Synthesis processes, photoluminescence mechanism, and toxicity of amorphous or polymeric carbon dots

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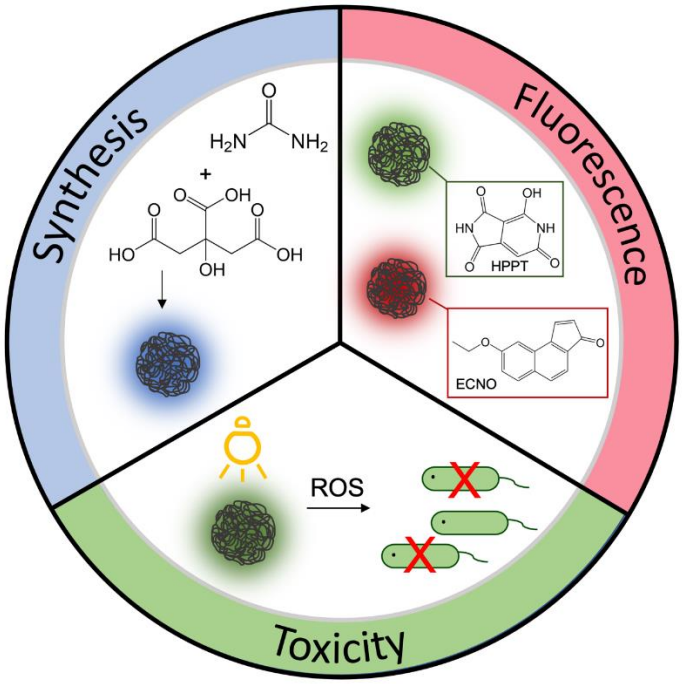
Conspectus

Fluorescence is the emission of light following photon absorption. This optical phenomenon has many applications in daily life, such as in LED lamps, forensics, and bioimaging. Traditionally, small molecule fluorophores were most common, but the types of molecules and particles with compelling fluorescent properties have expanded. For example, green fluorescent protein (GFP) was isolated from jellyfish and won the Nobel prize in 2008 due to its significant utility as a fluorescent biomarker. Using the intrinsic fluorescence of GFP, many previously invisible biological processes and substances can now be observed and studied. Other fluorescent materials have also been developed, greatly expanding the potential applications. Semiconductor quantum dots (QDs), which have bright fluorescence and a narrow bandwidth, are a popular choice for display technologies. However, QDs are made of heavy metal elements such as Cd and Se, which pose potential safety concerns to the environment and human health. Thus, new fluorescent organic materials are being developed to mitigate the toxicological concerns while maintaining the QD advantages.

One type of new material attracting great attention as an environmentally friendly substitute for semiconductor QDs are carbon dots (CDs). CDs have been developed with strong fluorescence, good photostability, and low toxicity using a variety of precursors, and some synthesis processes have good potential for scale-up. However, since they are made of a variety of materials and through different methods, the structure and properties of CDs can differ from preparation to preparation. There are three major types of CDs: graphene quantum dots (GQDs), carbon quantum dots (CQDs) and amorphous or polymeric carbon dots (PCDs). This account focuses on PCDs and its unique properties by comparing it with other CD types. The synthesis processes, fluorescence properties, fluorescence mechanisms, and toxicity are discussed below with an emphasis on the distinct attributes of PCDs.

PCDs can be synthesized from small molecules or polymers. They have an amorphous or crosslinked polymer structure with bright fluorescence. This fluorescence is possibly due to crosslink-enhanced emission or clusteroluminescence that arises from the through-space interactions of heteroatomic rich functional groups. Other fluorescence mechanisms of CDs, including distinct contributions from the carbon core and surface states may also contribute. The toxicological profiles of CDs are influenced by chemical composition, surface functionalization, and light illumination. CDs are generally thought to be of low toxicity, and this can be further improved by removing toxic by-products, functionalizing the surface, and reducing light exposure to minimize the generation of reactive oxygen species.



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work).[4] Machine learning was employed to predict the emission wavelength of CDs and achieved minimum mean average error of 25.8 nm. Reaction method and solvent were found to impact CDs' emissions more profoundly than reaction temperature and time.

Introduction

CDs were accidently discovered during the gel electrophoresis purification of carbon nanotubes in 2004 and were described as a type of impurity made of fluorescent nanoparticles (Figure 1A).[5] If we look back, the CDs were likely oxidized GQDs generated in the arc-discharged soot. Later in 2006, Sun et al. synthesized CDs via laser ablation of graphite, followed by surface passivation with polyethyleneimine (PEI). Interestingly, the bare carbon core right after the laser ablation did not exhibit detectable photoluminescence, and the surface passivation with the polymer is the key to the multicolor fluorescence (Figure 1B).[6] As of now, there are various types of CDs that have been synthesized, with both crystalline and amorphous structures. Due to the excellent fluorescence and biocompatibility, CDs have been explored extensively in a range of applications. However, since CDs can be made from a variety of precursors such as small molecules, polymers, graphitic materials, or biomass, the structure and the composition of CDs are very complex and variable. Thus, a number of photoluminescence mechanisms have been raised for different types of CDs. The complexity in CD structure and photoluminescence properties presents a barrier to logical design of CDs for various applications. Hence, there is a need to classify CDs into different categories and to better understand their shared traits and differences. PCDs have been increasingly recognized as a type of CDs that can be easily synthesized with a high quantum yield. In this account, we focus on PCDs by comparing it to other CDs to discuss their synthesis methods, the structural and physicochemical properties, and related toxicity effects.

