence emission: amine-rich content leads to red-shifted emission, while an abundance of pyridine nitrogen results in blue-shifted emission. By incorporating specific functional groups via a liquid solution, this method significantly enhances the luminescence characteristics of carbon dots. Several research reports underscore the potential of laser-ablated CQDs in practical applications. N-doped Micro-pore



Fig. 3. Top-down and Bottom-up processes for carbon dots synthesis. Schematic representation of CQDs production using laser ablation process, modified, and reprinted with permission of [42]. Schematic representation of arc-discharge method for production of carbon dots, reprinted with permission from Ref. [43]. Electrochemical oxidation of graphite for production of a colorless dispersion of CQDs in alkaline alcohol at 5V, reprinted with permission from Ref. [2]. Production of Nitrogen and Sulfur co-doped carbon dots using direct combustion technique, modified with permission from Ref. [44]. Schematic of the microwave pyrolysis process for the synthesis of CQDs, modified and reprinted with permission from Ref. [45].

CQDs achieved a quantum yield of 32.4 percent and a fluorescence lifespan of 6.56 ns, marked by stable and strong photoluminescence emission suitable for cellular staining and imaging, showing good internalization in various cells [53]. Dual Beam Laser Ablation created from low-cost carbon fabrics, these CQDs exhibited a 35.4 percent QY, making them suitable for cell bioimaging applications [54]. Despite its efficacy, the method requires a significant amount of raw materials and often results in CQDs with low QY due to an inability to control particle sizes precisely. Nonetheless, the rapidity and effectiveness of the laser ablation method, coupled with the tunability of the CQDs' surface state, make it a valuable tool in the CQD synthesis repertoire.

3.1.2. Acidic oxidation

The acidic oxidation method stands as a pivotal technique in the top-down approach for synthesizing CQDs, distinguished by its effectiveness in fragmenting bulk carbon into nanoparticles. This technique, besides reducing the size of carbon materials, concurrently integrates hydrophilic functional groups, such as hydroxyl or carboxyl, onto the surface of CODs, enhancing their dispersion in aqueous and biological environments. The acidic oxidation method commences with the use of a potent oxidizing mixture, typically comprising HNO₃, H₂SO₄, and NaClO₃. This blend is used to oxidize carbon nanoparticles derived from readily available sources such as Chinese ink, initiating the breakdown of bulk carbon structures [55]. The oxidized carbon material is then subjected to hydrothermal reactions with sources of nitrogen, sulfur, and selenium-namely, di-methyl formamide, sodium hydro-sulfide, and sodium selenide, respectively. This step importantly introduces heteroatoms, leading to the formation of doped CQDs, which exhibit enhanced photoluminescence performance and quantum yield compared to their undoped counterparts. Nitrogen doped CQDs, Sulfur doped CQDs, and Selenium doped CQDs synthesized using this technique display elongated fluorescence lifespans, improved photoluminescence performance, and favorable quantum yields, a testament to the effectiveness of acidic oxidation in creating high-quality, doped CQDs. The doped heteroatoms alter the electronic structure of CQDs, enhancing their electrocatalytic activity and application potential in electrochemistry [56]. Following the acidic oxidation procedure, CQDs have found extensive utility in electrocatalysis, cellular imaging, and as sensors. For instance, CQDs synthesized from coke via acidic oxidation demonstrated a yield of 9.2 %, showcasing their suitability for practical applications [57]. Moreover, targeted HIV treatment was explored using graphene quantum dots (GQDs) prepared through this method, with surface modification using reverse transcriptase inhibitors (RTIs) for effective drug delivery [58]. The acidic oxidation approach is celebrated for its simplicity, scalability, and effectiveness in producing CQDs on a large scale. One of the key advantages of this process is its capability to augment the water solubility of carbon dots, a feature that has led to its widespread application towards enhancing solubility. However, the use of strong oxidizing agents poses certain risks, including potential combustion or explosion, and complex post-processing steps are often required to achieve desired purity levels.

3.1.3. Arc discharge

The synthesis of Carbon Quantum Dots (CQDs) through the arc-discharge method represents a notable top-down approach, capitalizing on the reorganization of carbon molecules from bulk carbon sources under high-energy conditions. This process unfolds within a specialized setup—a sealed reactor equipped with an anodic electrode [59], creating a conducive environment for the high-temperature reaction necessary for the formation of CQDs. The arc-discharge method necessitates a sealed reactor environment where gas plasma can be initiated between electrodes. This plasma is critical for driving the breakdown and reorganization of carbon molecules from bulk sources. The application of an electric current generates temperatures up to 4,000 K within the reactor. Such extreme heat is instrumental in creating a plasma with sufficient energy to facilitate the desired molecular reorganization. Carbon vapor resulting from the plasma conditions congregates at the cathode, culminating in the formation of CQDs. During the synthesis process, carbon molecules disassembled from bulk sources are effectively reconstituted into nano-sized quantum dots. The utility of this method was particularly highlighted in the synthesis of single-walled carbon nanotubes (SWCNTs), where it inadvertently led to the generation of three distinct types of carbon nanoparticles. These nanoparticles exhibited comparative molecular masses and showcased prominent photoluminescence, underscoring the potential of arc-discharge in producing functional CQDs [60]. In practical implementations, the arc-discharge method has facilitated the production of CQDs with varying fluorescence emissions, including orange, blue-green, or yellow under a 365 nm light. Through post-synthesis treatment with HNO₃, the CQDs' surfaces were functionalized with hydrophilic carboxyl groups, enhancing their water solubility [61]. Notably, Chao-Mujica et al. utilized a submerged arc-discharge process to synthesize CQDs in water, yielding particles with a quantum yield of 16 %. These CQDs displayed significant fluorescence, emitting consistent bands within the spectrum of 320-340 nm (band A) and 400-410 nm (band B), making them viable for use as fluorescent biomarkers in in-vitro L929 murine fibroblasts studies [43]. Despite its promise, the arc-discharge method confronts certain challenges, particularly in yield and purity. The process typically generates CQDs with a relatively large average size $(\sim 18 \text{ nm})$, coupled with the production of complex components that complicate purification efforts. This complexity often leads to reduced yield and diminished fluorescence quantum efficiency. Consequently, the usage of this method is currently infrequent.

3.2. Bottom-up approaches

3.2.1. Solution routes

Hydrothermal and solvothermal synthesis methods constitute a critical bottom-up approach for producing Carbon Quantum Dots (CQDs), distinguished by their simplicity, efficiency, and the ability to yield high-quality CQDs with controlled size and excellent quantum yield. These methods notably involve the reaction of organic precursors within a high-pressure, high-temperature aqueous (hydrothermal) or solvent-based (solvothermal) environment, typically within a sealed reactor like a stainless-steel autoclave lined with Teflon [62]. The synthesis starts with dissolving small organic molecules in water or an appropriate organic solvent. This solution is then transferred to the autoclave, where it is subjected to elevated temperatures, enabling the organic molecules to merge and

initiate carbon seeding cores [63]. These cores progressively grow into CQDs with sizes generally less than 10 nm [64]. Among its many advantages, the hydrothermal method facilitates the synthesis of CQDs with uniform sizes and high quantum yields. The as-synthesized CQDs have found extensive applications across various domains, including bio-imaging, drug delivery, sensor development, and in the fabrication of solar cells and supercapacitors [65–67]. This method's eco-friendly and non-toxic nature, combined with its cost-effectiveness, positions it as a considerably advantageous approach for large-scale production. Through the adjustment of experimental conditions, such as the quantity of graphite nitrogen and selection of biomolecules rich in carbon and nitrogen, this method allows for the precise tuning of the photoluminescence properties, doping composition, and electronic architectures of CQDs [68]. High quantum yields of up to 80 %, equivalent to fluorescent dyes, have been achieved using hydrothermal methods, underscoring the potential of this approach in synthesizing CQDs for advanced applications [69]. Innovations have led to the synthesis of CQDs from a variety of biomass sources, such as banana peels and broccoli, demonstrating the method's versatility and sustainability [70,71]. Despite its numerous benefits, this method can sometimes yield CQDs with variable sizes and lower quantum yields, emphasizing the need for optimization in reaction conditions and precursor selection.

