Minireview

Carbon "quantum" dots for bioapplications

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Impact statement

Photoactive nanomaterials have generated much excitement in a number of research fields in biology and medicine. Carbon dots (CDots) as emerging photoactive nanomaterials that are high performance yet nontoxic have attracted considerable recent interest, with successful explorations for a variety of potential biological and medical applications. This mini-review highlights the unique and advantageous properties of CDots as relevant to the already demonstrated excellent promises for their serving as bioimaging probes and their potent light-activated antimicrobial function. The purpose is to simulate the interest of the experimental biology and medicine community to pursue more valuable and rewarding applications of these nanomaterials for broader and more significant impacts.

Abstract

Carbon "quantum" dots or carbon dots (CDots) exploit and enhance the intrinsic photoexcited state properties and processes of small carbon nanoparticles via effective nanoparticle surface passivation by chemical functionalization with organic species. The optical properties and photoinduced redox characteristics of CDots are competitive to those of established conventional semiconductor quantum dots and also fullerenes and other carbon nanomaterials. Highlighted here are major advances in the exploration of CDots for their serving as high-performance yet nontoxic fluorescence probes for one- and multi-photon bioimaging *in vitro* and *in vivo*, and for their uniquely potent antimicrobial function to inactivate effectively and efficiently some of the toughest bacterial pathogens and viruses under visible/natural or ambient light conditions. Opportunities and challenges in the further development of the CDots platform and related technologies are discussed.

Keywords: Carbon dots, quantum dots, photoexcited states, optical bioimaging, antimicrobial

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Introduction

Bioimaging that takes advantage of the highly sensitive nature of fluorescence has attracted increasing attention, with major advances. The advances have been enabled at least in part by the development of high-performance fluorescence probes derived from nanomaterials. In fact, generally, the rationale for the use of nanomaterials-derived fluorescence agents over established traditional dyes is now widely accepted in the literature.[1–4] Among the most popular has been the use of conventional semiconductor quantum dots (QDs) that are surface decorated with aqueous compatible moieties as fluorescence probes, such as the famous and now commercially available CdSe/ZnS core-shell nanostructures capped by selected organic molecules.[3,4] Similarly, bright and colorful fluorescence emissions have been found in other nanomaterials containing no conventional semiconductors, thus no toxic heavy metals and associated hazards and limitations. One of such fluorescence nanomaterials is carbon "quantum" dots or carbon dots (CDots, Figure 1).[5–7] Since the original report in 2006,[5] CDots and their derived or configuration-wise comparable materials/entities have emerged to represent a rapidly advancing and expanding research field.[7–12]

Similarly, photoactive nanomaterials for their advantageous characteristics have also been explored to address the growing threat of more infectious and deadly microorganisms, including multidrug-resistant (MDR) pathogens, since the same photoexcited states responsible for the superior optical properties are shared by other processes and activities, such as photodynamic effects.[13] Conventional semiconductor nanoparticles including especially colloidal TiO_2 and various QDs have been particularly popular for their photoinduced antimicrobial functions.[14–16] More recently, CDots have been

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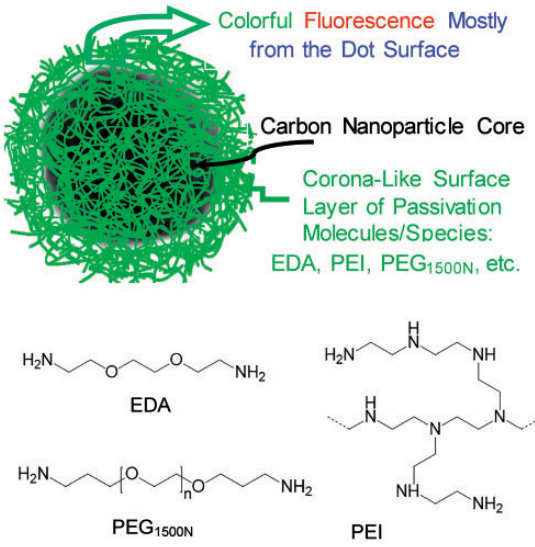

Figure 1. Upper: A cartoon illustration on the structure of typical CDots, with a solid small carbon nanoparticle core functionalized by organic species that form a surface layer similar to a soft corona. Lower: Chemical structures of the selected molecules for the carbon nanoparticle surface functionalization. (A color version of this figure is available in the online journal.)