Classification of carbon dots

The term carbon dot is an oversimplified designation used to define a series of nanosized carbon materials. In general, CDs have intrinsic photoluminescence, including fluorescence, phosphorescence, or electroluminescence. The terminology "CDs" include GQDs, CQDs and PCDs. However, it seems in recent years, GQDs have been made more distinct from other CDs. GQDs are composed of a single or several graphene layers, which are extensive aromatic structures. GQDs are mainly made from cutting down large graphitic materials. For the other CDs, one of the major defining differences is if they have a crystal lattice or not. If the materials contain crystal lattice, they are called CQDs; while for those that do not contain crystal lattice, they are amorphous or polymeric CDs (PCDs). Even though both GQDs and CQDs contain crystal lattices, GQDs refer to mostly a single layer or a few layers of graphene, and their thickness/height is usually less than 2 nm.[7] Generally GQDs have lateral dimensions larger than their thickness while CQDs are more spherical. The lattice fringes in diffraction spectra or images of single-layer GQDs are around 0.24 nm, which is the in-plane lattice spacing of graphene;[8] when there are multilayered graphene structures, lattice fringes of 0.334 nm are observed, corresponding to the inter-layer spacing of graphite.[9] Sometimes, other lattice constants are measured for CQDs, suggesting that the ordered structure is not from graphitic carbon.[10] PCDs are easy to distinguish from GQDs and CQDs since they do not show crystallinity in transmission electron microscopy (TEM), and thus they contain less ordered and conjugated structure than other CDs (Figure 1C). PCDs are crosslinked polymers composed mainly of C, N, O elements with sizes less than 20 nm. It is generally thought that these amorphous/polymeric CDs are synthesized at lower temperatures and thus they have the least carbonized structures.[11] Additionally PCDs have higher quantum yields compared to GQDs and CQDs.[12] It should be noted that nanostructures which are much larger, lack carbonization or a crosslinked structure, or do not possess intrinsic photoluminescence are not considered PCDs.

