

Carbon quantum dots in biomedical applications

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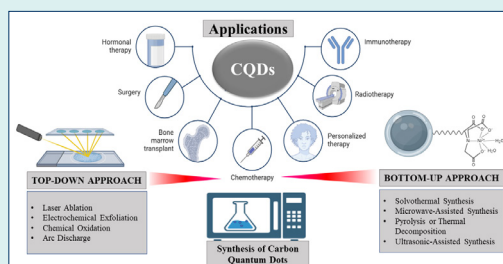
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Abstract

Carbon quantum dots (CQDs) have emerged as an important class of carbon-based fluorescent nanomaterials owing to their tunable photoluminescence, favorable aqueous dispersibility, and modifiable surface chemistry, which support diverse biomedical applications. This review critically summarizes recent advances in biofabricated CQDs with particular emphasis on green synthesis approaches, physicochemical characterization, surface engineering, and biomedical performance. Various synthesis strategies, including hydrothermal, microwave-assisted, electrochemical, and biomass-derived methods, are comparatively evaluated in terms of scalability, reproducibility, biocompatibility, and translational potential. In addition, the biomedical applications of CQDs in bioimaging, biosensing, targeted drug delivery, cancer theranostics, and antimicrobial platforms are comprehensively discussed with recent literature updates. The influence of surface functionalization on colloidal stability, targeting efficiency, cellular uptake, and biological interactions is also examined. Furthermore, current evidence from in vitro and in vivo studies regarding the biosafety and toxicity profiles of CQDs is critically analyzed. Despite significant progress, several challenges remain, including large-scale synthesis standardization, long-term toxicity evaluation, batch-to-batch reproducibility, and regulatory limitations associated with clinical translation. Future advancements in scalable fabrication techniques, multifunctional surface engineering, and safety assessment strategies may accelerate the development of CQDs-based nanomedicine platforms for diagnostic and therapeutic applications.



Introduction

Carbon quantum dots (CQDs) are an emerging class of zero-dimensional carbon-based fluorescent nanomaterials generally possessing particle sizes below 10 nm. Due to their tunable photoluminescence, favorable aqueous dispersibility, chemical stability, and modifiable surface chemistry, CQDs have attracted considerable attention in nanomedicine and biomedical research.¹ Compared with conventional semiconductor quantum dots, CQDs exhibit relatively lower toxicity, improved biocompatibility, and environmentally sustainable synthesis potential, making them attractive candidates for biological and clinical applications.²

CQDs can be synthesized through a variety of top-down and bottom-up approaches, including hydrothermal, solvothermal, microwave-assisted, electrochemical,

and green synthesis methods using biomass-derived precursors. Depending on their structural organization and physicochemical characteristics, carbon-based quantum dots have been classified into several categories, including graphene quantum dots (GQDs), carbonized polymer dots, crystalline C₃N dots, and chirality-induced carbon dots. Although CQDs and GQDs share certain optical features, they differ in crystallinity, electronic structure, and surface organization. In contrast to graphene oxide, which possesses a two-dimensional layered structure, CQDs are predominantly quasi-spherical nanoparticles exhibiting quantum confinement and surface-state-dependent fluorescence behavior.³

Surface functionalization and passivation play critical roles in improving the physicochemical and biological performance of CQDs. The incorporation of hydroxyl,



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carboxyl, amino, and carbonyl functional groups enhances water solubility, colloidal stability, fluorescence efficiency, and targeting capability. Furthermore, CQDs exhibit several advantageous optical properties, including excitation-dependent emission, up-conversion photoluminescence, fluorescence resonance energy transfer (FRET), and surface-enhanced Raman activity, thereby supporting their applications in imaging and sensing technologies.⁴

Recent advances in CQD research have significantly expanded their biomedical applications, including bioimaging, biosensing, molecular diagnostics, antimicrobial therapy, targeted drug delivery, and multifunctional theranostic systems.⁵ In particular, increasing interest has been directed toward green and biofabricated CQDs due to their eco-friendly synthesis processes and potentially improved biosafety profiles.⁶ Nevertheless, several challenges remain, including large-scale synthesis standardization, reproducibility, long-term toxicity evaluation, and clinical translation (Fig. 1).⁷

Although numerous reviews on carbon nanomaterials and fluorescent quantum dots are available, limited attention has been given to critically correlating synthesis strategies, surface engineering, toxicity behavior, and translational biomedical performance of CQDs. Therefore, the present review provides a comprehensive and critical overview of recent developments in CQDs synthesis, classification, characterization, functionalization, and biomedical applications, with particular emphasis on green fabrication approaches, diagnostic imaging, biosensing,

targeted drug delivery, and theranostic applications.⁸

Carbon quantum dots

CQDs are a promising class of nanomaterials that are mainly composed of a carbonized core and have heteroatom containing functional groups.⁹ These are generally grouped into carbon nanodots, polymer dots and graphene GQDs, and are of 10 nm or smaller particle size and typically have an sp^2 -hybridized carbon core-shell structure. There are several bottom-up and top-down methods for the synthesis of CQDs with different carbon precursors. Top-down approach is the physical or chemical breaking down of bulk carbon materials by laser ablation, chemical oxidation and electrochemical synthesis process, etc.¹⁰ The bottom-up approach, on the other hand, produces CQDs from molecular precursors through various processes including hydrothermal treatment, thermal carbonization, combustion and ultrasonic irradiation. Bottom-up approaches are more commonly employed than top-down because they are less expensive, less complicated, faster and less polluting. But the top-down methods may need more surface passivation and functionalization procedures to enhance optical properties. No matter the synthesis method, CQDs have tunable physicochemical and optical properties, which can find use in bioimaging, sensing, photocatalysis, and drug delivery. The particle size, crystallinity and surface chemistry vary according to the synthesis conditions like pH, temperature, type of precursor and reaction time. CQDs can be in amorphous (mixed sp^2 /