3.2.2. Microwave synthesis routes

Microwave irradiation stands out as a prominent bottom-up technique for the synthesis of Carbon Quantum Dots (CODs), distinguished by its direct, efficient approach and commercial viability [72]. This method leverages microwave irradiation to activate polar molecules within the solvent, catalyzing atomic-level heating without the need for a physical medium. The inherent simplicity, along with the capacity for rapid, high-yield reactions, positions this method as a green, time-efficient pathway to CQD production. Utilizing a modified commercial microwave setup, microwave irradiation promotes intense interaction between polar molecules, leading to immediate thermal effects at the atomic level. This process favors a prompt and selective yield through direct application of high-frequency electromagnetic radiation, significantly enhancing the reaction rate compared to traditional heating methods [73]. The method is celebrated for its rapid synthesis timeline, environmentally friendly nature, energy and time efficiency, minimal impurity incorporation, and precise control over size and temperature conditions, all of which contribute to high reproducibility and superior quality of synthesized CQDs. The produced CQDs have showcased excitation-dependent photoluminescence (PL) characteristics, with surface oxygen-containing groups enabling metal ion coordination [74]. This feature is particularly advantageous for developing carbon-based electrocatalysts. Exceptional quantum yields, as high as 82.7 %, indicate the method's efficiency, although purification processes may adjust these values [75]. Furthermore, microwave-assisted synthesis has been utilized to generate heteroatom-doped CQDs (e.g., N, S, P) with notable quantum yields, demonstrating the method's versatility in creating functional materials for a range of applications, including bio-imaging, drug delivery, and sensor fabrication [76-78]. CODs synthesized via microwave-assisted routes have emerged as potent tools in bioimaging due to their high fluorescence stability and low toxicity, offering significant advantages over conventional fluorescent dyes. For drug delivery applications, their surface functionality enables conjugation with therapeutic molecules, providing targeted delivery and controlled release mechanisms. The high sensitivity and selectivity of microwave-synthesized CODs have been exploited in the development of sensors for the detection of ions, biomolecules, and solar cells [79]. Their tunable surface properties facilitate specific interactions with analytes, allowing for the detection of trace amounts. Despite its convenience and eco-friendly profile, the primary limitation of the microwave route lies in the less controllable size distribution of the resulting CQDs.

3.2.3. Combustion routes

The combustion synthesis route provides a straightforward, scalable, and environmentally friendly methodology for the production of Carbon Quantum Dots (CQDs) [80]. This technique employs combustion reactions of suitable carbon-rich organic precursors to yield highly luminescent CQDs with controllable properties. The attractiveness of this method lies in its simplicity, the ability to precisely design starting molecules, and its cost-effectiveness, making it particularly suitable for bulk production [81]. The combustion method enables the rapid conversion of organic precursors into CQDs under high-temperature conditions. This process leads to the formation of CQDs with homogenous sizes and an abundance of functional groups, such as carboxyl groups, which are critical for enhancing their solubility and reactivity for further applications [82]. CQDs synthesized using the combustion route have shown significant promise in bio-imaging due to their high quantum yield and excellent photostability. Their surface functionality allows for easy bioconjugation with targeting molecules, making them ideal candidates for specific biological imaging and diagnostics tasks [44]. The unique photoluminescent properties of combustion-synthesized CQDs enable their use as sensitive and selective sensors for various analytes, including metal ions, pollutants, and biomolecules. The tunability of their optical properties through simple modifications in the combustion process allows for the development of specialized sensors with high specificity [83]. Besides its cost-effectiveness and simplicity, the combustion synthesis method is noted for its eco-friendliness and the ability to produce CODs with minimal impurities. The method facilitates a controlled design of CQDs, granting tailor-made solutions for a wide range of applications while ensuring consistent quality and reproducibility. Despite its many advantages, the combustion method faces challenges related to the precise control of CQD size and distribution. Moreover, the presence of small polycyclic aromatic hydrocarbons (PAHs) poses potential environmental concerns, necessitating careful management and optimization of combustion conditions [84].

3.2.4. Electrochemical routes

The electrochemical synthesis route presents an innovative and sustainable approach to producing Carbon Quantum Dots (CQDs), leveraging relatively simple electrochemical reactions to generate these nano-sized materials. This method is distinguished by its operational simplicity, ambient conditions (temperature and pressure), and the direct conversion of carbon precursors into CQDs without requiring complex instrumentation or hazardous chemicals. Electrochemical synthesis involves the application of an electrical

current to carbon precursors dissolved in an appropriate electrolyte solution. This process induces the oxidation or reduction of the carbon source, leading to the formation of CQDs [85]. The method is highly favored for its ability to tailor the size and photoluminescent properties of CQDs by adjusting the electrochemical parameters such as voltage, current, and time. CQDs synthesized through electrochemical methods exhibit excellent photostability and biocompatibility, making them ideal candidates for bioimaging applications. Their surface functional groups facilitate conjugation with biomarkers or targeting ligands, allowing for targeted imaging of specific cells or tissues with minimal toxicity. The unique optical and electronic properties of electrochemically synthesized CQDs, including their size-dependent fluorescence, have been exploited in developing sensors for detecting various analytes. These include ions, molecules, and environmental pollutants, with high sensitivity and selectivity owing to the customizable surface chemistry of CQDs. Through electrochemical carbonization, employing sodium citrate and urea in deionized water, CQDs capable of blue emission (\sim 2.4 nm in size) are generated. These CQDs demonstrate their utility in sensitive mercury (Hg²⁺) detection in wastewater, showcasing the method's application in environmental monitoring. A distinctive electrochemical setup comprising a three-electrode system-with a graphite working electrode, a platinum foil or wire as the counter electrode, and an Ag/AgCl reference electrode—facilitates the CQD synthesis [86]. Utilizing alkaline alcohol as the electrolyte, a potential of 5 V is applied to the graphite electrode for 3 h within a nitrogen atmosphere, achieving a colorless COD dispersion [87]. This electrochemical strategy, being environmentally gentle, cost-efficient, and highly reproducible, diverges from other methodologies by preventing electrode enlargement and dispensing with brown COD dispersion [88]. The quantum yield (QY) noted for exfoliated CQDs reaches up to 5.6 %, with nitrogen-doped variants (Fe–N-CQDs) showing a 7.5 % QY, indicating slightly lower levels compared to alternate methods [89, 90]. The electrochemical synthesis route is notably advantageous for its environmental friendliness, cost-effectiveness, and the capability for large-scale production. Additionally, this method offers precise control over the doping of CQDs with heteroatoms, enhancing their photoluminescence and electronic properties for advanced sensing and imaging applications [91]. While offering numerous benefits, the electrochemical synthesis of CQDs faces challenges regarding the uniformity of particle size distribution and controlling the extent of doping. Continuous efforts in optimizing reaction conditions and electrode materials are essential to overcome these hurdles and fully harness the potential of this synthesis route.