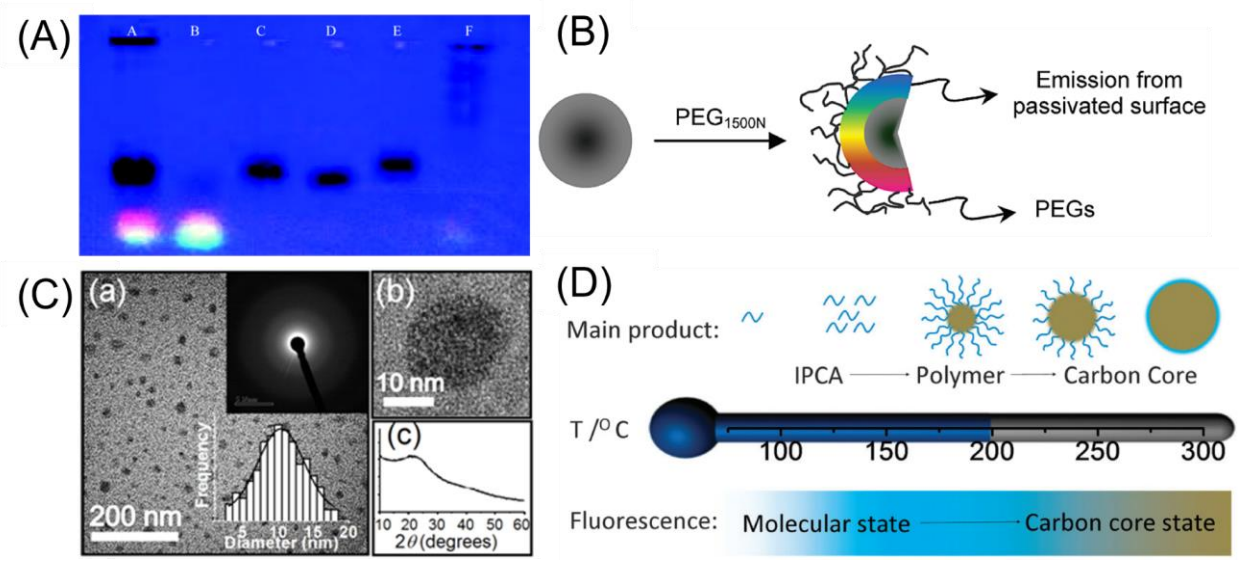

Figure 1. (A) Fluorescent CDs that were first isolated during gel electrophoresis of carbon nanotubes, adapted with permission from ref.[5]. Copyright 2004 American Chemical Society. (B) CDs that were first synthesized from the laser ablation of graphite and cement, followed by surface passivation, reproduced with permission from ref.[6]. Copyright 2006 American Chemical Society. (C) High resolution TEM images (a, b) and X-ray diffraction pattern (c) of PCDs that were synthesized via ultrasonication, reproduced with permission from ref.[13]. Copyright 2018 Abu Bakar Siddique et al. Published by Springer Nature under a Creative Commons CC BY License. (D) PCD transformation from molecular state to carbon core state at a range of temperatures, reproduced with permission from ref.[11]. Copyright 2015 Royal Society of Chemistry.

Synthesis of carbon dots

Generally, CDs can be synthesized using both top-down and bottom-up approaches. Top-down methods break down large carbonaceous materials, such as graphite, graphene oxide, and carbon fibers by acidic digestion, electrochemical methods, and so on.[14-15] In comparison, bottom-up methods heat small molecules to form large aromatic structures or crosslinked polymers. In general, GQDs are most frequently synthesized using top-down methods, while PCDs are produced mostly using bottom-up methods. CQDs can be synthesized from both top-down and bottom-up methods.

The advantages of top-down approaches include low-cost raw materials, simple treatments, and the potential to scale up; the disadvantages are that the cutting or exfoliation are not selective and that the strong acids/bases applied during cutting are hard to remove completely.[15] In GQD or CQD synthesis, nitric acid or sulfuric acid are frequently utilized to introduce carbonyl groups onto the carbon lattice, and then alkaline solutions are utilized for chemical cleavage and deoxygenation.[15]

Both CQDs and PCDs can be synthesized from the same range of precursors using bottom-up methods, such as polymers or small molecules. Yang and coworkers have attributed reaction temperature as a key factor in the synthesis of CQDs or PCDs.[11] They concluded that at lower temperatures, small molecule or polymer structures are likely to form, while at higher temperatures, the precursors or reaction medium start to carbonize to form carbon-only cores (Figure 1D).[11] Since CDs can be synthesized from a broad range of precursors and various experimental conditions in bottom-up approaches, it usually takes significant effort to optimize CDs with desired properties. With the increasing number of CD publications, machine learning has become a great tool to predict CD properties based on data available in the literature. Haynes and coworkers collected 407 literature examples of CDs synthesized from citric acid and urea or ethylenediamine. Classification models were used to predict the color, and regression models were used to predict the wavelength. Combining both models, it was feasible to achieve the minimum mean average

error of 25.8 nm in wavelength prediction. Meanwhile, from the analysis of the database, it was found that reaction method and solvent choice correlate most closely with the emission, rather than reaction temperature or time. Thus, big data analysis of CD synthesis holds great potential at this stage to reveal critical synthesis parameters and CD properties.[4]

Fluorescence

Fluorescence is the most important property of CDs. CDs are well-known for their high QYs, good photostability, and high biocompatibility, making them viable for many applications, especially in bioimaging.[16] All CDs, including GQDs, CQDs, and PCDs, can be synthesized to have emission throughout the visible spectrum from blue to red or near IR-I (700-900 nm).[17] A general survey of the literature suggests that it is more likely for GQDs and CQDs to have red emissions than PCDs. To evaluate this notion, we analyzed the literature in table 1 from reference.[18] Among the 52 CDs with red or multicolor emission, 78% of them have crystalline structure, while only 22% are amorphous or didn't include high resolution TEM images. This analysis reveals that crystalline structures likely contribute to longer-wavelength emission, perhaps originating from more conjugated structures, such as graphitic carbon or nitrogen-doped pyrene structures.[2, 19] Following this observation, a rational preparation of CDs for long wavelength emission is to use aromatic compounds or conjugated polymers as precursors for bottom-up approaches.[20] It is more likely for those precursors to cross-link and form larger conjugated structures in the synthesis. Additionally, some organic solvents such as formamide and dimethylformamide have been shown to contribute to the formation of red and multicolor CDs. It is possible that these solvents decompose and are incorporated into the CD structure, providing heteroatom doping.[20]

In general, CDs generated from top-down approaches have lower QYs compared to bottom-up approaches.[12] Thus it is not surprising that PCDs have higher QYs compared to other types of CDs. The high QYs are often related to molecular fluorophores associated with CDs that have not been completely carbonized. There are a number of reports about the synthesis of CDs with extremely high QYs. For example, blue CDs made from citric acid and tris(hydroxymethyl)aminomethane have been shown to have a QY of 93.3%.[21] There are also a few reports about high QY red-emitting CDs. CQDs made from tris(4-aminophenyl)amine showed red fluorescence with a QY of 84% in ethanol.[22] For a comprehensive summary of recent CD QYs, please refer to table 1-9 in ref.[23].