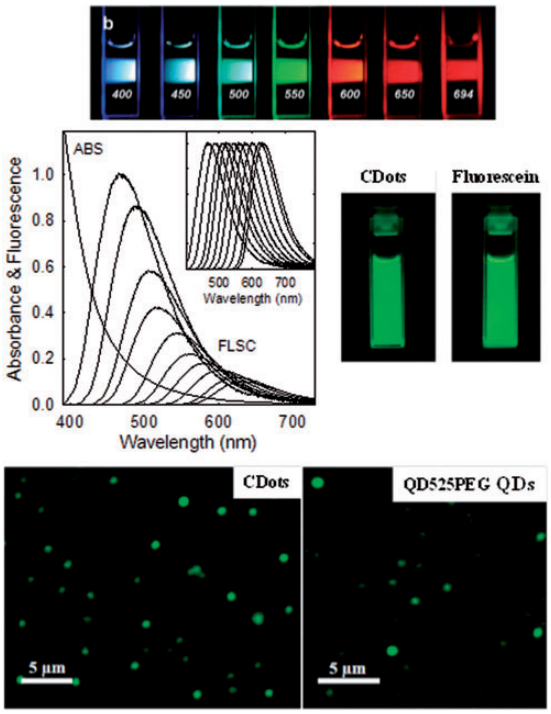

Figure 2. Upper: Aqueous solution of PEG_{1500N}-CDots excited at the indicated wavelengths and photographed directly.[5] Middle: (Left) Absorption (ABS) and fluorescence (FLSC) spectra of EDA-CDots in aqueous solution excited at (in the order of progressively lower peak intensity) 400 nm to 580 nm in 20 nm increments, with the corresponding normalized spectra shown in the inset; (Right) Monochromated light excited PEG_{1500N}-CDots and fluorescein solutions photographed through a 530 nm cutoff filter.[20] Lower: Fluorescence microscopy images (458 nm excitation) of PEG_{1500N}-CDots and Invitrogen (QD525PEG) CdSe/ZnS QDs.[22] (A color version of this figure is available in the online journal.)

demonstrated for their highly potent light-activated antimicrobial properties.[17,18]

CDots are generally defined as small carbon nanoparticles (CNPs) with effective surface passivation (Figure 1),[5,7] where the small refers to typical sizes of sub-10 nm. Conceptually, the intrinsic optical properties and photoexcited state characteristics of the small CNPs are realized and enhanced in CDots due to the effective passivation of the abundant surface defects, which is typically achieved via the surface chemical functionalization with organic molecules or biological species. In fact, small CNPs represent the zero-dimensional member in the family of nanoscale carbon allotropes, with their structural and edge defects also found in other members including carbon nanotubes and graphene nanosheets.[7,19,20] The same effective passivation of the defects in nanotubes and nanosheets results in similarly bright and colorful fluorescence emissions.[19–21] Thus, the discussion on the photoexcited state properties and processes of CDots and the associated functions is also applicable to the nanotubes and nanosheets.

CDots *versus* "Nano-carbon/organic hybrids"

CDots (Figure 1) were found originally for their bright and colorful fluorescence emissions (Figure 2).[5,6] Since then, the fluorescence brightness across the visible spectral region has been used as a convenient "qualifier/metric" in the preparation of dot samples.

The original synthesis of CDots was based on the surface functionalization of small CNPs with organic molecules or polymers in established chemical reactions, where the CNPs were harvested from carbon soot samples containing nanoscale particles.[5] Such a deliberate chemical functionalization approach with existing CNPs as precursors has yielded some of the best-performing CDots in terms of their fluorescence brightness or quantum yields (Φ_F).[22–24] For example, the oligomeric PEG diamine of molecular weight ~1500 (PEG_{1500N}) was used for CDots of multicolor fluorescence emissions, particularly bright in the green.[22] The as-prepared sample was found as a mixture of PEG_{1500N}-CDots with different levels of PEG_{1500N} functionalization on the dot surface and correspondingly different Φ_F values. The mixture could be fractionated on an aqueous gel column, with the most fluorescent fraction exhibiting a Φ_F value close to 60%,[22] competitive in performance to that of the commercially available semiconductor CdSe/ZnS QDs both in solution and at the individual dot level for the same green spectral region (Figure 2). Thus, PEG_{1500N}-CDots have served as a benchmark in the exploration for a number of potential applications of CDots.

PEG_{1500N} is not a small molecule, so in PEG_{1500N}-CDots, the surface of each dot is covered by a relatively thick organic layer, similar to a soft corona. To reduce the amount of surface organic moieties and thus overall dot sizes, small diamine molecules like 2,2′-(ethylenedioxy)bis (ethylamine) (EDA, 148 in molecular weight) were used successfully for the functionalization of small CNPs.[24] The resulting EDA-CDots are of similarly high optical performance, also considered as a benchmark. The EDA-CDots are overall smaller, with the average size comparable with that of green fluorescent protein,[24] amenable to various

applications including the use as ultra-small fluorescence probes for cell imaging.[25]

The chemical functionalization synthesis of CDots from pre-existing CNPs is hardly difficult or complex, but unfortunately not used as much as it should have been in the relevant research field. Instead, overwhelming majority of the reported syntheses were using "one-pot" thermal carbonization of organic precursors,[7–12] yielding samples that have been given different names from "carbon dots" and "carbon quantum dots" to "graphene quantum dots" or sometimes "polymer dots",[26,27] despite the fact that the same processing method and conditions for a fix set of precursors could only produce the same samples. Nevertheless, one way or another, the relevant reports have associated the samples from the carbonization synthesis with the originally reported CDots, which might be due mostly to the observation of similarly bright and colorful fluorescence emissions in the carbonization produced samples.[8–12,26] However, optical spectroscopic similarities between the differently synthesized materials are hardly sufficient conditions for equivalencies in chemical compositions and/or nanoscale structures between the materials. In fact, there have been lingering questions, which have largely been avoided or ignored in the relevant publications, on the actual structures of the "dots" in the carbonization produced samples and their relationship to the simple structure of the classically defined CDots (Figure 1), and also on why the intrinsically random and chaotic thermal carbonization conditions would result in the presumed well-ordered dot structures. More recent investigations and results have prompted increasing concerns on major or dominating spectroscopic contributions by molecular dyes or chromophores in the samples from thermal carbonization of organic precursors.[27–32] Especially alarming was the claimed success in the synthesis of "red/near-IR carbon dots" by using a handful of specific organic molecules as precursors, including citric acid mixtures with formamide or urea, under rather mild thermal carbonization conditions,[33] despite the obvious warning signs for the claims. Simply, how could the intrinsically indiscriminative thermal carbonization processing become so selective or sensitive to the choice of specific organic precursors. That should be a major troubling signal to those making the claims, namely that it was thermally induced chemical reactions instead of carbonization at work. Indeed, recent experimental evidence confirmed unambiguously that the red/near-IR absorptions and fluorescence emissions of the samples derived from citric acid mixtures with formamide or urea must be due to molecular dyes or chromophores, not associated with any nanoscale carbon entities in those samples, let alone any "carbon dots."[34,35]