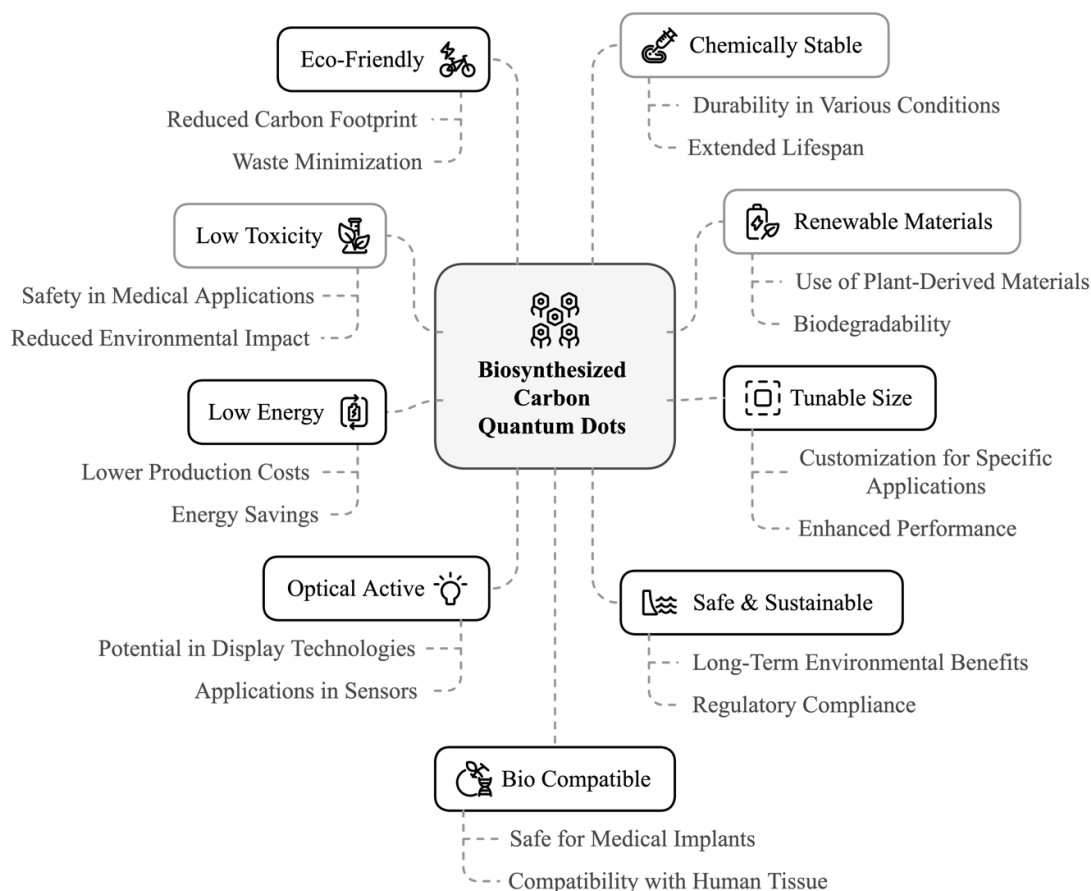


Fig. 1. Schematic representation of the key benefits and properties of biosynthesized carbon quantum dots.

sp³) and/or crystalline (dominantly sp²) form.¹¹ Generally, higher synthesis temperatures (above 300 °C) will tend to favor graphitization and lower temperatures will tend to produce amorphous carbon structure. Transmission electron microscopy (TEM), high-resolution TEM (HRTEM), scanning electron microscopy (SEM), Raman and x-ray diffraction (XRD) are the most typical methods used to characterize the core structure and morphology of CQDs. Selected area electron diffraction (SAED) patterns also support the crystallinity.¹² Surface functional groups include hydroxyl, carboxyl, carbonyl and amino groups, which can be analyzed by Fourier transform infrared (FTIR) spectroscopy, x-ray photoelectron spectroscopy (XPS), elemental analysis and nuclear magnetic resonance (NMR) spectroscopy. The optical absorption is assessed and occurrence of π - π^* and n - π^* transition is confirmed by UV-Visible spectroscopy. Surface charge and colloidal stability can be determined by zeta potential analysis, and surface area can be determined by N₂ adsorption techniques.¹³ Multiple surface functional groups are shown on a representative structure of CQDs in Fig. 2, which is responsible for the physicochemical and biological properties of CQDs.¹⁴

Characteristics of carbon quantum dots

CQDs can be chemically structured with different groups containing oxygen, amino groups and polymeric chains that greatly influence their optical properties. Such surface functionalities are important to control the photoluminescence properties,¹⁵ such as emission intensity, and wavelength tunability. The photoluminescence properties of CQDs are size- and surface-state-dependent, and can emit in the visible and in some cases near-infrared (NIR) wavelengths ideal for deep tissue bio-imaging.¹⁶ CQDs exhibit the absorption in UV and visible region where the characteristic transition is assigned to C=C, C=O, C-N and C-S bonds while the GQDs showed the absorption in the same regions. Surface passivation and

functionalization also have significant effects on the optical response of CQDs, which can change the surface electronic states and increase the efficiency of emission.¹⁷ While CQDs can emit blue, green and red fluorescence, controlling and uniform emission is difficult because of a range of size distributions and surface chemistries.¹⁸ The excitation-dependent fluorescence is observed in most of the CQDs, while the excitation-independent emission is observed in heteroatom-doped CQDs like N and S-co-doped CQDs. Their photoluminescence mechanisms have been explained by a variety of theoretical models, which are still being developed, such as quantum confinement effects, surface-state emission, and molecular-state fluorescence. The CQD emission behavior is also significantly affected by the solvent used in the synthesis, and surface chemistry variations in different media have a significant effect.¹⁹ Other parameters affecting the photoluminescence properties are the surface oxidation degree and molecular structure. In contrast to other carbon nanomaterials like graphene oxide, CQDs have showed low cytotoxicity and high biocompatibility, which are ideal for biomedical applications.²⁰ Likewise, GQDs exhibit superior *in vivo* safety, rapid renal clearance and promise applications in radiotherapy and drug delivery on account of their low toxicity and functional versatility.²¹ CQDs are highly photostable and are good for long term imaging or monitoring applications. The optical properties can be modified by different synthesis technique, hydrothermal, chemical oxidation and ultrasonic, to obtain desired emission profile for applications. The exact emission mechanism of CQDs is still not fully understood, and further investigation on the structure-property relationships is needed due to the extensive progress.²² However, their distinctive properties of tunable fluorescence, low-toxicity, and surface-modifiability, have led to diverse applications in bioimaging, biosensing, drug delivery, catalysis, flexible electronics, and optical devices (Fig. 3).²³ Additionally, CQDs can be modified to exhibit therapeutic effects and act as drug delivery vehicles for controlled release, improving the therapeutic uses of these materials. Based on the different synthesis approaches, the CQDs were classified and described in terms of their properties.²⁴