The emission can be excitation-dependent or -independent for all types of CDs. If the CDs are excitation-independent, it shows that the energy gap is well-defined and they have good uniformity in size and structure.[20] The excitation-dependent emission is often attributed to heterogeneity in size and complex chemical composition or surface defects in CDs.

Fluorescence mechanisms

Carbon core state

CDs are composed of carbon cores and various surface states. For GQDs and CQDs, carbon cores are usually crystalline, indicating a high degree of carbonization. PCDs have a more amorphous carbon core structure, and sometimes they are paracrystalline (which means that there are small sp^2 structures surrounded by polymers).[24] Pristine GQDs are composed of sp^2 carbon structures, with only carbon and hydrogen. Thus the luminescence of pristine GQDs comes from the carbon core where the electrons are confined in the sp^2 structures, leading to the quantum confinement effect. DFT calculations show that for pristine zigzag-edged GQDs with diameters from 0.46 to 2.31 nm, their emissions should span from the deep UV to the near infrared (figure 2A).[25] Mueller et al. synthesized GQDs made of 132 carbons that emit at 670 nm from solution chemistry, which aligns with the DFT calculations.[26] However, there are some discrepancies between the GQD sizes and the emission from other synthetic methods. For example, pristine GQDs synthesized by solution exfoliation of graphite nanoparticles are monolayered and oxygen defect-free. They are 4 nm in size and emit at 420 nm; however, calculations indicate that 4 nm GQDs should have near IR emission. Considering that these GQDs are synthesized using a top-down approach, it is

possible that they contain vacancies or interstitial atoms that impact their photoluminescence properties.[27] Indeed, pristine GQDs are challenging to synthesize and most CD syntheses generate imperfect carbon cores.

Surface state effect

A CD's surface state refers to the organic molecules or polymers wrapped around or bonded to the carbon core of CDs. In addition to emission from the carbon core, the surface state is sometimes thought to be an optical center as well. This can be indirectly observed as the emission from CDs shifts with solvent properties or based on the correlation between emission and surface oxygen or nitrogen percentage. Zhang et al. attributed the yellow/green emission (460-580 nm) of CQDs to the carbon core and red emissions (580-710 nm) from electron transitions on surface states, as shown in Figure 2B. They studied CD emissions in a range of chemical environments, temperature, and UV irradiation. The yellow/green emission seems to be independent of those changes, while emissions at the red region are quite sensitive. Furthermore, computational calculations showed that with the addition of carbonyl groups on the surface, CD band gaps decrease.[28]