The recent experimental evidence has also confirmed the suspicion by many researchers that there are major structural differences between the carbonization synthesized samples and the classically defined and synthesized CDots.[32,34] On the former, the samples prepared under the carbonization processing conditions in overwhelming majority of the studies reported in the literature must be more like nano-carbon/organic composites or "hybrids" (Figure 3), which are dominated by organic species in mixtures with only a small fraction of nanoscale carbon entities entangled or crosslinked in the organic matrix. As also illustrated in Figure 3,[35] at the very local level in ultra-small domains, the structural configuration in a carbonization synthesized sample might be analogous to the structure of the classically defined CDots, and the limited comparability in the nano-carbon/organic configuration might be responsible for the optical spectroscopic similarities discussed above. The spectroscopic similarities may serve to justify the designation of some carbonization synthesized samples as "carbon dots" for bioimaging, even though these same samples may not have some of the other photoinduced functions of the classically defined and synthesized CDots.[32] Moreover, cautions must be exercised in the use of the carbonization synthesized samples for bioimaging and/or in the interpretation of the results from bioimaging or the like, so as to be sure that the observed absorptions and fluorescence emissions are not significantly contaminated or even dominated by organic molecular dyes or

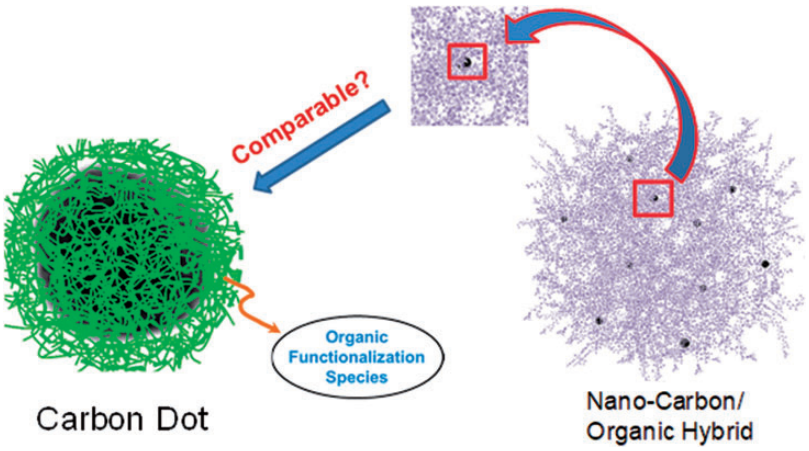

Figure 3. Cartoon illustrations on (1) the structure of "nano-carbon/organic hybrid" sample obtained from the carbonization of organic precursors under correct processing conditions, composed of mostly organic species (the crosslinked precursors and their thermal reaction products) and a small amount of nanoscale carbon materials, and (2) the possible comparability between the structure of CDots and the highlighted ultra-small domain in the hybrid sample. (A color version of this figure is available in the online journal.)

chromophores produced in the indiscriminative thermal carbonization processing.

Optical bioimaging

CDots with their bright visible fluorescence emissions have been used as probes for fluorescence imaging *in vitro* and *in vivo*, yielding promising results.[7–12] For the dot samples synthesized by using pre-existing small CNPs for surface chemical functionalization, thus adhering closely to the definition of CDots, their derived fluorescence probes and associated imaging results are specific to the optical properties of CDots, completely free from any contributions or contaminations of molecular dyes or chromophores. This is obviously not the case with the fluorescent samples obtained from the popular carbonization processing of organic precursors, in which the presence or dominance of molecular dyes or chromophores represents such a serious issue that may conceptually and fundamentally defeat the purpose of nanomaterials for bioimaging. Thus, the representative examples highlighted here focus only on the fluorescence bioimaging with CDots from the chemical functionalization synthesis, because if some of the carbonization synthesized samples are indeed structurally and property-wise comparable to the CDots highlighted, their imaging results and the associated conclusions should be the same anyway.