3.2.5. Functionalization

Carbon dots (CDs) are distinguished by their nanoscale dimensions and highly varied microstructure, which significantly



Fig. 4. (a)Relation of the energy gap with the number of fused aromatic rings of graphene oxide, reproduced with permission from Ref. [101]. (b) The structure of different sizes of GQDs along with their edge conformation whether zig-zag(ZZ) or arm-chair (AM) edge type, the integer next to the edge type represents the number of aromatic chains. The energy bandgap and exciton binding energy decreases as the size of GQDs increases, reprinted with permission from Ref. [102]. (c) The relation of energy gaps with the amount of surface oxidation of carbon dots, reprinted with permission from Ref. [103]. (d) Excitation wavelength independent photoluminescence as the color varies red to blue the size is reduced from 8 nm to 1 nm, reproduced with permission from Ref. [99]. (e) Predicted energy level diagrams for graphene with different functional groups, along with schematic illustrations of the chemical structures used for theoretical calculations. Isosurfaces depict the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), reprinted with permission from Ref. [104].

contribute to their utility across various domains including biomedicine. The quantum size, arguably their most defining characteristic, is inherently linked to the chosen synthesis method, influenced by factors such as the precursor ratio, duration of reaction, temperature, choice of solvent, among others [92–96].

Modifying the surface state of CDs spans several approaches. These strategies encompass heteroatom doping, surface functionalization, and surface passivation, each contributing uniquely to the CD's properties [97]. Heteroatom doping involves substituting carbon atoms within the sp2/sp3 network with other elements like N, B, S, P, or halogens, altering their electronic structure [92]. Surface functionalization incorporates covalently bonded groups to the carbon base, enriching the CDs with functional groups that enhance their reactivity and interaction with external entities, Fig. 4a. Meanwhile, surface passivation involves coating the carbon core with agents such as polyethylene glycol (PEG), tuning the CDs' surface properties. These surface modifications directly influence the CDs' energy gap, affecting their performance [97]. The quantum confinement effect and surface energy trap mechanism are generally recognized for controlling this energy gap, with the former relating to the non-zero bandgap induced by excitons within graphene fragments and the latter highlighting the role of surface states on the energy gap of CDs [98]. From Fig. 4b, the size of conjugated-domains and the edge sites are pivotal in determining the energy gap, with an increase in the size of conjugated domains correlating to a narrower gap [99]. Conversely, the edge site—particularly in zigzag-edged GQDs—can substantially impact the energy gap due to localized states [100].

Surface defects, often resulting from oxidation, act as exciton capture centers, narrowing the band gap and enhancing the CDs' photoluminescence as illustrated in Fig. 4c, d [105]. Furthermore, the incorporation of oxygen atoms or other heteroatoms with high electronegativity values into the sp2-domain can also modify the band gap, with nitrogen-containing groups being notably popular for CD functionalization [104,106,107]. From Fig. 4e, functional groups like *o*-phenylenediamine, azo, or p-methyl red, have been shown to lower energy levels, affecting the CDs' HOMO/LUMO energy levels through strong orbital interactions [108]. Surface passivation, particularly with polymer molecules, is an effective method to enhance the fluorescence emissions of CDs, eliminating surface dangling bonds which could otherwise trap electrons in a non-radiative manner [109]. The incorporation of polymer molecules also contributes to the CDs' stability and performance, underpinning their potential in various applications from bio-imaging to sensing [97].

Lastly, the chirality of CDs, imparted through chiral synthesis or assembly, influences their physicochemical properties, offering unique advantages in fields such as biomedicine [110]. The emergence of computational models and artificial intelligence in CD design promises a streamlined approach to optimizing CDs' synthesis and application, potentially revolutionizing their production and utility [111].

The functionalization of CDs plays a crucial role in tailoring their physiochemical properties for specific applications, particularly in biomedicine. Through strategic alterations in their surface state and leveraging the quantum confinement effect and surface energy traps, CDs' performance in bioimaging, biosensing, and drug delivery can be significantly enhanced, offering a versatile platform for innovation in nanotechnology applications.

4. Properties of carbon dots

4.1. Structural properties

The structural properties of CDs significantly influence their potential applications, notably in biomedicine. Research indicates CDs typically possess a core-shell structure, albeit without a distinctly demarcated boundary between the carbon core and the surrounding polymer shell [112]. The core commonly features polycrystalline nanodomains comprised of small carbon clusters encased within amorphous regions [113]. These subdomains can exhibit a conjugated π -structure or adopt a diamond-like configuration, determinable through lattice imaging via transmission electron microscopy (TEM). The polymer surface of CDs imparts specific characteristics to them, with polymer side chains identifiable using techniques like atomic force microscopy (AFM) and dynamic light scattering (DLS). A comparative analysis of particle sizes helps confirm the presence of these side chains, while small-angle X-ray scattering (SAXS) modes validate the core-shell architecture [114,115]. Research in this area delineates two prevalent structures: some CDs display an amorphous core-shell structure characterized by mixed sp2/sp3 hybridization, while others boast a graphitic crystalline structure predominantly consisting of sp2 carbon [116,117]. This dichotomy in structural foundation underlines the versatile nature of CDs, with each structure imparting distinct physicochemical properties that influence their application scope. For instance, Carbon Nanodots (CNDs) often exhibit an amorphous, quasi-spherical shape, underscoring the diversity in CD forms. Both the quantum confinement phenomenon and the presence of surface-active groups distinctly affect the energy band gap of CNDs. CNDs' lattice constants fall midway between graphene and graphite lattice, suggesting a unique hybridization pattern. The presence of oxygen- and amino-based functional groups on their surface denoting an external sp3 and an internal sp2 configuration further enriches the surface chemistry of CNDs [118].

Despite the predominant dot-like structures, advancements in precursor selection and synthesis methodologies have enabled the creation of CDs with varied sizes and morphologies, including triangular structures, ribbons, and rods [119]. Utilizing symmetrical precursors such as phloroglucinol, along with concentrated sulfuric acid in an ethanol solution as a catalyst, allows for controlled sizing of CDs. This approach leverages the high reactivity of hydrogen atoms, positioned at meta locations activated by electron-donating hydroxyl groups, to achieve unique morphologies like the triangular structure. The phenomenon of quantum confinement dictates that emission wavelengths shift red with increasing size [93]. Conversely, utilizing precursors with cyano groups can induce quantum band bending, creating carbon quantum rings or ribbons of varying diameters and demonstrating size-dependent bandgap variation [120].

The fabrication process introduces a range of functional groups such as -OH, -COOH, -CHO, -NH2, and -SH onto the CDs' surface,

which can differ based on the precursors used [121-123]. These functional groups directly impact the CDs' properties; for instance, oxygen-containing groups render the CDs negatively charged, while nitrogen-containing groups impart a positive charge, influencing their passivation capabilities. Quantification of surface functional groups, such as amino groups, is typically achieved by ninhydrin colorimetry, with lysine as a standard. Carboxyl group content can be determined through Boehm titration, involving a titration of CDs with NaHCO₃ solution followed by HCl, with pH as the endpoint [124].