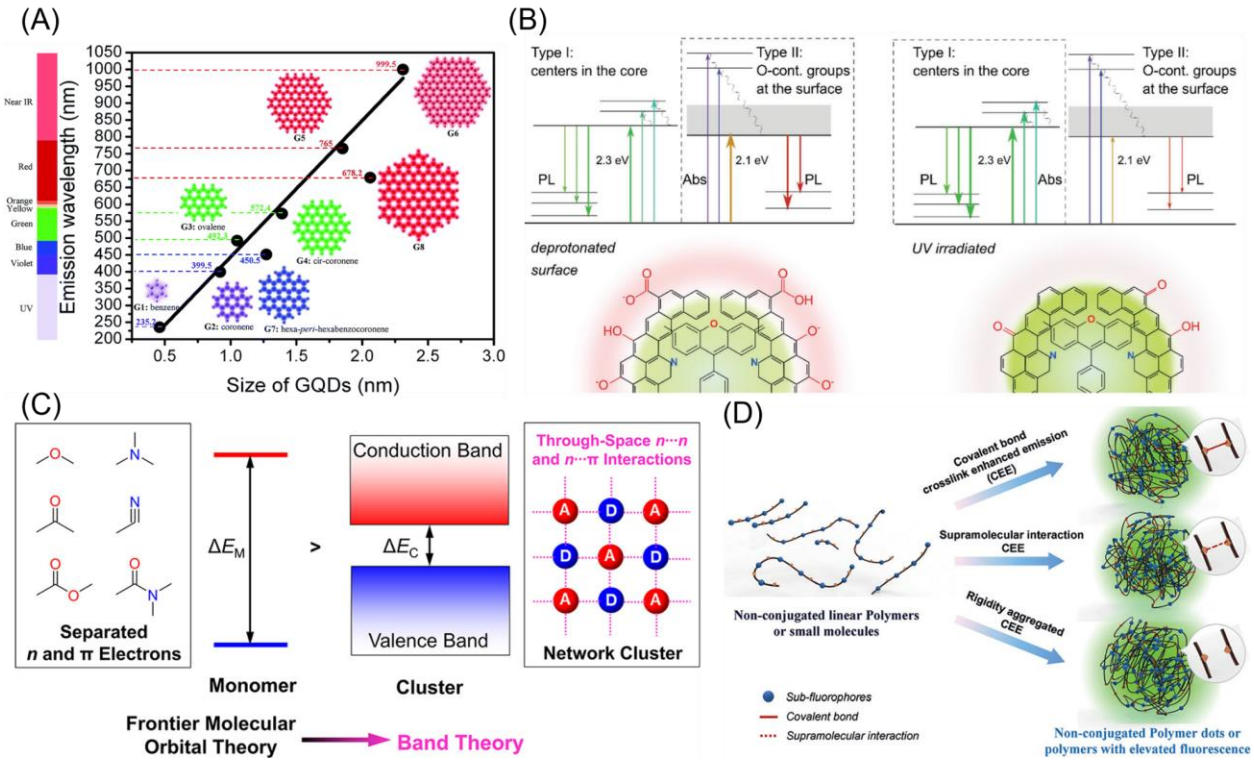

Figure 2. (A) The emission wavelength and the corresponding size of GQDs as calculated by time dependent-density functional theory (TD-DFT), reprinted with permission from ref.[25]. Copyright 2014 Royal Chemical Society. (B) The emission of CDs is attributed to oxygen-containing surface groups (left panel); after UV irradiation, the optical center of CDs is a carbon core (right panel) , reprinted with permission from ref.[28] Copyright 2022 John Wiley and Sons. (C) Unconjugated clusteroluminogens form a network cluster in an aggregated state, leading to a smaller energy gap, reprinted with permission from ref.[29]. Copyright 2021 Zhang, H. and Tang, B. Published by American Chemical Society under a Creative Commons 4.0 license. (D) Non-conjugated polymers induce crosslink-enhanced emission from covalent linkages, supramolecular interactions, or rigid aggregates, reprinted with permission from ref.[30]. Copyright 2015 John Wiley and Sons.

As for PCDs, both the carbon core and surface state could contribute to their emission; however, the crosslink-enhanced emission or clusteroluminescence have become more recognized in recent years in the discussion of the photoluminescence mechanism PCDs. In addition, molecular fluorophores generated during CD synthesis remains a possible source of fluorescence.

Clusteroluminescence

Since most of the CD precursors have heteroatomic functional groups such as -COOH, -NH_2, and -OH, it is likely that the polymer structure in amorphous CDs includes heteroatoms, and thus, becomes fluorescent due to clusteroluminescence. Different from traditional aromatic fluorophores, clusteroluminescence is induced by the subunits (clusterolumigens or CLgens) which are non-conjugated and contain multiple heteroatoms such as O and N (Figure 2C).[29] Conventional planar fluorophores usually suffer from aggregation-induced quenching, where π stacking effects impede electron transitions, limiting their potential applications. Interestingly, when CLgens aggregate or cluster, higher photoluminescence occurs. The fluorescence comes from through-space interactions, such as n···n interactions or n···π interactions.[29] Vallan *et al.* proposed that the blue fluorescence of CDs comes from charge transfer within the entangled polyamide chain. Extensive DFT calculations were conducted on various sized polyamide clusters which involve both intra and intermolecular hydrogen bonds. They found that the charge transfer occurs from the HOMO on the amide to the LUMO on the carboxylic groups. One important condition for this fluorescence to occur is that those molecular orbitals must be confined very specifically in rigid polymer structures so that nonradiative relaxations are restricted.[31] Additionally, previous work from Haynes and coworkers identified a nonaromatic fluorophore: 5-oxopyrrolydine-3-carboxylic acid from the citric acid and urea CD synthesis.[1] The fluorophore was found to have aggregation-enhanced emission when dispersed in different ratios of DMSO and THF. As the proportion of THF increased, more molecular clusters formed, resulting in increased fluorescence intensity. DFT calculations suggested that the fluorescence originated from the -COOH group on a single fluorophore. The group proposed that the through-space intermolecular n···n or n···π interactions play a critical role in the fluorescence enhancement when the fluorophores are confined within the polymer structure of CDs. Additionally, the fluorophore also had excitation-dependent emission characteristics, especially in the solid state, which is a general feature for CLgens and also a commonly found spectral characteristic for CDs.[1] This property is believed to result from the variety of CLgen cluster structures that experience different extent of electron delocalization and thus, different energy gaps.[29]

Similarly, Yang and coworkers proposed a crosslink-enhanced emission effect, which is similar to clusteroluminescence but focuses more on the rigidity of the polymer network (Figure 2D).[30, 32] The Yang group synthesized PCDs from poly(vinyl alcohol)[33] and polyethyleneimine[34]. They attributed the fluorescence to the crosslinked polymer structures where the vibration and rotation of subchromophores were restricted.[34] Also similar, Hedstrand and coworkers have focused on non-traditional intrinsic luminescence (NTIL) based on the fact that both NTIL and CDs tend to emit in the blue and green.[35] NTIL describes the fluorescence from the confinement of functionalized moieties that are electron rich and heteroatomic, in the absence of aromatic or π conjugated structures.[35] Looking forward, simulations on the molecular clusters are needed to elucidate the structures and explain the fundamental photophysical mechanisms of enhanced photoluminescence.