Cell labeling/imaging

CDots are nontoxic to cells at concentration levels much higher than those commonly used in fluorescence labeling and imaging.[36–38] Since the original investigation suggesting that CDots could be readily taken up by cells to reside primarily in the cytoplasm, with only minor penetration into the cell nucleus,[5] many subsequent studies have demonstrated similar cell internalization of CDots. Unlike conventional semiconductor QDs, whose fluorescence colors change with dot sizes, the fluorescence emissions of CDots are associated with the passivated surface defects of CNPs, no meaningful dot size dependence of the emission color. Thus, ultra-small CDots of sizes comparable with or smaller than fluorescent proteins may be developed and used for fluorescence cell imaging with minimal interference to the cellular functions.[24,25]

CDots have extremely large two-photon absorption cross-sections in the near-IR, more than 40,000 Goeppert-Mayer units ($1\ GM = 10^{-50}\,cm^4\ s/photon$).[39] The two-photon excitation of CDots in the near-IR results in bright visible fluorescence emissions, making them among the best two-photon fluorescence probes.[39–41] In fact, the same CDots could be used as fluorescence probes for cell imaging by using either normal (one-photon) excitation in the visible or two-photon excitation in the near-IR, as confirmed experimentally on optical microscope of both capabilities.

It has also been demonstrated that CDots can serve as cell imaging probes in super-resolution microscopy based on stimulated emission depletion (STED), achieving resolution down to ~70 nm (Figure 4).[42]

CDots have been used for fluorescence labeling of live cells.[41] For stem cells, the fluorescence label may be passed on to the next generations. A major advantage of CDots for the cell labeling and bioimaging/labeling in general is the excellent versatility and flexibility with the dot surface functionalities. For example, the often-used organic functionalization molecules for the more effective surface passivation of small CNPs to achieve high fluorescence performances for the resulting CDots, such as diamines or polyimine oligomers (Figure 1), decorate the dot surface with amino moieties. These surface functional groups can readily be conjugated with molecular or biological species for various purposes from cellular uptakes, including the penetration into the cell nucleus,[43] to specific targeting *in vitro* and *in vivo*. The imaging/labeling applications go beyond mammalian cells to include also plant cells, bacteria, and fungi.

Fluorescence imaging *in vivo*

CDots for their nontoxic nature while high in fluorescence performance are particularly attractive as optical probes

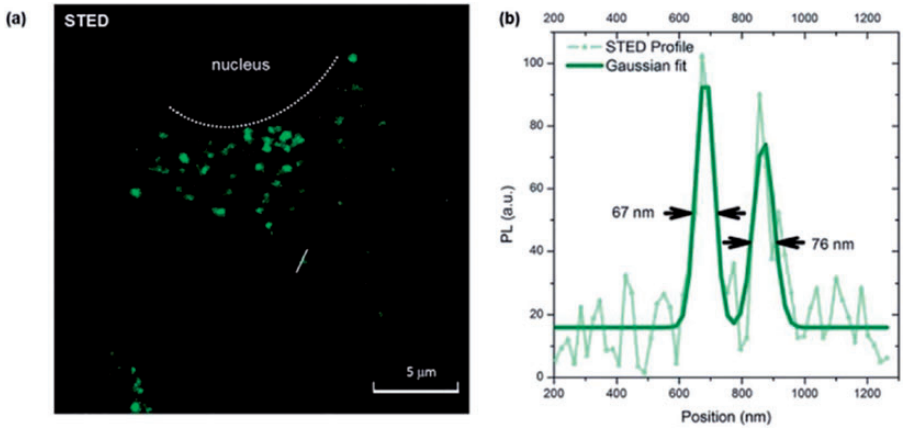

Figure 4. Results from the live cell imaging with PEG_{1500N}-CDots by STED microscopy. (a) STED imaging of the CDots in MCF-7 cells incubated with the CDots for 48 h, with the white dotted line delineating the nuclear region. (b) The line profile (light-colored line with triangles) and the Gaussian fit (dark line) of the STED signals corresponding to the images marked with the white line in (a). A FWHM resolution of 67 and 76 nm was obtained.[42] (A color version of this figure is available in the online journal.)

in vivo.[7–12] As demonstrated experimentally, the CDots of sizes around 5 nm in diameter have a residence time of less than 6 h in mice following intravenous injection, predominantly via the renal excretion.[36,44] The observed fluorescence imaging performances of the CDots in mice are competitive to those of the commercially available CdSe/ZnS QDs,[44,45] and for the latter, the hazard associated with the toxic heavy metal cadmium has generally been considered prohibitive for any *in vivo* uses.[46] Among the investigations on CDots for fluorescence imaging *in vivo*, the comprehensive study by Huang *et al*.[47] about a decade ago remains highly relevant as a benchmark.

In that study,[47] the brightly green fluorescent PEG_{1500N}-CDots were conjugated with the fluorescence dye ZW800 for its strong emissions in the red/near-IR, and the resulting probes were used for imaging *in vivo* and *ex vivo* (Figure 5). The ZW800-conjugated CDots were efficiently and rapidly excreted from the body after injection in different routes. Post intravenous injection, for example, there were some probes found in liver, spleen, and lungs within an hour. Very bright fluorescence was observed in kidneys, and the urine excretion was confirmed. All injection pathways led to meaningful tumor uptakes.[47] For FRET probes to extend the emission color to longer wavelengths, the CDots were conjugated with the fluorescence dye Ce6 to allow blue excitation (430 nm) and red fluorescence emissions (668 nm) via FRET.[48] After intravenous injection, the accumulation of the probes at the tumor site was detected, and the laser excitation of the probes in the mice could significantly suppress the tumor growth.[48]