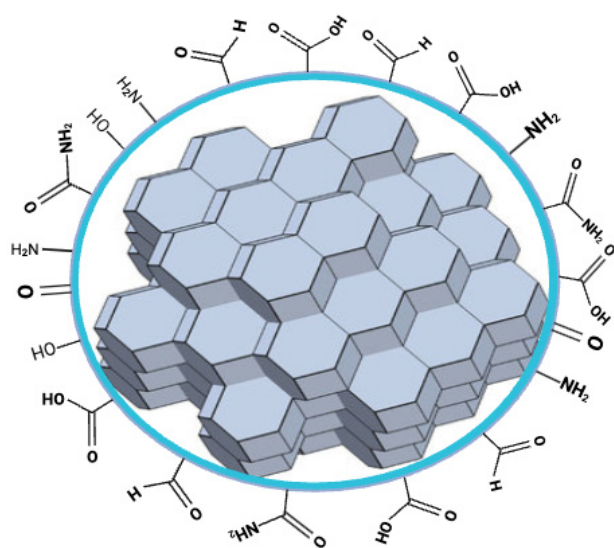


Fig. 2. Diverse functional groups and Surface modification of carbon quantum dots in their usual structure, influencing their unique qualities and uses.

Synthesis techniques of carbon quantum dots

Synthesis of CQDs (Fig. 4) can be achieved through various methods, each offering distinct advantages in terms of simplicity, scalability, and tunability of CQD properties. The most commonly used synthesis approaches include hydrothermal methods, microwave-assisted synthesis, and electrochemical techniques.²⁵

There are several methods for synthesizing CQDs that have different advantages that include scalability, low cost, short reaction time, and tunability of physicochemical properties. Commonly used techniques are hydrothermal synthesis, microwave-assisted synthesis, electrochemical methods and laser ablation methods.²⁶⁻²⁸ The electrochemical synthesis is a simple and environmentally friendly bottom-up method that allows the control of the production of CQDs with

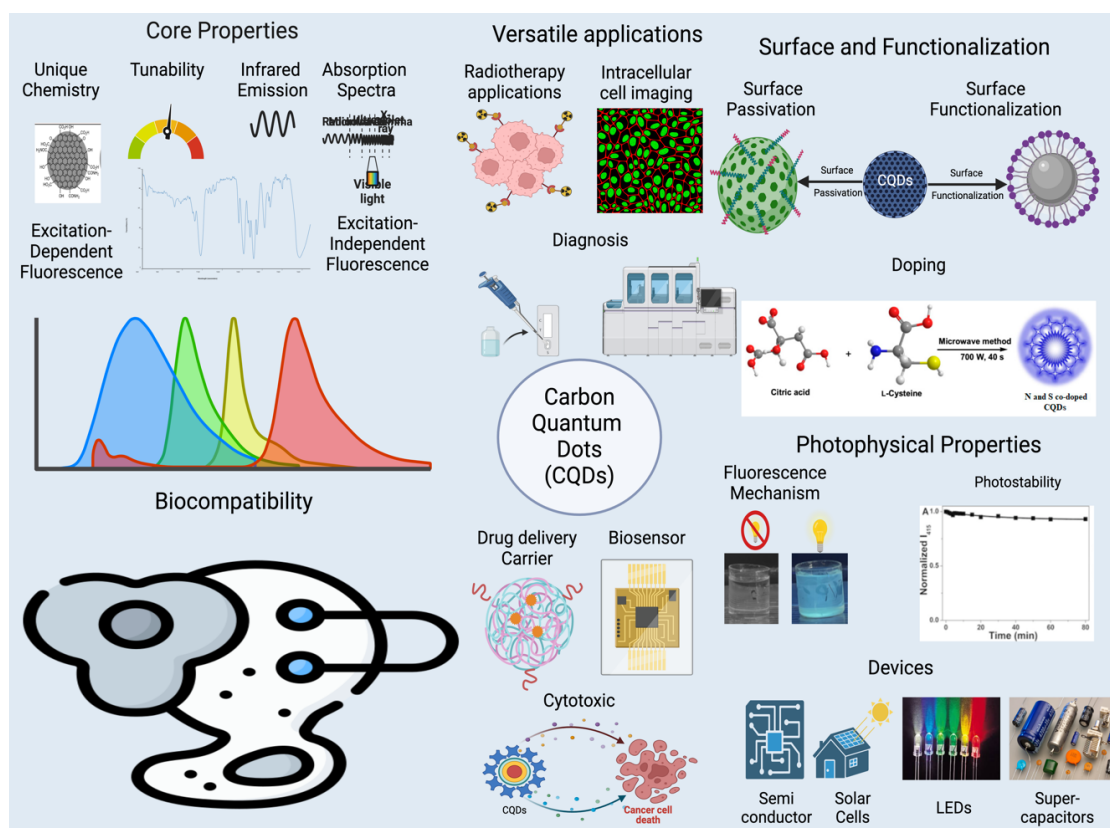


Fig. 3. Schematic representation of key properties, surface engineering, and biomedical and device applications of carbon quantum dots (CQDs).

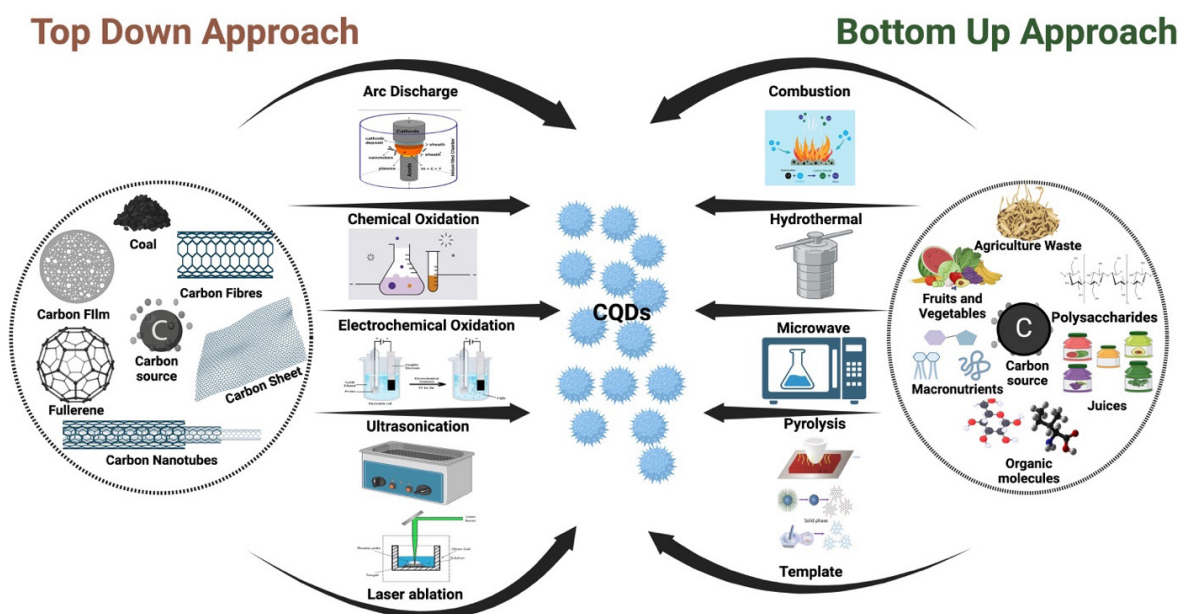


Fig. 4. A wide variety of different methods is used to produce Carbon Quantum Dots such as hydrothermal, microwave-assisted, electrochemical and laser ablation techniques. They all have their own benefits in making their own properties and applications.