Molecular fluorophore

Molecular fluorophores have been discussed widely as a likely component of PCDs, especially those prepared using bottom-up routes. A number of molecular fluorophores have been identified so far for PCDs as well as CQDs and GQDs. A blue fluorophore, 1,2,3,-tetrahydro-5-oxo-imidazo[1,2-*a*]pyridine-7-carboxylic acid (IPCA), has been identified in citric acid and ethylenediamine CDs.[11] Similar structures such as 1-(2-aminoethyl)-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]-pyridine-7-carboxylic acid (AEIOP) etc. have been identified.[36] Green fluorophores such as 4-hydroxy-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione (HPPT), and even red fluorophores like 8-ethoxy-3H-cyclopenta[a]naphthalen-3-one (ECNO) have also been separated and characterized.[37-38] Thus, it is critical to always include a thorough purification process to fully remove free molecular fluorophores before optical characterization of CDs. Continuing to identify fluorophores helps the CD community to elucidate more of the possible reaction routes in CD synthesis. As for the relationship of fluorophores and CDs, some have proposed that CDs are an aggregate of molecular fluorophores,[39] while others have suggested that the fluorophores are attached to a polyamide backbone to make up the CDs.[2] For example, Haynes and coworkers have proposed a few aromatic molecules and pyrene structures to be the fluorescence origin of multicolor PCDs. TD-DFT calculations showed hydrogen bonding and stacking effects have a pronounced effect on the fluorescence properties of these chromophores. Additionally, the association with the polyamide backbone would red shift the emission of those fluorescence centers.[2] This work has provided insights on the interaction of fluorophores with the polymer backbone

of PCDs, and future efforts are needed to understand other possible relationships between the fluorophores and the polymer structure, and also how different structures influence the fluorescence properties. This will require more computational simulations on other models or reverse engineering CDs by attaching fluorophores to polymers of known structures.

Toxicity

CDs have many applications in imaging, theranostics, agriculture, electrochemistry, sensing, and more.[11] Thus, understanding any toxicity imposed directly on biological organisms intentionally exposed to CDs, is critical. In general, CDs are assumed to have low toxicity and high biocompatibility;[40] however, due to being an extremely broad class of nanoparticles, labeling the entire class as non-toxic can be dangerous.[41] CDs vary greatly in terms of reaction conditions, methods, and reagents used, resulting in different sizes, surface charges and chemistry, and chemical compositions, which can all impact toxicity.

This review will highlight work done on general CD toxicity and assessing the measured toxicological impacts of PCDs. The factors identified as most broadly influencing toxicity can be grouped into the following categories: (i) chemical composition, (ii) surface charge and functionalization, and (iii) light irradiation. Dose is another important factor in determining CD toxicity, however, since toxicity in general is typically dose-dependent, dose will be included as relevant to the other factors.

Chemical composition (reagents and separations)

The chemical composition of CDs is largely determined by the small molecules and/or polymers used in bottom-up syntheses and any doped heteroatoms. Typically, the chemical composition of CDs is adjusted to optimize desired properties for a relevant application; however, more recently, research has begun assessing how those adjustments may impact toxicity.

Cailotto et. al. synthesized CDs from glucose (G-CDs), fructose (F-CDs), and ascorbic acid (A-CDs).[42] The resulting CDs had varying characteristics, where G-CDs and F-CDs showed graphitic nature, while A-CDs were amorphous. There were notable differences in viability towards HeLa cells following CD exposure; G-CDs showed minimal toxicity at all concentrations (1.95 μg/mL - 1 mg/mL), F-CDs exhibited high toxicity at all concentrations, and A-CDs showed toxicity only at concentrations >0.25 mg/mL. Additional GC-MS and NMR analysis revealed furan-like molecules, which are known to be carcinogenic, in F-CDs. The slight difference in precursor composition despite using the same reaction scheme shows how the chemical transformations that occur during CD synthesis are important to consider and can greatly impact toxicity.

Research by Haynes and coworkers has investigated toxicity of PCDs, specifically phosphorus doping effects[3] and the toxicity of different CD color fractions.[2] To understand the effects of phosphorus doping, four CD series were synthesized: citric acid CDs (CACDs), phosphorous-doped citric acid CDs (CA-P-CDs), malic acid CDs (MACDs), and phosphorous-doped malic acid CDs (MA-P-CDs), where phosphorus was doped at increasing amounts (labeled CDs-1 to 4).[3] In most cases, after 1 hr of CD exposure at 5 mg/mL, there were no toxic effects from the CDs towards *Shewanella oneidensis* MR-1 (*S. oneidensis*), and rather in many cases, bacteria formed more colonies in the presence of CDs. An increase in bacterial growth was most prominent in CA-P-CDs-2 and MA-P-CDs-2, and in general the phosphorus-doped CDs yielded higher cell viability than their undoped counterparts. Increased bacterial colony formation suggests it is possible for *S. oneidensis* to use PCDs as a nutrient source, or that other by-products in the PCDs could promote colony formation. One notable exception to this was the MA-P-CDs-4, which had the highest amount of phosphorus added and showed extremely high toxicity, with nearly all bacterial colonies eradicated. The exact cause of MA-P-CD-4 toxicity remains unknown; additional metabolomic studies or toxicity experiments on additional organisms may illuminate the cause of this toxicity.