CNPs are intrinsically more absorptive in the blue/near-UV than in the red/near-IR, with the observed absorption spectrum showing the progressively decreasing absorptivities toward longer wavelengths (Figure 2). The surface chemical functionalization or more generally modification of small CNPs for CDots does not alter the intrinsic absorption profile in any meaningful ways. Consequently, CDots without any help of molecular dyes or chromophores are weaker absorbers and correspondingly weaker emitters in the red/near-IR,[49] the spectral region favorable to bioimaging for higher tissue transparency. The extremely high two-photon cross-sections of CDots in the red/near-IR provide a solution for the desired fluorescence imaging to address the limitation at the absorption side by two-photon excitation, but not at the emission side. As discussed in the previous section, the carbonization produced "red/near-IR carbon dots" claimed in some literature reports were simply crosslinked organic materials structurally incorporated with molecular dyes or chromophores that were generated in chemical reactions under the thermal processing conditions intended for carbonization.[34,35] In fact, instead of the uncontrolled random introduction of molecular dyes or chromophores, the deliberate conjugation of the classical CDots with specifically selected red/near-IR organic dyes represents a viable strategy for bioimaging needs, as demonstrated by Huang *et al*.[47] Even with the thermal carbonization approach, it should be more targeted and controllable to include explicitly some specifically selected organic dyes in the precursor mixtures to be processed, at least for the obvious advantage of being able to design the resulting fluorescence probes to cover the desired spectral region.[50,51] The same by-design principle should be applicable to the near-IR II window as well, namely to choose purposely the dyes or chromophores to be incorporated in

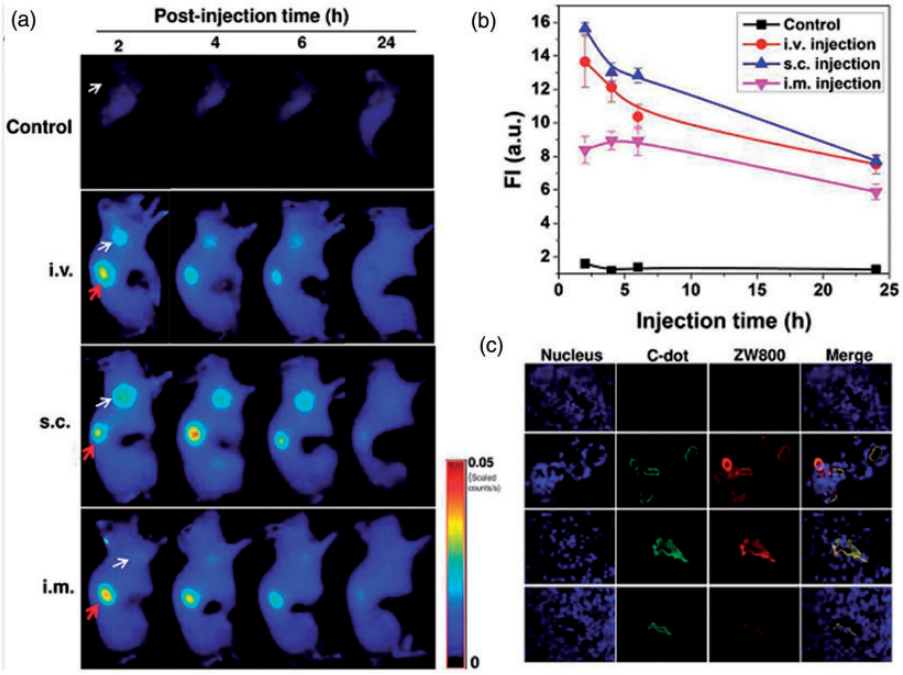

Figure 5. The tumor uptake of the PEG_{1500N}-CDots – ZW800 conjugates after different routes of injection. (a) NIR fluorescence images of SCC-7 tumor-bearing mice acquired at 2, 4, 6, and 24 h post-injection: control (without injection), i.v. injection; s.c. injection; i.m. injection (white arrow indicates tumor; red arrow indicates kidney). (b) Tumor ROI analysis. Fluorescence signal unit: $\times 10^8$ photons/cm^2/s. (c) *Ex vivo* fluorescence images derived from the emission of the CDots – ZW800 conjugates were acquired to confirm tumor uptake of the conjugates.[47] (A color version of this figure is available in the online journal.)

carbonization produced samples, instead of having whatever dye-like species in colored precursors (watermelon,[52] for example) randomly dictate the covered wavelengths.

Antimicrobial activities

Semiconductor nanoparticles are known for their photoinduced antimicrobial activities. Among the most popular are colloidal TiO_2, requiring UV light excitation, and conventional semiconductor QDs.[14–16] CDots resemble nanoscale semiconductors in terms of possessing the same or comparable photoexcited state properties and redox characteristics, exhibiting potent photocatalytic activities.[53] Similarly, CDots have been explored with major success for their visible/natural light-activated antimicrobial function.[17,18,54–57]