tunable size and surface characteristics. The electrochemical oxidation or reduction approach involves the application of an electrical potential to carbon precursors, followed by the formation of CQDs.²⁹ However, for some materials or electrolytes, and the addition of other purification processes including dialysis or chromatography may be necessary to produce monodispersed nanoparticles.³⁰

Electrochemical routes have been successfully employed by employing different precursors like o-phenylenediamine, in which the formation of CQD involves oxidation process and polymerization process.³¹ One of the most common bottom-up approaches to the preparation of CQDs is named hydrothermal synthesis. Here, the organic precursors or biomass-derived materials

are carbonized and surface passivated simultaneously by treating in closed autoclaves under high temperature and pressure.³² A wide range of carbon sources such as plant extracts, food waste, and organic acids have been utilized. The sizes, crystallinity and optical properties of CQD are strongly dependent on the reaction parameters such as temperature, pH, and precursor composition. Hydrothermal methods are especially notable for their simplicity, green chemistry attributes, and their ability to impart heteroatoms to create a range of tunable fluorescent CQDs.³³ The microwave-assisted synthesis is faster than the conventional synthesis and the process is energy-saving.³⁴ Microwave irradiation will boost reaction rate, with time of synthesis being considerably reduced with high yields. Simple precursors like glucose and ammonia solutions have been employed to prepare Nitrogen doped CQDs by this approach with great success. The method can also be applied to biomass and waste-based materials, which is ideal for sustainable CQD production.³⁵ The resulting CQD generally shows high fluorescence, and can be tuned to become biosensors and bioimaging agents.³⁶

CQDs can be synthesized by laser ablation, a physical top-down approach, in which carbon materials are ablated with high-energy laser pulses in a liquid medium.³⁷ During the process, nanoscale carbon fragments are generated which are stabilized to form CQDs with tunable size and optical features.³⁸ The final characteristics depend greatly upon the laser parameters like: wavelength, pulse duration, energy density, etc. This approach provides high purity synthesis of CQDs and enables in situ surface modification, which is beneficial for further applications,

such as advanced photonic and biomedical applications.³⁸ Overall, the synthesis method is a key factor in shaping the structural, optical and surface properties of CQDs that directly affect their applications such as bioimaging, sensing, drug delivery, and catalysis.³⁹ Fig. 5 shows a typical summary of synthesis strategies for CQD.⁴⁰

Chemical precipitation process is widely adopted for the synthesis of CQDs where controlled chemical reaction of carbon-based precursor molecules to produce nanocarbon particles.⁴¹ It is a simple, cheap and scalable method applicable for large-scale production of CQD. In thermal processes, known as pyrolysis and carbonization, CQDs are produced at high temperatures without oxygen, using carbon-rich starting materials.⁴² The carbon source may be biomass, polymers, organic compounds or biowaste. The CQDs thus obtained generally have many surface functionalities and structural properties, which are suitable for sensing, imaging and energy storage applications.⁴³ The ultrasonic-assisted synthesis method uses ultrasonic energy to break down carbon-rich precursors, which further disperses the precursors and increases the uniform production of CQDs. This technique is easily performed, efficient and allows control of particle size and surface properties.⁴⁴ In template-assisted synthesis, the synthesis of CQDs is controlled by using porous matrices, polymers or surfactants to control the size and morphology of the CQD. CQDs can be *in-situ* synthesized in templates or can be synthesized using a template as a scaffold to control the structural features in applications such as catalysis and sensing.⁴⁴ To improve the stability, solubility and performance of CQDs, post-synthesis surface passivation

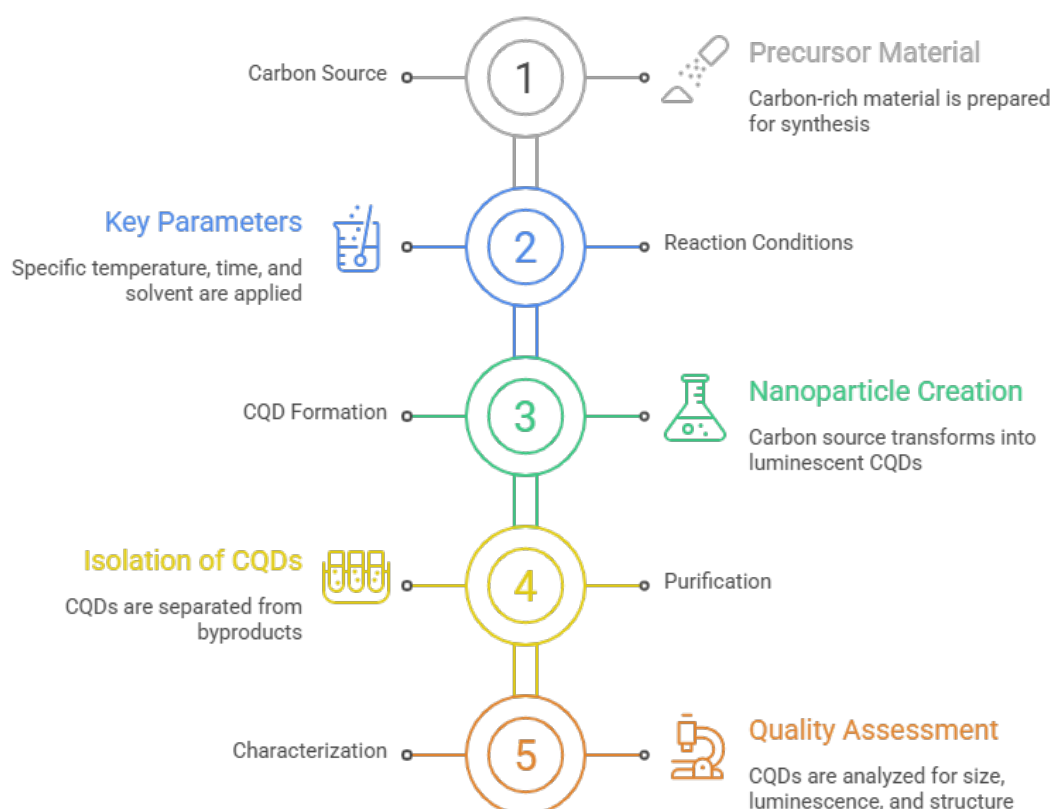


Fig. 5. Schematic representation of the single-step synthesis of CQDs.