To investigate the toxicity of different color fractions of PCDs, blue, green, and red CD color fractions (CD-B, CD-G, and CD-R, respectively) were separated from a single CPD synthesis and used in toxicity studies on rainbow trout epithelial cells.[2] Cell viability studies showed low toxicity for all color fractions after 24 hours except for CD-B at the highest concentration used (0.1 mg/mL). Cellular distribution experiments with CD-B and CD-R showed

CD-B localized in the mitochondria and resulted in enlarged lysosomes, which was not true of CD-R. It is possible chemical composition and size differences between the color fractions resulted in CD-B having broader cellular distribution and impaired lysosomal function, resulting in greater toxicity.

Each of the examples above investigated how changes in CD chemical composition impacts toxicity. While many of the CDs explored were minimally toxic, especially at lower concentrations, when toxicity was observed it typically resulted from an unexpected or unknown source. Even when using benign reagents, undesirable by-products can form and induce high levels of toxicity,[3, 42] and at the same time it is possible for precursors to be more toxic than the resulting CDs.[43-44] Slight changes in synthetic methods or purification steps can result in large changes to toxicity, emphasizing the need for complete toxicity studies on all new CDs synthesized before being used in further applications.

Functionalization and surface chemistry

Carbon dot surface chemistry dictates the chemical interactions CDs can have with the surrounding environment and thus greatly impacts toxicity. Since functionalizing CDs changes the surface chemistry by attaching ligands or other molecules of interest, these changes can play a critical role in determining toxicity.

For example, functionalization can be used to create CDs with targeted cellular affinity and lower overall toxicity.[45] Li et. al. developed CDs functionalized with PEG and transferrin (Tf) to load doxorubicin (Dox), a widely used chemotherapeutic drug used for various cancers. The role of Tf was to ensure that the CDs were only internalized by cancerous cells. As desired, GQD-PEG-Tf@Dox showed toxicity towards cancerous tumor (MCF-7) cells; however, they were not toxic towards non-cancerous cells since the surface Tf only facilitated CD internalization in cancerous cells (Figure 3B).

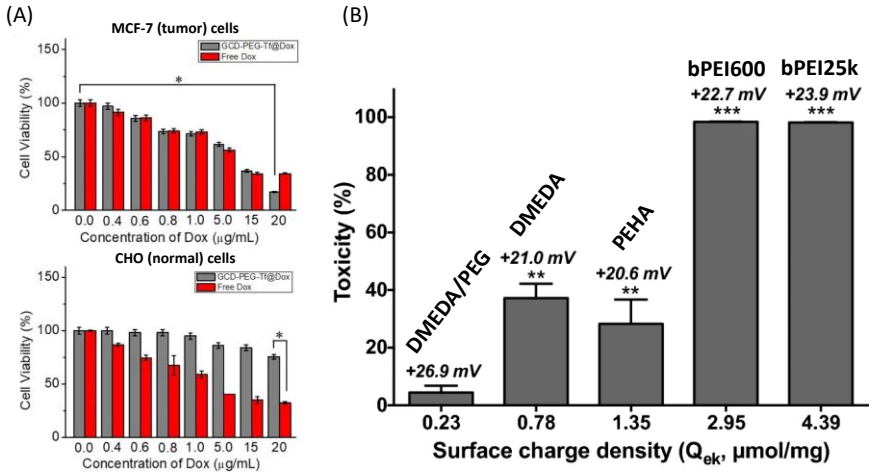

Figure 3. (A) GQDs functionalized with ligands that target cancerous cell types can be used for Dox drug delivery and reduce toxicity to non-cancerous cells. Figure reprinted with permission from ref.[45]. Copyright 2021 American Chemical Society. (B) Surface charge density impacts toxicity at 200 μg/L, the polymers used for functionalization and zeta potential of the CDs are included above toxicity (%). Figure adapted from ref.[46] Copyright 2021 Weiss, M. et al. Published by Springer Nature under a Creative Commons 4.0 license.