From Fig. 5a, the structural integrity of CDs, spanning amorphous to graphitic crystalline configurations, is pivotal for their application efficiency. Surface functionalization imparts negative or positive charges to CDs, affecting their passivation capabilities and interaction potential with biological targets. Understanding the presence and relative abundance of such functional groups is vital for harnessing CDs in biosensing, imaging, and therapeutic applications, necessitating comprehensive analytical characterizations through TEM, XRD, and conventional titration methods.

The intimate understanding of CDs' structure-property relationships is crucial for their effective utilization in biology and biomedicine. As we delve into the optical properties and assess the minimal toxicity of CDs, their compatibility for bio applications becomes evident. Their structural configurations — whether graphitic or amorphous, adorned with various functional groups — play a key role in defining their use in biomedicine and sensing, paving the way for innovative applications that leverage their unique nanostructure.

4.2. Optical properties

The elucidation of Carbon Dots' (CDs) optical characteristics in Fig. 5b illuminates a fascinating interplay between their quantum effects and structural heterogeneity. Their absorption spectrum predominantly lies within the near-ultraviolet region, spanning 230–270 nm, traced back to π to π^* transitions of aromatic phenyl rings [127]. This fundamental attribute underscores the CDs' intrinsic photophysical behavior and establishes a basis for their varied application in spectroscopy and imaging. In contrast, the absorption intensity dims within the visible to near-infrared (NIR) regions 280–350 nm, attributable to $n-\pi^*$ transitions involving C–C or C=O bonds, reflecting the structural diversity and compositional variability of CDs, see Fig. 1 [128].

From Fig. 5c, upon light excitation, CDs exhibit a range of photoluminescence (PL) phenomena influenced by several underlying mechanisms: the quantum confinement effect, surface states, molecular states, and the crosslink enhanced emission (CEE) effect [103, 129–131]. The mechanisms highlighting quantum confinement and surface states notably imply that as the band gap narrows, there



Fig. 5. (a) Properties of Carbon Dots, reproduced with permission from Ref. [125]. (b) UV–Vis absorbance spectrum showcasing the π to π^* and n- π^* transitions, with strong absorption of ultraviolet light extended all the way to the near-infrared (NIR) region, reproduced with permission from Ref. [126]. (c) The aqueous solution of PEG-CQDs: (a) illuminated at 400 nm and imaged through bandpass filters of varying wavelengths, as indicated, and (b) illuminated at specified wavelengths and directly photographed. (c) Displays absorbance and luminescence emission spectra of propionylethyleneimine-co-ethyleneimine (PPEI-EI) CQDs in an aqueous solution, with excitation wavelengths progressively increasing from 400 nm in 20 nm increments. Emission spectral intensities are normalized to quantum yields (inset: normalized to spectral peaks, adapted with permission from Ref. [97].

emerges a shift in the emission wavelength. From Fig. 6a, the molecular state mechanism diverges, attributing the PL not to the carbon core but rather to organic fluorophores adorning the CD surface, evident particularly at moderate temperatures where carbogenic aggregation becomes pronounced [130]. The innovative CEE effect, illustrated in Fig. 6b, delineates how sub-fluorophores tethered on the CD surface, when immobilized by a crosslinking structure or carbon core, contribute to amplified radiative transitions, showcasing the role of structural dynamics in modulating CDs' luminescent output [132].

Advancements in the study of CDs' optical behaviors, such as up-conversion and phosphorescence, underscore their burgeoning role in bioimaging and security [134,135]. Up-conversion (Fig. 6c), or anti-Stokes photoluminescence, signifies CDs' capacity to emit photons of a higher frequency after absorbing multiple photons of a lower frequency, marking a significant stride toward optimizing in-vivo imaging techniques for enhanced resolution and depth penetration [136,133]. Similarly, Fig. 6d illustrates the unique phosphorescence properties of CDs, manifesting even at room temperature given specific structural prerequisites, unlock new dimensions in applications ranging from anti-counterfeiting measures to novel luminescent markers.

The variegated absorption characteristics of CDs, spanning the UV to the visible spectrum, are intricately tied to the synthetic origins and choice of precursor materials. This spectrum delineation, stemming from π - π * or n- π * transitions, underlines the impact of CDs' π conjugated domain sizes, assortment of surface functional groups, and the elemental composition of the carbon core on their optical profiles [137,22].

CDs' photoluminescence, a key attribute for their widespread application in the biomedical field, is subject to influence from an array of factors including size, morphology, synthesis route, and surface passivation. Techniques like hydrothermal carbonization and microwave synthesis have been identified as pivotal in enhancing the photoluminescent properties of CDs, underpinning their significant role in biomedical imaging and diagnostics. Passivation strategies employing agents like polyethylene glycol (PEG) and polyethyleneimine (PEI) not only bolster the PL of CDs but also refine their stability and compatibility for biological applications [97, 138].

Introducing chirality into CDs through specific additives like L-cysteine further augments their light stability and enables precise imaging endeavors, highlighting dynamic intracellular processes. This chiral manipulation accentuates the nuanced interaction of CDs with biological systems, enhancing their efficacy and safety as imaging agents [139,140].

The complex optical properties of CDs, spanning a comprehensive spectral range of absorption and emission, are foundational to their multifaceted applications in science and technology. Grounded in quantum confinement, precise surface modifications, and structural adaptations, these properties not only broaden CDs' applicability across bioimaging and sensing but also beckon continuous



Fig. 6. (a) Illustration depicts the photoluminescence (PL) intensity arising from both a carbon core and organic fluorophores at varying carbonization temperatures. Reproduced with permission from Ref. [94] (b) Diagram illustrates the Charge-Excited Emission (CEE) effect mechanism of pristine polyethyleneimine (PEI) and corresponding photoluminescent dots (PDs). Reproduced with permission from Ref. [38]. (c) Represents the up-conversion photoluminescence (PL) nature of carbon dots (CDs). Reproduced with permission from Ref. [129]. (d) Digital photo captures the Room-Temperature Phosphorescence (RTP) phenomenon of CDs post cessation of UV lamp irradiation (365 nm). Reproduced with permission from Ref. [133].

exploration and optimization. As research progresses, the potential of CDs to revolutionize fields from nanomedicine to optical engineering becomes increasingly tangible, heralding a new era of discovery and innovation.

4.3. Toxicity

The assessment of Carbon Dots' (CDs) toxicity is an essential aspect of their development, especially considering their burgeoning applications in the biomedical realm. The evaluation employs both in vitro and in vivo methodologies, aiming to thoroughly understand the impact of CDs on biological systems before their integration into human health applications.

In vitro studies leverage assays such as MTT and WST-1, wherein cultured cells are exposed to varying concentrations of CDs to determine their viability in comparison to both control groups—those not exposed to CDs and those exposed to known toxic substances. These assays, pivotal for establishing a baseline toxicity profile, measure metabolic activity as a proxy for cell health and viability. Zhang et al. has been instrumental in demonstrating how CDs interact at the cellular level, shedding light on their cytocompatibility and potential cytotoxic effects under lab-controlled conditions [141].

Expanding the toxicity evaluation, in vivo studies typically involve the administration of CDs to model organisms, such as mice or zebrafish, followed by comprehensive analyses including blood, hematological, and inflammatory markers across critical organs (liver, spleen, kidney). This approach allows researchers to gauge the systemic impacts of CDs beyond cellular effects, providing insights into how these nanomaterials interact within a living organism [142].