and functionalization are frequently used.⁴⁵ These changes add functional groups, making them more useful for biomedicine and technology.⁴⁶ Microorganisms, plants, and fungi-based biosynthesis of CQDs is an eco-friendly and sustainable process. Biosynthesized CQDs are known to have varied properties depending upon the source and culture conditions and they have been extensively explored for use in bioimaging, drug delivery, and sensing.⁴⁷ The green synthesis methods are used as natural resources and wastes material as carbon sources and provided significant environmental and economic benefits. These methods are less toxic, less expensive and less polluting than standard chemical processes, and can be used to produce large quantities of the material.⁴⁸ Different types of biowaste such as animal proteins (crustacean shell, hair, skin and manure) and industrial (paper mill wastes, petroleum coke and plastic wastes) have been employed in the synthesis of CQDs with good efficiency.⁴⁹ The strategies not only help enable waste valorization, but also help with sustainable nanomaterial production (Table 1).⁵⁰⁻⁵⁵

Comparative analysis of CQDs synthesis methods

Despite its simplicity and environmental friendliness, hydrothermal synthesis is still the most commonly used approach, but there are problems with reproducibility caused by variations in the precursors.⁶⁸ The microwave-assisted synthesis can significantly shorten the reaction time and energy consumption, but it can be difficult to ensure that the particles have a uniform characteristic (Table 2).⁶⁹ Electrochemical techniques provide better control of particle size and surface characteristics, but need to be highly instrumented.⁷⁰ Advantage of laser ablation is that there are no chemical contaminants in the generated CQDs, but the drawback is the low production amount and high operating cost.⁷¹ Pyrolysis is an economically attractive process for large scale processing, but is often plagued by inadequate control of the particle morphology and surface functionalization.⁷² So far, none of the synthesis methods can meet all these demands for reproducibility, scalability, low cost and biomedical applicability, which calls for optimization and standardization of the synthesis (Table 2).⁷³

Characterization of carbon quantum dots

It is crucial to characterize the properties of CQDs in order to understand their influence on the biochemical performance and their physicochemical properties (Table 3) and (Fig. 6). The particle size is a critical parameter for controlling tissue penetration, circulation time, and cellular internalization.⁷⁹ The size of the particles can vary significantly depending on the particle synthesis method and precursor source, and CQDs are typically considered to be quasi-spherical particles in the size range of 2-10 nm.⁸⁰ The surface charge of CQDs is usually determined by zeta potential and it is related to the stability in dispersion and aggregation of CQDs with biological membranes. Surface functional groups that have been determined using Fourier transform infrared spectroscopy (FTIR), X-ray

photoelectron spectroscopy (XPS), and elemental analyses play a key role in aqueous solubility, biocompatibility, drug conjugations, and targeting.⁸¹⁻⁸⁴ The excitation-dependent fluorescent properties of CQDs are controlled by particle size, surface states and functional groups, which allows the fluorescence emission to be tuned for diagnostic applications in particular in bioimaging and biosensing.⁸⁵⁻⁸⁸ UV-Vis spectroscopy also gives an insight into the electronic transitions and surface modification that can influence the optical performances.⁸⁹⁻⁹⁰ Characterization, a critical task for elucidating the relationship between CQD physicochemical properties and biomedical applications such as drug delivery, bioimaging, biosensing, and therapeutic use, is not just an analytical requirement but also influences the degree of graphitization and crystallinity of CQDs.⁹¹

Diagnostic applications

The functional characteristics of CQDs are closely associated with their structural properties, including surface functional groups, electron density distribution⁹⁹⁻¹¹⁰ and the energy gap between the conduction and valence bands.¹¹¹⁻¹²¹ Surface functional groups such as carboxyl, hydroxyl, amino, and carbonyl groups play significant roles in determining the chemical reactivity, solubility, and interactions of CQDs with other molecules or ions.¹²²⁻¹²⁵ These functional groups may either originate during synthesis or be introduced through surface modification.¹²⁶⁻¹³⁰ The presence of these functional groups provides CQDs with a high affinity for various biomolecules, making them highly suitable for applications in bioimaging and drug delivery.¹³¹⁻¹³⁵ In addition, surface functional groups contribute to the photoluminescence properties of CQDs by acting as surface states that trap excitons or facilitate non-radiative recombination processes. As a result, they influence both the emission intensity and emission wavelength of CQDs.¹³⁶⁻¹³⁸ The distribution of electron density within CQDs is another critical factor affecting their electronic and optical properties. CQDs generally possess an sp^2 -hybridized carbon core embedded within an sp^3 -hybridized environment. This structural arrangement gives rise to quantum confinement effects, which strongly influence their optical behavior upon light excitation. The size of the sp^2 domains directly affects the bandgap energy and is closely related to the extent of π -conjugation. An increased bandgap typically results in blue-shifted emission, whereas a reduced bandgap leads to red-shifted emission.^{139,140} Furthermore, heteroatoms such as nitrogen, sulfur, and phosphorus can be incorporated into the carbon lattice to modify the electron density and introduce additional electronic states within the bandgap. These dopants create localized energy states capable of trapping electrons and holes, thereby altering the photoluminescence characteristics and enhancing the quantum yield of CQDs.¹⁴¹ Doping also modifies the chemical properties of CQDs, including their catalytic and sensing capabilities, by introducing active sites that can interact with different analytes.¹⁴² The bandgap energy

Table 1. Reported synthesis methods of carbon quantum dots with precursors, properties, applications, and key findings