As found in the initial study by Meziani *et al.*,[17] EDA-CDots exposed to ambient light in a biosafety cabinet could inactivate *E. coli* cells. The inactivation was more effective with the use of visible light from a commercial LED lamp in a light box, killing 4 logs of *E. coli* cells in 30 min. In subsequent investigations, the photoinduced antimicrobial activities of CDots were correlated with their photoexcited state properties, which for CDots are best measured by the observed fluorescence quantum yields (Φ_F). Phenomenologically and mechanistically, the difference in photophysical properties between small CNPs and their derived CDots is such that in the latter the effective nanoparticle surface passivation by organic functionalization "protects" the photoexcited states from immediate deactivation, thus enabling the subsequent excited state processes. These processes are reflected by the bright fluorescence emissions of CDots, with the observed Φ_F values considerably larger than those of the solvent suspended small CNPs.[58] In fact, the effectiveness in the nanoparticle surface passivation by organic functionalization can be correlated with the observed Φ_F values of the resulting CDots, namely different EDA-CDots samples have different Φ_F values, depending on how well the small CNPs are functionalized by EDA molecules in the samples. It was shown that the more fluorescent dot samples, thus larger observed Φ_F values, were more effective in the photoinduced antimicrobial activities under otherwise the same or comparable conditions.[54]

CDots coupled with visible/natural light exposure have shown great promise in the inactivation of multidrug-resistant (MDR) bacterial pathogens. In the study using several MDR *Enterococcus* strains as representative targets,[56] the samples containing 10^7–10^8 CFU/mL of the *Enterococcus* cells were treated with the CDots of oligomeric polyethylenimine (PEI, Figure 1) for surface functionalization, thus PEI-CDots, and the exposure to visible light from a commercially acquired household LED lamp. The results showed that the meaningful inactivation of more than 1 log could be achieved at PEI-CDots concentrations as low as 0.12 μM_{DOTS}, where M_{DOTS} denotes the molar concentration based on the number of dots. Under the same household LED light conditions, the complete eradication of the *Enterococcus* cells could be achieved at the dot concentration of 1.2 μM_{DOTS} (Figure 6).[56]

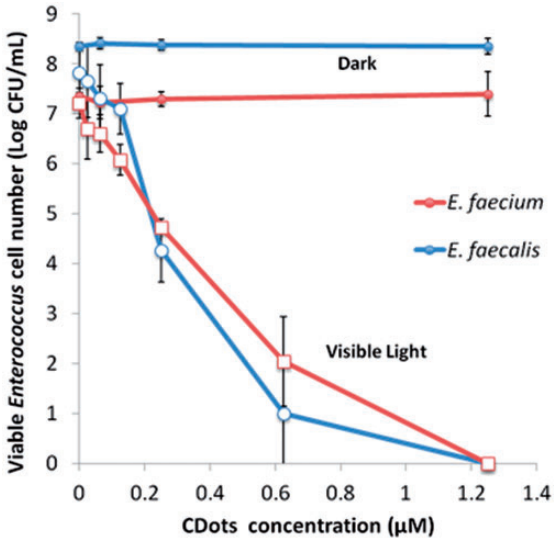

Figure 6. The logarithmic viable cell numbers in the samples upon the treatment with different concentrations of PEI-CDots under visible light for 1 h, along with the samples treated with the same CDots in dark.[56] (A color version of this figure is available in the online journal.)

Biologically, the inactivation of bacterial cells by light-activated CDots is associated with significant damages to the cells, typically accompanied by the lipid peroxidation, a process of multiple steps in which lipid carbon–carbon double bonds are attacked by free radicals and/or strong oxidants.[59] The lipid peroxidation produces lipid hydroperoxides (LOOH) and various aldehydes including especially malondialdehyde (MDA) that is commonly used for the quantification of the lipid peroxidation.[60,61] For the MDR *Enterococcus* cells inactivated by PEI-CDots with visible light, the MDA levels in the cells quantified by the thiobarbituric acid reactive substances assay (TBARS) were much higher than those in the controls, suggesting substantial lipid peroxidation.[57] The significant damages to the cell membrane by the light-activated CDots were confirmed more directly in the assessment with the live/dead bacterial viability kit containing two nucleic acid dyes to stain the live and dead cells for their significant differences in the membrane permeability.[57]

MDR bacterial pathogens are known for their resilience to antibacterial agents, including those based on photodynamic effects, such as traditional molecular photosensitizers or even established conventional semiconductor QDs, and consequently such pathogens are generally much more difficult to kill. Thus, the observed high potency of the light-activated CDots against the representative MDR pathogens is special, which may be attributed to the unique photoexcited state properties and redox characteristics of CDots.[53,57] There is growing evidence suggesting that CDots are hardly the fancier version of photosensitizers represented by known dye molecules, much more potent instead, and they are also superior to other photoactive nanomaterials in the light-activated antimicrobial function.

Mechanistically, upon the photoexcitation of CDots, there must be ultrafast charge transfers and separation for the formation of electrons and holes, which are trapped at

the various passivated surface defect sites of the core CNPs (Figure 7).[56,57] These separated redox pairs should be highly reactive, conceptually analogous to the charge separated species found in some conventional semiconductor QDs following photoexcitation, denoted as "light-activated redox species" (LARS),[16] except that the separated redox pairs in CDots are apparently more lethal in terms of their major contributions to the observed antimicrobial activities.[57]