A pivotal factor influencing the toxicity of CDs is their physicochemical properties, such as surface charge, concentration, and photolysis products. Positive surface charges, as illustrated in Fig. 7a, on CDs have been identified as contributing to their toxicity, with increased surface charge density correlating with oxidative stress and inflammatory response induction [143]. These findings suggest a direct relationship between the electrostatic properties of CDs and their biocompatibility or toxicity.

Interestingly, in vitro experiments on HeLa cells using reactive red 2 (RR2) as a precursor for CD synthesis have shown that CDs derived from RR2 exhibit significantly higher cell viability than RR2 itself, indicating that the synthesis process can reduce the toxicity profile of the starting materials, see Fig. 7b. This outcome, echoed in in vivo studies using zebrafish, further supports the concept that CDs possess reduced cytotoxicity, enhancing their safety profile for preclinical applications [146,147].

Moreover, the bio-distribution of CDs in mammals as showcased in Fig. 7c, assessed through radiolabeling techniques, suggests an efficient excretion pathway via the kidneys and feces, with negligible accumulation within the body. This finding, indicative of low in vivo toxicity, contrasts with certain materials that persist within biological systems, potentially leading to adverse effects [148].

A comprehensive analysis underscores that while most CDs exhibit low toxicity or are non-toxic, their toxicity is intricately linked to their individual physicochemical characteristics. Surface charge, photolysis behavior, and concentration notably stand out as critical factors shaping their biocompatibility.



Fig. 7. (a) Investigating the influence of surface charge on the toxicity of carbon dots (CDs). Reproduced under the terms of the CC-BY 4.0 license [143]. (b) Assessment of the toxicity of CDs photolysis products. Reproduced under the terms of the CC-BY 4.0 license [144]. (c) Examination of CDs metabolism in mice. Reproduced with permission from Ref. [145].

This detailed exploration of CDs' toxicity, incorporating various evaluation methods and considering the influence of physicochemical properties, forms a foundation for their safe application in biomedicine. Given their minimal toxicity, coupled with unique optical and physicochemical properties, CDs emerge as promising candidates for bioimaging, therapeutic delivery, and other biomedical applications, pending further research to precisely tailor their characteristics for specific uses while ensuring safety and efficacy.

4.4. Electrochemical properties

The electrochemical behavior of Carbon Dots (CDs) is crucial across a broad spectrum of applications, integrally connected to their carbon core and surface characteristics. Their electrochemical attributes are primarily shaped by the quantum confinement effect alongside surface energy traps, presenting distinctions from the 2D graphene layers [98,100]. CDs exhibit versatility as both electron acceptors and donors, where electron-hole pairs generated upon photon excitation can be transferred to various entities, thus underlining the exploration of the intricate tie between their electronic properties and their carbon core.

Research efforts have underscored how the size of Graphene Quantum Dots (GQDs) significantly influences the trapping of single electrons between the GQD surface and its core [149]. Modifications in the electric field can alter the energy gaps of GQDs, attributed to GQDs' spatial anisotropy [150]. Furthermore, configurations of GQD edges examined through tunneling spectroscopy highlight that zig-zag edge states confer a metallic nature to zig-zag-edged GQDs, owing to distinct electronic structures facilitated by their edge configurations [151].

Contrarily, CDs with amorphous structures showcase subpar electrochemical performance. This might relate to their elemental makeup and the prevalence of sp3-carbon atoms, which impact their electronic configuration less markedly compared to a sp2-network [152]. Enhancing CDs' electrochemical performance might thus lean more effectively towards heteroatom doping and surface functionalization than carbon core design alone.

In the realm of electron transfer, the introduction of electron-donating heteroatoms like nitrogen could potentially accelerate the rate of heterogeneous electron transfer. Conversely, doping with electron-withdrawing elements such as halogens might reduce electron transfer rates [151]. The excessive presence of oxygen functional groups on CDs could also decelerate electron transfer due to the disruption of the conductive sp2 carbon network [25]. Emerging studies explore dual-/multi-element co-doping's impact on CDs' electron transfer rates, highlighting their synergistic effect. Metal dopants notably enhance CDs' charge transfer capabilities, with copper (Cu) doping substantially increasing the electric conductivity of CDs. This Cu-ligand bonding ostensibly optimizes intra-CD charge transfer, markedly enhancing both electron-accepting and donating capacities [153].

The emergence of Fluorescence Resonance Energy Transfer within CDs, encompassing the interplay between two fluorescent



Fig. 8. (a) Illustration of surface-passivated green fluorescence carbon quantum dots (CQDs), wherein positively charged ions (Fe^{3+}) interact with the negatively charged surface of the CQDs for sensing confirmation via fluorescence quenching mechanism [160]. (b) Explanation of the process involving oxygen extracting electrons from metal oxide, resulting in decreased conductivity, and the subsequent injection of electrons into the oxide in the presence of a reducing agent (R), leading to increased conductivity. Reproduced with permission from Ref. [161]. (c) Schematic representation of an electrochemical DNA biosensor. Reproduced with permission from Ref. [162].

entities spaced around 10 nm apart, underpins CDs' application in sensor technology by facilitating energy transfer from a donor to an acceptor molecule, enhancing the acceptor's fluorescence while potentially quenching the donor's [154]. Mitigating fluorescence quenching remains a focal point of investigation, with strategies such as boron atom doping to introduce electronic defects, inhibiting charge transfer, or integration within matrices like poly(vinyl alcohol) or starch to maintain fluorescence integrity [155–157].

This intricate dance of electrochemical properties, grounded in the quantum and surface states of CDs, opens vistas for further exploration and innovation in sensor technology, bioimaging, and beyond, emphasizing the nuanced yet potent role of CDs in advancing scientific and technological frontiers.

5. Applications of CDs

5.1. Sensors

Carbon Quantum Dots (CQDs) have recently gained attention for their application in developing sensor systems capable of detecting substances at extremely low concentrations, down to pico- or femtomolar levels. Among the advantages CQDs offer are their straightforward manufacturing process, cost-effectiveness, ease of functionalization, exceptional luminosity, high quantum yield (QY), chemical stability, and limited toxicity [158,159]. Fig. 8a illustrates that in the presence of specific metal ions such as Fe³⁺, CQD sensors operate by either quenching or inhibiting the absorption spectrum of the CQDs, a process potentially grounded in charge transfer mechanisms that lead to diminished fluorescence emission [160].

Various quenching mechanisms within CQDs include static quenching, dynamic quenching, the inner filter effect, photo-induced electron transfer, and fluorescence resonance energy transfer [163]. Static quenching occurs when an external quencher forms a non-fluorescent complex with the CQDs, potentially altering their emission spectrum [164]. This interaction might be compromised at elevated temperatures, reducing the efficacy of static quenching. Conversely, dynamic quenching involves the return of CQDs from an excited state to the ground state through energy transfer between the CQDs and the quencher, not affecting the optical absorbance of CQDs [163].

Fluorescence resonance energy transfer, a notable process, involves the transfer of energy from a higher-energy donor QD to a lower-energy acceptor when in proximal contact, often leading to a decrease in donor fluorescence in favor of enhanced acceptor emission. The introduction of a quencher can initially suppress the emission of QDs, which may be reversible if the quencher interacts competitively with a target molecule near the analyte, illustrating the adaptability of CQD-based sensors [165].