Precursor/ Source	Synthesis Method	Size/ Morphology	Optical properties	Functional groups	Applications	Key findings	References
<i>Aloe barbadensis</i> Miller (Aloe vera) extract	Microwave-assisted reflux	< 5 nm, spherical, amorphous	Blue fluorescence, excitation-independent, QY = 31%	OH groups contributed to uniform dispersion	Photocatalysis (eosin yellow degradation), Bioimaging, Anti-cancer (MCF-7 cell line)	98.55% dye degradation in 80 min; 100% in 100 min; Cytotoxicity (IC ₅₀ = 52.2 µg/mL); Cellular internalization; Anti-proliferative effect in breast cancer cells	56
Coal (bulk carbon source)	Facile, green top-down exfoliation (without strong oxidizing acids)	-	Fluorescent CDs (visible-light responsive)	-	Photocatalysis (organic dye degradation), Environmental remediation, Solar energy conversion/storage potential	High yield; scalable synthesis; avoids toxic gases & tedious purification; environmentally safer alternative to conventional acid treatment	57
<i>Azadirachta indica</i> (Neem leaves)	One-pot hydrothermal (green synthesis)	-	High fluorescence QY = 27.2%	-	Peroxidase-mimetic activity; Colorimetric detection of H ₂ O ₂ and Ascorbic Acid (AA); Real fruit sample analysis	Peroxidase-like catalytic activity follows Michaelis–Menten & ping-pong kinetics; H ₂ O ₂ detection (LOD = 0.035 mmol/L, range 0.1–0.5 mmol/L); AA detection (LOD = 1.773 µM, range 5–40 µM); Good sensitivity in real fruits	58
Flowers of <i>Abelmoschus manihot</i>	One-pot hydrothermal	-	Strong blue emission, QY 30.8%	Not explicitly doped; functional groups from plant biomass	Detection of 2,4,6-trinitrophenol (TNP); cellular imaging	High selectivity and sensitivity for TNP (LOD 5 nM); successful detection in tap/river water (recoveries 92.6–113%, RSD < 5%); excellent biocompatibility; cytotoxicity negligible; Hela cell imaging demonstrated	59
Flowers of <i>Osmanthus fragrans</i>	Green hydrothermal	-	Strong blue fluorescence at 410 nm (excitation 340 nm), QY ≈ 18.53%	Functional groups from biomass; likely –OH, –COOH	Detection of Fe ³⁺ and ascorbic acid; cell imaging	Highly selective and sensitive detection of Fe ³⁺ (LOD 5 nM, linear 10 nM–50 µM); “off–on” detection of AA (LOD 5 µM); recoveries 93.76–113.80% in water/serum; low cytotoxicity and good biocompatibility; effective fluorescence imaging in living cells	60
Grass	Hydrothermal	Tiny and uniform particles (SEM/TEM/AFM); morphology confirmed	High photoluminescence intensity	Nitrogen-doped (N-CQDs); surface functional groups from biomass	Removal of organic dyes (Acid Blue, Acid Red, Eosin Y, Eriochrome Black T, Methyl orange, Methylene blue); adsorption of heavy metals (Cd ²⁺ , Pb ²⁺)	Efficient photocatalytic degradation of dyes under radiation; adsorption of Cd ²⁺ (37%) and Pb ²⁺ (75%); low-cost green precursor; potential for water purification applications	61
Green tea leaf residue	Pyrolysis at high temperature + oxidation by concentrated H ₂ SO ₄	-	High fluorescence; fluorescence stable to pH, ionic strength, UV; temperature-sensitive (15–65 °C); quantum yield (14.8%)	N, S co-doped	Fluorescent sensor for gefitinib	High fluorescence; quenching by gefitinib via inner-filter effect; linear detection range 0.1–20 µg/mL; sustainable use of tea leaf waste; potential for drug sensing applications	62

Table 1. Continued.

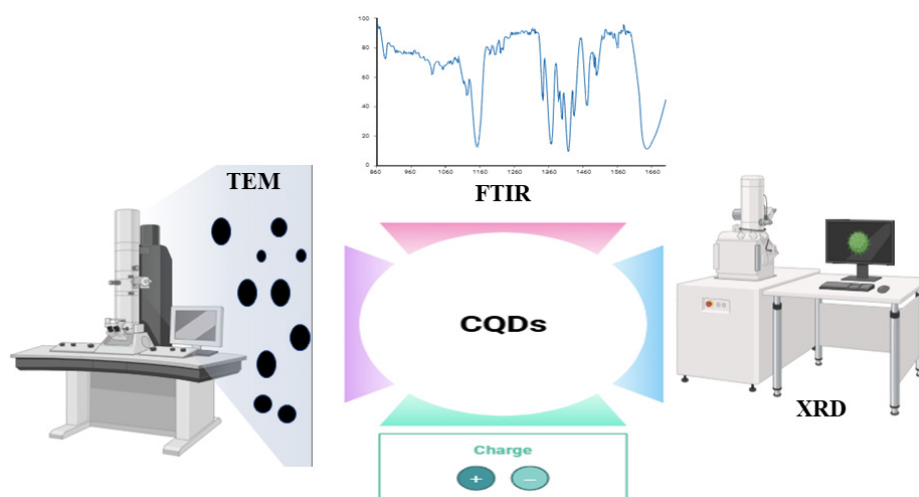
Precursor/Source	Synthesis Method	Size/Morphology	Optical properties	Functional groups	Applications	Key findings	References
Green algae (<i>Halimeda opuntia</i>)	Pyrolysis	Characterized by HR-TEM (size not specified)	Fluorescence studied via emission spectra; excellent optical properties	Functional groups on CQD surface (not detailed)	Energy storage (supercapacitors), optoelectronics	Specific capacitance of 311 F g ⁻¹ ; CQDs show good electrochemical performance and optical properties; low-cost, green synthesis from biomass; potential use in energy conversion and storage applications	63
Honey	Hydrothermal	Characterized by HR-TEM (size not specified)	High fluorescence (QY ≈ 19.8%), excellent photostability	COOH, OH, NH ₂ groups on CD surface	Sensing (Fe ³⁺ detection), bioimaging, coding	CDs synthesized entirely via green method; high QY, non-toxic, stable; Fe ³⁺ detection in linear range 5.0 × 10 ⁻⁹ –1.0 × 10 ⁻⁴ M with LOD 1.7 × 10 ⁻⁹ M; aggregation-based fluorescence quenching; successful cell imaging and coding applications	64
Jackfruit seeds	Facile, green, one-step microwave-assisted method	water-soluble N-CDs	High photoluminescence, QY 17.91%, high photostability, stable > 180 days	Nitrogen-doped (N-CDs), surface functional groups inferred	Au ³⁺ detection in aqueous media, in vitro multicolor cell imaging, gold nanoparticle synthesis	N-CDs exhibit excellent solubility, low cytotoxicity (90% viability at 0–2 mg/mL in A549 cells), fluorescence stable over wide pH/ionic strength; Au ³⁺ sensed selectively with LOD 239 nM; N-CDs act as reducing agents for AuNP synthesis	65
Kelp (natural kelp juice)	Hydrothermal carbonization; surface modification with PEI; conjugation with fluorescein isothiocyanate (FITC)	-	Fluorescent carbon dots; dual-signal and ratiometric FL behavior	Polyethylenimine (PEI)-modified; FITC-conjugated	Sensing and imaging of pH and Cu ²⁺ in real samples (yogurt, human serum, glass bottles, leaves, latent fingerprints)	CDs-FITC composites enable visual, ratiometric, and dual-signal detection of pH and Cu ²⁺ with high feasibility, accuracy, and stability; suitable for environmental, food, and biological samples	66
Bacteria (probiotic and pathogenic strains)	Pyrolytic carbonization at varying temperatures	-	QY negligible below 200 °C; poor water dispersibility above 220 °C	Amide functionalities inherited from bacterial surface proteins; heterocyclic aromatic-carbon structures; nitrogen content correlated with protein content	Generation of reactive oxygen species (ROS) for potential probiotic-assisted therapy, food supplementation	CQDs inherit chemical properties from source bacteria; surface proteins control ROS generation; probiotic-derived CQDs show highest ROS activity; offers alternative to using live bacteria in applications	67