For imaging applications, CD functionalization can increase quantum yields and alter emission wavelengths to give a better performing nanoparticle (NP); however, this can also come with increased toxicity. Wu et. al. synthesized CDs for selective lysosomal imaging through functionalization with a morpholine derivative (ML) and used polyethylenimine (PEI) to increase quantum yields.[47] The optically best performing CDs, CDs-PEI-ML, showed greater toxicity towards HeLa cells at all concentrations (< 900 μg/mL) in comparison to the bare CDs (CDs) and to the functionalized CDs without PEI (CDs-ML). The greater toxicity of CDs containing PEI could be a result of their positive surface charge, as cationic NPs typically show greater toxicity because of their ability to bind to negatively charged biological entities.[48]

How functionalization alters the physicochemical characteristics of the CDs is important to understanding CD toxicity and decreasing the likelihood of synthesizing highly toxic CDs. Surface charge is known to influence toxicity in other NP systems. However, for PCDs, the answer is less straightforward. Sachdev et. al. synthesized CDs from PEI (CD-PEI) and PEG (CD-PEG), which had positive and negative surface charges, respectively.[49] For both normal (BHK-21) and cancerous (A549) cells, CD-PEG resulted in lower cell viability compared to CD-PEI. They hypothesized that surface density may play a role in the observed toxicity. A large-scale study conducted to better understand the relationship between PCD physiochemical properties and toxicity suggested surface charge density as a main factor influencing toxicity,[41] corroborating the results observed by Sachdev et. al.[49]

The Pons group conducted a study to explicitly study the role of surface charge density by synthesizing five cationic CDs with similar zeta potentials but increasing surface charge densities.[46] The two CDs with the highest charge densities induced oxidative stress and an immune response in mice, and were dramatically more toxic in comparison to CDs with a lower charge density (figure 3B). At 200 μg/mL, cell viability dropped to 0% after exposure to the two CDs with the highest charge densities, while CDs with lowest charge density did not impact cell viability at all.

Overall, many components of surface chemistry influence CD toxicity making it difficult to predict safety from a single characteristic. Inherent toxicity of the ligands used for functionalization and how the physiochemical characteristics impact CD affinity for cells both play a role in final CD toxicity. However, as with chemical composition, there are certainly exceptions and measures to be taken to reduce toxicity, such as functionalization with multiple ligands[46] and incorporating ligands that target specific cells.[45, 47] The specific physiochemical characteristics that impact PCD toxicity need to be further investigated to confidently determine what properties are directly correlated with toxicity.

Light irradiation

For the most part, CD toxicity has been explored in relation to different CD compositions and surface functionalization. However, another consideration for toxicity is how CDs transform over time, whether in biological settings during applications or in the environment because of the increased production, use, and disposal of CDs. This is important because many of the proposed CD applications involve light (bioimaging, light-emitting diodes, photovoltaic cells, etc),[11] and sun exposure is relevant in environmental conditions.

CDs have been shown to produce reactive oxygen species (ROS) upon light irradiation, a known cause of CD-induced toxicity.[40] On occasion, this property is harnessed for applications where light activates antibacterial and antifungal properties in CDs.[50-51] In these cases, neither light nor CD exposure alone produce the same antibacterial and antifungal effects as the combination. Bagheri et. al. observed a positive correlation between toxicity and the length of time yeast cells were exposed to PCDs under light, where the concentration of intracellular ROS increased with increasing irradiation time.[51]

In addition to light-induced ROS generation by CDs, by-products of CD degradation are also a potential toxicity concern.[52-54] Both PCDs and GQDs rapidly transform under natural sunlight, producing lower molecular weight compounds. Decomposition and photobleaching is likely driven by CD-generated ROS from light exposure, resulting in self-degradation. The rate of decomposition can vary greatly based on the structural features present within CDs as a result of different synthetic methods and precursors.[53-54]

Overall, light-induced toxicity of CDs results from the combination of CD-generated ROS and toxic byproducts formed during photodegradation. Based on current research, ROS generation and potentially toxic byproduct formation is a possibility regardless of CD classification or composition. This further emphasizes the need for thorough toxicity studies on CDs not only in pristine conditions, but how they may transform following inevitable environmental exposure. It is critical to thoroughly examine the toxicity of this new class of nanoparticle before employing wildly and risking damage to the environment.

Conclusion and Outlook

In summary, CDs are emerging fluorescent nanomaterials that possess characteristics in common with both fluorescent dyes and semiconductor QDs. As CD studies have advanced, the field is revealing more detail about CD structure and the origin of their photoluminescence. Since CDs have various structures and several proposed photoluminescence mechanisms, this work addresses basic concepts in CDs, starting with categorization of different types of CDs. With a primary focus on PCDs, much progress has been made to characterize relevant fluorescence mechanisms. Contributions from both the carbon core and surface states have been discussed widely as the fluorescence mechanisms for CDs, though they are more likely relevant for other classes of CDs. More relevant mechanisms to understand PCD optical properties include: clusteroluminescence, crosslink-enhanced emission, and non-traditional intrinsic luminescence. All three of these proposed mechanisms for PCDs point to the critical role of clustering of non-conjugated subfluorophore units that are rich in heteroatoms. Further exploration of the exact structure of the clusters of those subfluorophores are needed to understand the related electronic transitions. In terms of toxicity, CDs are generally found to be benign at low concentrations; however, it is important to keep in mind that the toxicity profile for a new CD made with different methods or precursors should still be assessed. With the diverse structures and interesting properties, the applications of CDs in the future will extend to many areas such as photocatalysis, green chemistry, and solar cells, in addition to traditional bioimaging.

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