The inner filter effect comes into play when an absorber within the sensor setup captures photons, influencing both the excitation and emission of light. This effect necessitates the presence of an absorber and a fluorophore within the system, where the absorber's spectrum overlaps with the fluorescence emission or excitation spectrum, allowing the absorber to regulate the fluorescence output. Accurate absorption responsive to the system's analyte concentration is essential for constructing inner filter effect-based detection systems [166].

Leveraging CQDs in sensors has enabled the detection of a diverse array of analytes including metal ions, acids, water contaminants, drugs, among others. This versatility positions CQD-based sensors as promising candidates for high-performance yet precise sensing across multiple application domains, underscoring the breadth of potential CQDs hold in advancing sensor technology.

5.1.1. Sensors for environmental monitoring

The necessity for gas sensor devices to monitor air pollution and safeguard human health has grown in recent years, pressing the demand for innovative approaches beyond traditional sensor technologies. The integration of small semiconductor structures, particularly Carbon Quantum Dots (CQDs), has revolutionized this domain by leveraging their unique electrical and optical properties. These properties arise from the confinement of charge transfer across one or more spatial dimensions [167].

Recent advancements in research have positioned Quantum Dots (QDs) as pivotal materials in the development of chemical sensors, due to their encapsulated electron-hole pairs within all three dimensions. This unique characteristic of QDs has propelled them to the forefront of sensor technology, showcasing their potential as a novel group of materials for chemi-resistive systems. The capability of QDs' structures to offer substantial sensing abilities underscores their emerging role in detection system frameworks [168]. However, the journey of fully understanding the influence of synthesis techniques and additional materials on QDs' sensing properties is ongoing. It has become essential to investigate materials' sensitivity and selectivity based on the modifications in the band-gap of QDs when adjusting their diameters.

Gas adsorptions on the semiconductor surface are divided into physisorption and chemisorption. Physisorption is governed by the van der Waals forces, a dipolar attraction that is relatively weak between solid materials and gas molecules, typically generating heat below 6 kcal/mol. Conversely, chemisorption features a robust interaction leading to charge transfer between the solid surface and gaseous species, with a heat production exceeding 15 kcal/mol. The physisorption process diminishes with increasing temperature, giving way to chemisorption at elevated temperatures [169].

The interaction of semiconductor materials with oxygen is a pivotal factor in detecting gases in the atmosphere. Gas sensors usually operate within a temperature range from ambient temperature to 500 °C [170]. Within this temperature spectrum, oxygen ions adsorbed in molecular (O^2) and atomic (O^-) forms can capture surface electrons. Below 150 °C, molecular ions predominate, while above this temperature, atomic forms take precedence, affecting the semiconductor's charge conduction and creating a depletion region and a Schottky barrier at grain boundaries, see Fig. 8b [170].

Exploring practical applications, reports have highlighted innovative modifications like silica aerogel surface modification with CQDs for precise and sensitive NO_2 gas detection [171]. Furthermore, fabrications like the ZnO/CQD composite and

Z.A. Qureshi et al.

hydroxyl-modified GQDs have demonstrated enhanced sensitivity and selectivity towards gases like NO and NH₃, respectively [172, 173]. These advancements not only reflect the versatility of CQDs in sensor applications but also highlight the ongoing quest to optimize their physicochemical properties for superior performance in gas sensing.

5.1.2. Fluorescent sensors

Fluorescent sensors are recognized for their capability to identify a wide range of metal ions and other chemical entities [174,175]. Traditional techniques for detecting metal ions or chemical species, such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry, though accurate, are often constrained by high costs, lack of portability, and complexity, limiting their immediate applicability in field conditions. Conversely, sensors based on fluorescence principles offer a more viable alternative due to their cost-effectiveness, ease of use, and notably high selectivity and sensitivity towards metal ions detection.

The inherent fluorescence properties of Carbon Quantum Dots (CQDs) in aqueous solutions exemplify their potential as sensitive detection tools. The fluorescence emission intensity from CQDs can vary or become quenched in the presence of certain quenchers in the solution, a phenomenon resultant from energy or charge exchange interactions between the CQDs and the quenching agents [176].

A critical advantage of using CQD-based fluorescence sensors is their remarkably low limit of detection (LOD) values, translating to ultra-sensitivity. Experimental conditions demonstrating this involve introducing heavy metal ions like Hg, Cd, and Pb into the aqueous solution containing CQDs. The differentiated response of CQDs in the presence of these ions underscores their potent selectivity towards Fe^{3+} ions, highlighting the tailored response of synthesized CQDs towards specific ions in solution [177].

Comparative analyses of facile synthesis methods for creating both doped and undoped CQDs from diverse organic and inorganic precursors reveal their efficacy in selective heavy metal ion detection. These CQDs have been reported to exhibit substantial quantum yield (QY) values ranging from 25 % to 50.78 %, coupled with impressively low detection figures (ranging from 19 μ M to 10 nM), showcasing their effectiveness and potential in sensitive detection applications [178–180].

5.1.3. Luminescence sensors

Luminescence is a type of light emission that occurs as a result of a chemical reaction between two reactants, leading to the release of light as an intermediary or product transitions back to its ground state [181,182]. Owing to its straightforward implementation and heightened sensitivity, luminescence has found significant applications in the realm of analytical chemistry [183]. Commonly employed luminant reagents include luminol, potassium permanganate, peroxalate, and lucigenin, known for their rigorous examination in various studies. Despite their widespread use, these conventional reagents encounter limitations like elevated costs, potential toxicity, and compromised sensitivity.

The equipment required for luminescence assessments is notably minimalistic, as the method does not necessitate excitation through UV–visible spectra or specialized spectral resolving devices. The measurement capitalizes on light emitted as a byproduct of the chemical reaction, rendering this technique highly sensitive due to its ability to detect minute quantities of light amidst minimal background interference. However, it's noteworthy that the limit of detection in analyses is predominantly influenced by the purity of the reagents more than the sensitivity of the detectors or sensors employed.

Carbon Quantum Dots (CQDs) present advancements in enhancing luminescence systems through various mechanisms: firstly, by concentrating the excited luminescence emitter, thus augmenting the luminescence emission; secondly, by functioning as a catalytic agent within the reaction; and thirdly, by their intrinsic capability to initiate a chemiluminescence response. The initiation of emission by CQDs may be attributed to the eradication of electron and hole-injected CQDs. Particularly, CQDs demonstrate the ability to catalyze the reaction of luminol without the need for additional oxidants [184]. This phenomenon may stem from interactions between CQDs and luminol and the formation of an active transition complex facilitating accelerated electron exchange in the oxygen-luminol chemiluminescence reaction.

Surface modifications of the dots, especially the introduction of carboxyl and carbonyl groups, have been shown to influence not only the luminescent emission but also the catalytic efficacy of CQDs. Enhancing CQDs' catalytic performance is achievable through strategic functionalization of their surfaces [185].

Moreover, CQDs can significantly amplify the otherwise weak luminescent intensity of the H_2O_2 –HSO₃– system, by as much as 60 times, through the generation of excited-state CQDs (CQDs*). This enhancement results from the annihilation of positively and negatively charged CQDs via electron transfer. The interaction with radicals such as •OH, •SO3–, SO4•–, and •O2– anions, generated through the reaction of H_2O_2 with HSO₃–, enables CQDs to acquire negatively and positively charged surfaces.