In the same mechanistic framework, the radiative recombination of the redox pairs results in the emissive excited states, whose decays include the observed characteristic fluorescence of CDots and the generation of traditional reactive oxygen species (ROS), such as singlet molecular oxygen and radical ions. The generation of ROS by traditional molecular dye photosensitizers is considered as the photodynamic effect of the dye molecules, and the ROS are generally credited for the observed antimicrobial activities of the photosensitizers.[62] Similarly, for example, the presence of ROS in the MDR *Enterococcus* cells treated with PEI-CDots under visible light were confirmed and quantified by using dihydrorhodamine 123, a commonly used probe that could be oxidized by ROS to convert to brightly fluorescent rhodamine 123 for detection and quantification.[63,64] The results showed that the intracellular ROS levels thus determined in the treated cells were many times of those in the untreated controls.[57] However, the ROS produced in the emissive excited states of CDots contributed only the minor part of the observed antimicrobial activities, as found experimentally on the basis of the ROS scavenging effect.[57] In the experiments, the popular ROS scavenger L-histidine was used to "quench" the killing of the MDR *Enterococcus* cells in the treatment of PEI-CDots under visible light. As shown in Figure 8, the viable cell numbers decreased from the starting 2.3×10^8 CFU/mL to 2.4×10^4 CFU/mL (99.99% reduction) or to 1.3×10^7 CFU/mL (~94% reduction) in the absence or presence of the scavenger L-histidine, respectively, suggesting that the scavenger could provide some meaningful yet rather limited protection of the MDR *Enterococcus* cells from the action of the light-activated CDots. On the other hand, the decrease in the intracellular ROS level from without to with the scavenger under the same treatment conditions was much more substantial, by more than 50% (Figure 8). The results provide strong evidence for the notion that mechanistically the observed highly effective and efficient antibacterial outcomes must be due to a combination of two different kinds of reactive species produced in the photoexcited CDots: the separated redox pairs formed upon photoexcitation and the "classical" ROS produced in the emissive excited states, with the former, which could not be "quenched" by commonly used scavengers, contributing more substantially to the observed antimicrobial outcomes.[57]

The antimicrobial activities of the separated redox pairs are not unique to photoexcited CDots, but uniquely effective for those of CDots. Conceptually and to a significant extent mechanistically analogous reactive species LARS in photoexcited conventional semiconductor QDs were also credited for their contributions to the killing of bacterial cells,[16] but the contributions were not as major and effective as those found with photoexcited CDots. A logical conclusion is that light-activated CDots with the combined actions by the two kinds of highly reactive species are uniquely potent antimicrobial agents, where particularly valuable is the effective activation of CDots by visible light, including also natural and ambient indoor light conditions.[17,54–57]

The light-activated CDots have also been demonstrated for their effective inactivation of viruses.[18,65,66] For MS2

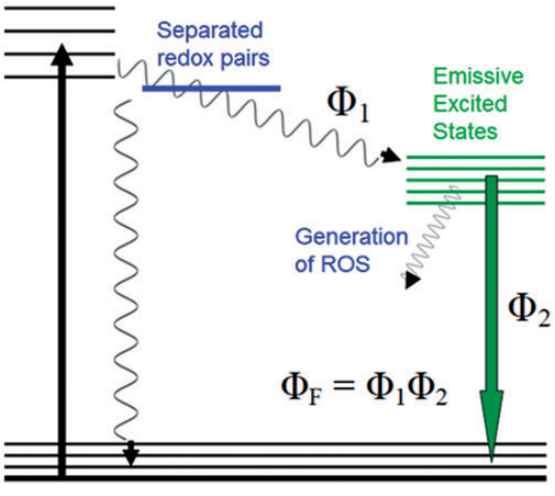

Figure 7. A state energy diagram on the photoexcited states and processes of CDots, highlighting the two sets of highly reactive species: the separated redox pairs from the initial charge transfer and separation; and the ROS generation as a part of the nonradiative deactivation of the emissive excited states. Φ_F denotes fluorescence quantum yields.[57] (A color version of this figure is available in the online journal.)

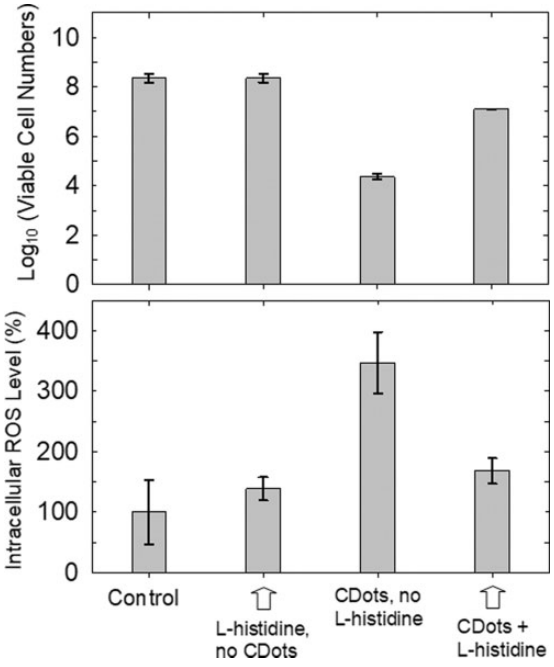

Figure 8. Effects of the common ROS scavenger L-histidine (30 mM) in treatments of the *Enterococcus* cells with 1.2 μM_{DOTS} PEI-CDots under visible light for 1 h: (upper) on the protection of the cells from inactivation in terms of viable cell reduction (CFU/mL) in $\log_{10}$ scale; and (lower) on the intracellular ROS generation.[57]