Table 2. Comparison of common carbon quantum dot synthesis methods

Synthesis method	Advantages	Limitations	Scalability potential
Hydrothermal	Simple, eco-friendly, suitable for biomass precursors	Long reaction time, batch variability	Moderate ⁷⁴
Microwave-Assisted	Rapid synthesis, energy efficient	Particle size heterogeneity	High ⁷⁵
Electrochemical	Good control over particle properties, high purity	Requires specialized equipment	Moderate ⁷⁶
Laser Ablation	High purity products, no chemical reagents	Expensive instrumentation, low yield	Low ⁷⁷
Pyrolysis	Cost-effective, suitable for large-scale production	Poor control over particle size and surface chemistry	High ⁷⁸

Table 3. Physicochemical characterization of CQDs derived from different precursor materials, highlighting the analytical techniques employed and the resulting size, optical properties, surface chemistry, and structural features relevant to biomedical applications

Precursor/Synthesis	Techniques used	Size/Morphology	Optical properties	Surface chemistry	Structure (XRD)
Folic acid CDs	UV–Vis, FL, FTIR, XRD	2–6 nm	Blue–green emission	NH ₂ , COOH	Graphitic domains ⁹²
Green CDs	UV–Vis, FL, XRD	3–7 nm	Stable emission	Oxygen-rich	Amorphous ⁹³
Resveratrol CDs	FTIR, TEM, FL	~4 nm	Strong emission	Phenolic groups	Amorphous ⁹⁴
Citric acid + cysteine	UV–Vis, FL	2–4 nm	Bright emission	NH ₂	Amorphous ⁹⁵
Cumin CDs	FTIR, FL, TEM	4–7 nm	Strong fluorescence	COOH, OH	Amorphous ⁹⁶
Lycii Fructus CDs	UV–Vis, FL	~4 nm	Multicolor	Oxygen groups	Amorphous ⁹⁷
Curcumin CDs	UV–Vis, FL, TEM	2–5 nm	Multicolor emission	Phenolic groups	Amorphous ⁹⁸

**Fig. 6.** Characterization techniques of carbon quantum dots.

between the conduction and valence bands is one of the most important parameters governing the electronic and optical properties of CQDs. This bandgap can be influenced by particle size, surface passivation, functional groups, and synthesis method.¹⁴³ For example, hydrothermal treatment, laser ablation, and microwave irradiation can produce CQDs with distinct electronic structures, resulting in variations in their optical behavior. Surface passivation using organic molecules or polymers can further improve photoluminescence efficiency by reducing surface defects that act as non-radiative recombination centers.^{144,145} In summary, the functional properties of CQDs arise from a complex interplay among surface functional groups, electron density distribution, and bandgap energy. Surface functional groups regulate chemical interactions and contribute to photoluminescence through surface-state emission.¹⁴⁶⁻¹⁴⁸ Electron density distribution, influenced by sp^2/sp^3 hybridization and heteroatom doping, governs quantum confinement effects and photophysical behavior.¹⁴⁹ Additionally, particle size, surface passivation, and synthesis strategy collectively determine the optical absorption and emission properties of CQDs. These characteristics enable a wide range of applications, including bioimaging, sensing, photocatalysis, and light-emitting devices. By carefully tuning these parameters, researchers can design CQDs with tailored properties for specific technological and biomedical applications.¹⁵⁰

Bioimaging is one of the most promising diagnostic

applications of CQDs.¹⁵¹ Their strong and tunable fluorescence properties have positioned CQDs at the forefront of imaging technologies. Due to their excellent biocompatibility and low toxicity, CQDs are considered highly suitable for both *in vitro* and *in vivo* imaging of cells and tissues.¹⁵² PEGylated CQDs have been successfully applied in the confocal microscopic imaging of *Escherichia coli*, representing a significant advancement in microbial detection.¹⁵³ *In vivo* studies involving subcutaneous injection in mice further demonstrated the potential of PEGylated CQDs as multimodal bioimaging agents capable of producing both green and red fluorescence emissions.^{154,155}

Moreover, the development of Gd(III)-doped CQDs has enabled the integration of multiple imaging modalities, such as magnetic resonance imaging (MRI) and optical imaging.¹⁵⁶ These CQDs exhibit dual fluorescence and MRI contrast properties, thereby enhancing diagnostic accuracy and imaging resolution.¹⁵⁷ The incorporation of CQDs into bioimaging technologies offers high-resolution imaging capabilities and provides new opportunities for detailed disease investigation and biomedical diagnostics.¹⁵⁸ Although CQDs have demonstrated considerable potential in bioimaging, biosensing, drug delivery, and theranostic applications, their clinical translation remains limited. Most CQD-based systems are currently evaluated in preclinical animal studies. To date, no CQD-based therapeutic or diagnostic product has received regulatory

approval, and only limited translational investigations have been reported. Table 4 summarizes the current clinical and translational status of CQD technologies.¹⁵⁹