Exploiting CQDs in CL sensors has facilitated the detection of a diverse range of substances including but not limited to 4-nitrophenol, Cu^{2+} , Fe^{2+} , bisphenol A, indomethacin, hydrogen peroxide, dopamine, Cr(VI), and adrenaline in water [186]. These sensors demonstrate the versatile applicability of CQDs-enhanced chemiluminescence in detecting various compounds with high sensitivity and specificity.

Recent advancements in electrochemiluminescence systems have leveraged a variety of nanostructures, such as graphene quantum dots, polymer dots, nanocrystals, and inorganic nanocomposites, for diverse applications [187]. Traditional nanomaterials utilized in these systems, including quantum dots composed of heavy metals like CdS, CdSe, CdTe, and CdTe/ZnS, however, are limited due to their toxicity, lower biocompatibility, and, in some cases, reduced sensitivity [188]. Carbon Quantum Dots have emerged as a superior alternative, offering a blend of exceptional properties ideal for electrochemiluminescence applications.

CQDs distinguish themselves through their remarkable optical attributes, such as photoluminescence and chemiluminescence, attributed to their straightforward synthesis, chemical inertness, facile functionalization, reduced toxicity, and simple labeling processes. These characteristics position CQDs as advantageous electrochemiluminescence luminophores. For example in Fig. 8c–a dual fluorescence and electrochemical DNA sensor has been developed utilizing an indium-tin oxide electrode modified with curcumin and

GQDs, targeting APOe4 DNA for the detection of Alzheimer's and coronary artery diseases. The GQD-ITO surface underwent electropolymerization of the curcumin molecule, displaying both fluorescent and electrochemical properties. A covalently attached aminosubstituted DNA probe was immobilized using a malonic acid spacer via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/Nhydroxysuccinimide (EDC/NHS) chemistry [162].

CQDs have been suggested to stimulate electrochemiluminescence emission through an electron-transfer mechanism involving the annihilation of positively and negatively charged CQDs [189]. This process significantly enhances electrochemiluminescence activity, making CQDs suitable for implementation in electrochemiluminescence systems alongside other luminophores, such as perylenete-tracarboxylic acid (PTCA), in the development of highly sensitive immunosensors. Such sensors, incorporating CQDs and PTCA, have demonstrated remarkable sensitivity, with a limit of detection reaching as low as 0.00026 fg ml⁻¹ [190].

Mechanical milling of CQDs has resulted in diverse surface states, directly influencing their electrochemiluminescence emission properties. The inclusion of potassium persulfate as an electron donor significantly amplifies the electrochemiluminescence response due to the contribution of dual-wavelength electrochemiluminescence peaks linked to the synthesized CQDs' surface states.

The integration of CQDs in ECL systems signifies a significant leap forward in sensor technology, offering high sensitivity, enhanced signal amplification, and broadened detection capabilities for various species. This progress highlights the potential of CQDs as luminophores in cutting-edge sensor design, driven by their unique properties and the innovative strategies employed to harness their capabilities.



Fig. 9. Advanced Applications and Synthesis Pathways of Carbon dots for Bioimaging: (a) This panel provides a schematic overview of the strategic synthesis route of HBIE-CNDs, specifically engineered for efficient, wash-free cellular nucleus imaging. The diagram illustrates the stepwise hydrothermal reaction process that combines folic acid with m-phenylenediamine, yielding HBIE-CNDs that exhibit distinct fluorescence properties in hydrogen-bonding versus non-hydrogen-bonding environments, underpinning their application in nucleus-targeted fluorescence microscopy. Reproduced with permission from Ref. [196]. (b) The illustration details the hydrothermal synthesis of CP-CNDs from Congo red and p-phenyl-enediamine in a single-step process. The figure highlights CP-CNDs' roles in conducting comprehensive toxicity evaluations and facilitating high-contrast in vitro and in vivo nucleolus imaging, showcasing their dual functionality in biomedical research and their potential as a tool for understanding nucleolar dynamics and assessing the cytotoxic impact of various compounds. Reproduced with permission from Ref. [198]. (c) This panel represents the application of carbon dots' specificity towards RNA-rich nucleolar regions, permitting the detailed visualization of nucleolar structures and associated molecular processes at the single-molecule level, thereby elucidating complex intracellular dynamics. (d) This section displays a SRRF microscopy image of TPP CNDs. The vivid imagery obtained through SRRF microscopy reveals the intricate mitochondrial network and morphology under physiological conditions, illustrating the application of TPP CNDs in the detailed observation of mitochondrial fission, fusion, and overall dynamics within a cellular context. Reproduced with permission from Ref. [199].

5.2. Imaging

Grasping the significance of surface functional groups and core size on nanomaterials is pivotal for their application in bioimaging. Research indicates that nanomaterials exhibit optimal cellular uptake at a size of approximately 50 nm, enhancing their efficacy and absorption rate by cells. It's observed that the absorption rate of nanomaterials tapers off with either an increase in size from about 70 to 240 nm or a decrease to sizes around 15–30 nm, pointing to the existence of an optimal size range for cellular uptake [191–193]. Notably, nanomaterials within the 30–50 nm size range demonstrate a proficient binding affinity to cellular membrane receptors, facilitating their internalization through receptor-mediated endocytosis. It is also established that the specificity of carbon nanodots to cellular organelles such as lysosomes and mitochondria can be attributed to the surface functional groups they possess, such as morpholine groups for lysosomes and triphenyl phosphonium ions for mitochondria [194,195].

In recent studies, a unique form of carbon nanodots through hydrothermal synthesis merging folic acid with m-phenylenediamine aimed at nuclear imaging was developed. Intriguingly, these dots exhibited distinct emission behaviors—green emission in hydrogenbonding environments and blue emission otherwise. Based on these observations, hydrogen-bonding-induced emission was proposed as a novel fluorescence sensing technique. With ultrasmall size, superior biocompatibility, and significant Stokes shift in water, the synthesized dots penetrate living cells seamlessly. They display a marked increase in green fluorescence upon interaction with DNA and RNA as illustrated in Fig. 9a, leveraging the nucleic acid's prevalence in the nucleus for efficient, wash-free subcellular imaging in a fluorescence turn-on mode, holding promise for bioanalytical and clinical applications focusing on nucleus imaging [196]. Tong et al. highlighted the potential of carbon nanodots synthesized via a single-step hydrothermal process using Rose Bengal and branched Polyethylenimine for lysosome imaging. The resulting carbon nano dots showcased low phototoxicity, a narrow emission bandwidth, high fluorescence quantum yield of 90.49 %, and negligible cytotoxicity. Unique to this combination is the innate lysosome targeting ability that negates the necessity for further ligand modifications, ensuring prolonged binding up to 48 h. The synthesis brings forth an ultrahigh photoluminescence efficiency and minimal cytotoxicity, attributable to the dehalogenation cross-linking and structural rearrangement during synthesis, which curtails intersystem crossing and nonradiative transitions while maintaining ideal NH2 functional group presence for cellular targeting. The synthesized carbon dots prepared from rose Bengal and functionalized with branched polyethylenimine, courtesy of their stellar attributes, emerge as superior candidate for lysosome imaging [197]. This paradigm of facile production paves the way for developing ultrabright functional nanomaterial indicators, offering expansive avenues for bioimaging advancements.