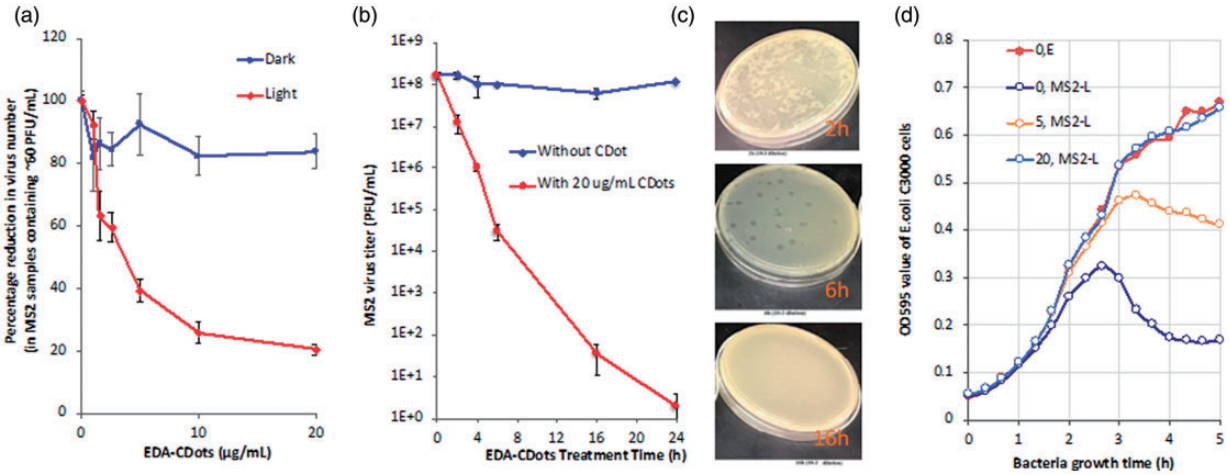

Figure 9. (a) Percentage reduction in virus titers when MS2 samples containing low virus titers (<100 PFU/mL) were treated with different concentrations of EDA-CDots under visible light for 1 h. (b) Reduction in virus titers when MS2 samples containing high virus titers (~10^8 PFU/mL) were treated with 20 μg/mL EDA-CDots for different visible light exposure times. (c) Images of the representative plates of MS2 plaque forming units on the lawn of *E. coli* C3000 after the MS2 samples were treated with 20 μg/mL EDA-CDots under visible light for 2, 6, and 16 h. (d) The growth curves of *E. coli* C3000 when they were infected with untreated and CDots-treated MS2 samples.[66] (A color version of this figure is available in the online journal.)

bacteriophage as a model, which is a commonly used surrogate for a number of small RNA viruses including Norovirus and more recently SARS-CoV-2, EDA-CDots with photoexcitation under common household visible light conditions were shown to be able to effectively inactivate MS2 under different conditions (Figure 9).[66]

Biologically, the light-activated CDots could damage the integrity of MS2 phage, capsid proteins, and viral genomic RNA. On the degradation of the genomic RNA specifically, the results from the gel assay showed no clear band of the total genomic RNA for all of the MS2 samples treated by EDA-CDots with visible light. The extent of degradation also increased with the increasing CDots concentrations used in the treatment with visible light.[66]

Summary and perspectives

CDots are simply surface functionalized small CNPs, which represent the nanoscale carbon allotrope at the zero-dimension. Because of the surface chemical functionalization, the core CNPs in CDots are protected for their photoexcited states not to subject to immediate deactivation, and as a result their intrinsically rich photoexcited state, properties and processes are revealed, realized, and/or enhanced for productive functions and/or uses. Among the most promising and useful are the bright and colorful fluorescence emissions and the uniquely potent photoinduced antimicrobial function of CDots, as highlighted above. The former enables CDots to serve as high-performance yet nontoxic fluorescence probes for one- and multi-photon bioimaging *in vitro* and *in vivo*, competitive to not only traditional molecular dyes but also conventional semiconductor QDs. The latter leverages the lethal actions of a unique collection of multiple highly reactive species in the photoexcited CDots, thus the ability to inactivate effectively and efficiently some of the toughest bacterial pathogens and viruses under visible and natural/ambient light conditions. Further rapid advances in the investigation of CDots for bioimaging and antimicrobial uses, including the associated mechanistic elucidation and technological development, can be envisaged. Other promising potential applications of CDots in biology and medicine include biosensing, drug delivery, photodynamic therapy, and other theranostics, on which readers are referred to relevant reviews.

On the relationship and differences between classically defined and synthesized CDots and the carbonization produced nano-carbon/organic hybrids, the latter if prepared correctly by using appropriate processing conditions to eliminate any significant contamination of molecular dyes or chromophores may be comparable with the former in some optical properties and related functions. Unfortunately, it is generally a very tough challenge to make a judgment on whether and/or how much the results of the carbonization synthesized samples are affected by the possible or likely presence of molecular dyes or chromophores that are produced in the thermal processing intended for carbonization. Extreme cautions must be exercised in the interpretation and extrapolation of those results and the making of related conclusions.

AUTHORS' CONTRIBUTIONS

All authors participated in the preparation of this mini-review. The final editing and proofread were done by LY and Y-PS, and the references were finalized by DY.

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