Limitations of current biomedical studies

While CQDs have shown great promise in the fields of bioimaging, drug delivery, biosensing, antimicrobial therapy, and cancer therapy, there are still some obstacles to overcome before they can be used in clinical settings.¹⁶⁵ The potential applications of CQDs in bioimaging, drug delivery, biosensing, antimicrobial therapies and cancer therapies have all been demonstrated, but some challenges remain before they can be used clinically. The biological long-term effects are not well understood since most reported studies are either *in vitro* studies or short-term animal experiments. Rigorous biodistribution, biodegradation, pharmacokinetics, immunogenicity and chronic toxicity studies are still not available.¹⁶⁶ Additionally, the physicochemical properties and biological activity of the different preparations often vary as a result of experimental variations in synthesis technique, precursor materials and surface modification, and thus comparisons between different studies are often made difficult.¹⁶⁷ The safety and efficacy of these products is further complicated by the fact that there are no uniform characterization protocols and regulatory guidelines. To move CQD-based technologies forward into clinical practice, it is crucial to overcome these limitations by conducting rigorous preclinical studies, standardized testing methods, and well-designed clinical investigations.¹⁶⁸

Challenges and limitations

Reproducibility challenges

The one hurdle in studying CQD is its non-reproducibility due to the different synthesis protocol. The precursor composition, reaction temperature, reaction pH, reaction time, purification and surface functionalization often lead to many differences in particle sizes, fluorescence characteristics, surface chemistry and biological performance.¹⁶⁹⁻¹⁷² This variability from batch to batch makes it hard to compare the results across studies and to develop uniform quality control standards. Further research is needed to construct and characterize harmonized procedures for increased reproducibility and clinical translation.¹⁷³⁻¹⁷⁶

Scalability challenges

Although significant advances have been made in the production of CQDs in the laboratory, industrial

production is still difficult. In industrial-scale production, maintaining uniform particle characteristics is sometimes challenging for many synthesis methods that have stringent requirements for the control of reaction parameters. Besides that, there are various hurdles for commercialization such as purification processes, quality assurance, production cost, and regulatory compliance. Scalable, cost-effective and reproducible manufacturing is essential for the translation of CQD-based biomedical products to be successful.¹⁷⁷

Critical challenges, research gaps and future perspectives

Critically evaluate challenges and gaps in research and their future directions. Although CQDs have made remarkable progress in their synthesis and biomedical applications, there remain some challenges that hinder their clinical translation. Standardization of synthesis protocols is one of the main concerns.¹⁷⁸ Because of the different precursor materials, reaction conditions, purification processes, and functionalization strategies, batch-to-batch variability can occur in the particles, in their fluorescence properties, and in their performance in biological applications. This makes it difficult to make comparisons between studies and results are not always reproducible. There's another crucial question: how scalable is it? Many of these methods have been proved to have promising laboratory scale results, but are not easily scalable to production scale and do not provide consistent quality and physicochemical properties. Scalable and cost-effective production technologies are still needed for future commercialization. Moreover, majority of the biomedical research on CQDs has been carried out *in vitro* and over short time period on animal experiments.¹⁷⁹ Full evaluations of long-term toxicity, biodistribution, biodegradability, immunogenicity and pharmacokinetic properties remain to be done. There are no standardized safety assessment protocols which makes regulatory approval and clinical acceptance difficult. Another research gap is the lack of knowledge regarding CQD-biological interactions mechanisms. Surface chemistry, heteroatom doping and functionalization markedly affect cellular uptake, intracellular trafficking and therapeutic efficacy but the mechanisms involved are still poorly understood. More research is needed to develop standard synthesis procedures, establish safe profiles, enhance production of large quantities of the drug, and to perform complete preclinical and clinical trials. The use of artificial intelligence in the context of biomedical applications would be complemented by advanced surface engineering and multifunctional theranostic platforms, all of which can further hasten the development of CQD-based technologies.¹⁸⁰⁻¹⁸⁴

Table 4. Overview of clinical studies associated including NIH identifiers, biomedical applications and clinical phases

NIH identifier	Applications	Phase
NCT03794960	Immunological detection of specific autoantigen tolerance	Diabetes mellitus Type I ¹⁶⁰
NCT04138342	Bioimaging/anticancer	Phase I ¹⁶¹
NCT03794973	Immunological detection of specific autoantigen tolerance	Phase II ¹⁶²
NCT03895437	Immunological detection of specific autoantigen tolerance	Phase II ¹⁶³
NCT04590872	Detection of CD8+ autoreactive T-cells	Phase I ¹⁶⁴

Conclusion

CQDs are the new promising class of nanomaterials which are characterized by their unique optical characteristics, good biocompatibility, tunable surface and their versatile biomedical applications. These applications and their potential in bioimaging, biosensing, drug delivery, antimicrobial therapy, cancer theranostics and tissue engineering underscore the importance of nanotubes in nanomedicine. The synthesis of CQDs via different methods, such as hydrothermal, microwave, electrochemical, laser ablation, and pyrolytic methods, has facilitated the progress of CQDs with precisely engineered physicochemical properties towards a wide range of biomedical applications. Despite all these advances, some challenges still remain that hinder the clinical translation of CQD-based technologies. There are still some critical concerns, including variability in the synthesis protocols, lack of long-term toxicity and biodistribution studies, and limited manufacturing options for large-scale production. In addition, the lack of uniformity in the characterization methods and regulatory guidelines makes safety and efficacy evaluation and quality control between studies difficult. Future studies should aim for reproducible synthesis methods, standardized evaluation systems and extensive preclinical and clinical studies. The focus should also be increased on the understanding of the interaction between CQD and biology, long term safety profile, and regulatory requirements for clinical implementation of CQD. However, CQDs will become even more important in the future in the field of the next generation of diagnostic and therapeutic platforms with the continued development of nanotechnology and surface engineering and the creation of multifunctional theranostic designs. To fully realize the clinical potential of carbon quantum dots in biomedical applications, it will be essential to address the current scientific and translational challenges.

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Competing Interests

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.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created

or analyzed in this study.

Declaration of AI-assisted Tools in the Writing Procedure

The authors declare that ChatGPT (OpenAI; <https://chat.openai.com/>) was used solely for language refinement, grammar correction, and improvement of clarity and readability of the manuscript. The AI tools did not generate scientific content, interpret data, perform analyses, or influence the study design, results, or conclusions. All intellectual content, data interpretation, and scientific responsibility remain entirely with the authors.

Ethical Approval

This article does not contain any studies involving human participants or animals performed by any of the authors. Therefore, ethical approval was not required.

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