Xin et al. have developed a carbon dot-based probe, targeting mitochondria with exceptional long-term tracking capabilities. Utilizing a solvothermal procedure with m-diethylaminophenol enabled the production of mitochondria-targeted nano dots, emitting light at 565 nm. The targeting attribute of CNDs is inherently linked to the surface functional groups, determined by the reaction precursors used. The carbon dot-based probe showcased remarkable stability, potent mitochondrial targeting, and a high quantum yield of 12.61 %, marking them as effective tools for bioimaging. Notably, the carbon dots accumulated in mitochondria, demonstrated by a high colocalization coefficient up to 0.90, thanks to the surface abundance of hydroxyl and ammonium cations, maintaining this targeting even post fixation. Additionally, their impressive photostability and compatibility in various conditions makes them suitable for long-term dynamic monitoring of mitochondrial functions in living cells without interference from other cellular components [200]. Xu et al. synthesis of bright red emissive carbon dots through hydrothermal reaction of Congo red with p-phenylenediamine demonstrates the versatility of CNDs. These red nano dots exhibit fluorescence that is both polarity-dependent and excitation-independent, allowing for in vivo imaging of zebrafish and Caenorhabditis elegans (C. elegans), alongside robust, wash-free nucleolus imaging in living cells, see Fig. 9b [198]. Li et al. presented photostable and amphiphilic carbon dots with vivid orange fluorescence through hydrothermal treatment of phenylalanine and phenylenediamine. Demonstrating high brightness, exceptional photostability under UV light, and selective targeting for intracellular endoplasmic reticulum, these CNDs provided insights into reconstitution during cell division [201]. Liu et al. introduced sulfur-nitrogen co-doped carbon dots with specific sensitivity to glutathione, important for Golgi apparatus imaging. With a linear response to glutathione and notable fluorescence stability, the co-doped carbon dots emerge as promising probes for simultaneous glutathione detection and Golgi imaging in living cells, highlighting their significant optical properties and minimal cytotoxicity [202]. The advancement of chiral carbon quantum dots, abundant with cysteine residues, underscores the innovative approach in targeting subcellular organelles like the Golgi apparatus. Exhibiting high quantum vield, photostability, and biocompatibility, chiral carbon dots unique structural properties enable long-term in situ Golgi imaging, contributing to the understanding of cellular processes during viral infections [203].

Carbon Nanodots have risen as viable candidates for tagging biological targets within cells due to their distinctive optical properties and lack of substantial toxicity. Efforts to synthesize red-emitting carbon nano dots aim at direct labeling without additional conjugation steps, enhancing the application of carbon dots in bioimaging [204,205]. For instance, photoblinking carbon dots specifically for visualizing mitochondria-HS– interactions via stochastic optical reconstruction microscopy were developed utilizing a simple hydrothermal method with 3-diethylaminophenol and tartaric acid. These CDs displayed selectivity towards mitochondria and HS–, attributed to the electrostatic interactions facilitated by the positive surface charge of the CDs, as inferred from their zeta potential measurements [206].

A carbon dot variant resembling a protein structure was also reported, integrating fluorescent blinking domains alongside RNAbinding motifs for enhanced nucleolar ultrastructure imaging. This innovation yields multidimensional insights for distinguishing cell types, a significant leap in super-resolution imaging methods previously unattainable through traditional fluorescence techniques [207]. Fig. 9c exhibits yellow-orange emissive CDs synthesized from urea and citric acid for detailed nucleolus morphology observation through single-molecule localization microscopy (SMLM), highlighting the CDs' specificity towards RNA molecules and thus their accumulation in the nucleolus [208]. Synthesis of highly photostable and water-soluble CDs using a one-step method with 3-(carboxy-propyl)triphenylphosphonium bromide and *o*-phenylenediamine showcases superior photostability compared to commercial dyes (Fig. 9d). The contribution to mitochondrial dynamics imaging, utilizing super-resolution radial fluctuations microscopy (SRRF), underscores the role of these CNDs in unveiling cellular processes under various conditions [199]. Li et al. synthesis of high-fluorescence quantum yield, low toxicity, antiphotobleaching, and water-soluble carbon dots via doping with fluorine and nitrogen represents a milestone for high-resolution visualizations using stimulated emission depletion microscopy [209]. These CDs demonstrate the potential for intricate imaging of cellular structures, proving their efficacy for long-term or in vivo applications. In another venture, Li et al. synthesized nitrogen-doped carbon dots adept at live-cell imaging of lysosomes and mitochondria. The synthesis, grounded in a simple hydrothermal process, led to water-soluble N-doped carbon dots validated for their biocompatibility and optical resilience [209]. Such attributes confirm the carbon dots utility as probes for stimulated emission depletion imaging, achieving unprecedented resolutions within organelle visualizations inside living cells. Each of these developments underscores the pivotal role of carbon dots in pushing the boundaries of bioimaging, facilitating detailed organelle studies, and advancing our understanding of cellular behaviors, all while maintaining a commitment to biocompatibility and photostability.

6. Conclusion

Carbon Dots (CDs) were initially discovered during the purification processes of single-walled carbon nanotubes. Subsequent explorations have witnessed several researchers engaging in the synthesis and development of CDs through a myriad of techniques. Exhibiting a suite of remarkable properties such as excellent biocompatibility, reduced toxicity, inherent fluorescence, straightforward synthesis, robust electrical conductivity, and cost-effectiveness, CDs have found significant applications across diverse fields including sensors, biomedical science, optoelectronics, and energy sectors. Despite their potential, achieving expectations and scaling up production to industrial levels remains a challenge.

Future advancements in the production of CDs necessitate the refinement of synthetic methodologies, incorporating precise elemental doping, and optimizing surface passivation techniques. For instance, electrically conductive doping of CDs has paved the way for their integration into microfluidic systems or as components of fluorescent conducting inks for sensors and microelectronic devices. The dual functionality of optical fluorescence and electrical conductivity positions CDs as viable materials in drug delivery systems, leveraging their biocompatibility. These CDs act as carriers, allowing for the targeted delivery of therapeutic agents, traceable via continuous fluorescence emission. Furthermore, when doped with electromagnetic or magnetically responsive materials, CDs can facilitate targeted drug delivery under the control of external magnetic forces, enhancing the precision of therapeutics.

In the realm of energy storage, the development of CD-based nanohybrids aims to construct supercapacitors with superior power density and energy capacity. This vision for CDs' future development underscores their burgeoning potential across several cutting-edge applications, including bio-sensing and organic solar cells.

Although current knowledge on CD synthesis and their prospective applications in various domains is robust, continued research is imperative. Challenges persist in fabricating CDs through simple, environmentally friendly methods that yield materials with specific structures and sizes tailored for advanced applications. The quest for CDs with high quantum yields (QY) and well-defined geometric, compositional, and structural integrity is critical, as variability in size can lead to a broad range of properties.

Moreover, many aspects of CDs' nanoscale chemical and physical properties remain unexplored, hindering their full potential in areas such as energy storage. The rapid evolution of raw material sources and the lack of standardization pose significant obstacles to the industrialization and wider commercialization of CDs.

To unlock the vast potential of these indispensable carbon materials, future endeavors must focus on developing cost-effective, innovative synthesis techniques and exploring novel applications. Advancing our understanding and overcoming current limitations will undoubtedly enable CDs to play an increasingly pivotal role in analytical, bio-analytical sciences, and beyond, promising an exciting avenue for future research and technological innovation.

CRediT authorship contribution statement

Zawar Alam Qureshi: Writing – review & editing, Writing – original draft. Hanan Dabash: Writing – original draft. Deepalekshmi Ponnamma: Writing – review & editing. M.K.G. Abbas: Writing – review & editing, Supervision.

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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