Applications of carbon dots on tumour theranostics

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Abstract
Carbon dots (CDs) have been extensively investigated as prime candidates for developing tumour theranostic platform due to their tunable fluorescence emission and excitation, good photostability, high water solubility and biocompatibility. Herein, we will update the recent research and latest development on the preparation, hydrophilicity, biotoxicity and optical property of CDs, as well as their applications in multimodal bioimaging and anticancer drug delivery. We will mainly highlight their applications in in vivo imaging and tumour-targeted theranostics with a special emphasis on large amino acid-mimicking carbon quantum dots-based tumour theranostics through a large neutral amino acid transporter 1 (LAT1)-mediated targeted strategy. Finally, we will also discuss the current status, key challenges and future directions of CDs in tumour theranostics. We expect that this review will summarize recent advances to inspire more researches on CDs for biomedical and clinical applications in the near future.

KEYWORDS
brain tumours, carbon dots, large neutral amino acid transporter 1, tumour theranostics

1 | INTRODUCTION

Cancer has become one of the most severe diseases affecting public health around the world due to the high incidence and mortality rate.[1] The current technologies for the diagnosis and therapy of tumours are usually limited by their inability to selectively target and direct drug to the cancer cells. Novel tumour-targeted strategies are urgently needed to overcome these biological barriers in cancer diagnosis and therapy.[2,3] Various different tumour-targeted strategies have been developed on the basis of enhanced permeability and retention (EPR) effect and receptor-mediated pathway.[4] Recent studies have demonstrated that each of these strategies has both advantages and disadvantages.[5,6] The clinical usefulness of drug delivery based on EPR effect is significantly limited due to the relatively low tumour accumulation, which leads to a reduced efficacy of anticancer drugs.[4] Receptor-based targeting of therapeutics relies on the specific receptors that are only over-expressed on the cell membrane of tumour cells rather than in both tumour and normal cells. Cancer cells of different genotypes and phenotypes may not be targeted by the same ligand.[7,8] Consequently, there is an urgent need to develop an effective strategy that enables selective treatment to tumours, regardless of their origin and location to meet the needs of emerging diagnostic methods and treatments.[5,6] One of the best approaches to achieve tumour-selective imaging and targeted drug
delivery is to leverage specific carrier transporters that are overexpressed in cancer cells, such as glucose transporters and large neutral amino acid transporter 1 (LAT1).\textsuperscript{[9–12]} Particularly, LAT1, which mainly mediates the transport of large neutral amino acids, has gained considerable importance in the area of anticancer research since it has been shown to be overexpressed in various tumours. More importantly, LAT1-based strategy exhibits a great potential in brain tumour treatment because it has also been found to be overexpressed in blood-brain barrier (BBB).\textsuperscript{[13]}

Recently, tumour theranostics with integrated tumour diagnostic and therapeutic functions has gained considerable importance and emerged as one of the most efficient strategies for cancer treatment.\textsuperscript{[14–17]} The diagnostic function within the system provides specific biological information of tumours, while the therapeutic function specializes in tumour-directed drug delivery.\textsuperscript{[18–20]} Newly emerging theraonostic agents have been explored based on multicomponent inorganic and organic nanomaterials.\textsuperscript{[21–25]} Recent researches on tumour theranostics with fewer components and better clinical efficacy have gained much research interest.\textsuperscript{[26]} In comparison to existing theraonostic agents, such as polyester micelles, lipidosome and metal-organic framework (MOF)-based nanomedicine, carbon dots (CDs) possess a number of advantages, including stable photoluminescence, biocompatibility, low cytotoxicity and facile functionalization.\textsuperscript{[27–29]}

CDs are a type of zero-dimensional carbon-based nanomaterials, exhibiting high crystallinity with the lattice spacing of 0.34 and 0.21 nm corresponding to the (002) and (100) lattice planes of graphite\textsuperscript{[30–32]} They can be divided into two classic types, one is carbon quantum dots (CQDs) with excitation-independent luminescence originating from the quantum confinement effect, the other one is CDs with excitation-dependent luminescence due to surface defects.\textsuperscript{[33–35]} In 2004, CDs were discovered for the first time during the preparation of single-walled carbon nanotubes.\textsuperscript{[36]} Since their first cell imaging application in 2011, CDs as a new type of carbon-based nanomaterials have attracted much attention in biomedical applications.\textsuperscript{[37]} They also have been recognized as effective tumour theranostic agents for both in vivo imaging and tumour therapy.\textsuperscript{[38–41]}

There have already been several reviews focusing on the diverse aspects of CDs, such as preparation, optical property, sensing and imaging capability, the purpose of this review is to give an update of the current research status of CDs on tumour theranostics, aiming at delivering critical perspectives to inspire future researches on CDs for biomedical applications.\textsuperscript{[42–48]} This review covers the latest advances in the preparation, hydrophilicity, biotoxicity and optical property of CDs, as well as the achievement from their original use as bioimaging probes to multifunctional tumour theranostic systems (Figure 1). Finally, the remaining challenges and future prospects of CD-based theranostics are also proposed.

## 2 | PREPARATION OF CDs

The development of synthetic route and subsequent functionalization plays a fundamental role in developing CDs towards biomedical applications. In particular, the characteristics of CDs are required to meet the demand of tumour theranostic applications, including their uniform structure, mass production and diverse functions. The investigation on the synthetic and functionalization strategies of CDs will also be very helpful to optimize the production of CDs.\textsuperscript{[49,50]} Based on carbon source for the preparation of CDs, the synthetic approaches of CDs can be generally divided into bottom-up and top-down strategies.\textsuperscript{[51]} The small aromatic molecules are commonly employed for a bottom-up strategy through the hydrothermal/solvothermal synthesis of CDs. Then, it undergoes pyrolysis and the formation of carbonized core with different degrees of carbonization.\textsuperscript{[52–54]} The hydrothermal/solvothermal methods have been widely developed due to the fast, easily scalable, low-cost and environment-friendly production. Nonetheless, they also often suffer from drawbacks that requiring the complicated purification process to obtain CDs with uniform structures, such as dialysis and column chromatography.\textsuperscript{[55]} The top-down approaches are accomplished via either chemical or physical techniques to cut down bulk carbon sources (eg, graphite rods, carbon fibres and fullerenes) into smaller units with nanoparticle size less than 10 nm.\textsuperscript{[56,57]} The typical example of these approaches includes the synthesis of graphene quantum dots (GQDs) using electrochemical treatment of graphite rods.\textsuperscript{[58,59]} In comparison to the top-down strategy, the bottom-up strategy can deliver CDs with a uniform size and structure for biological applications.

Several strategies have been already developed for modulating the structure of CDs in order to optimize their properties for biomedical applications, such as heteroatom doping and surface modification.\textsuperscript{[60–62]} The heteroatom doping approaches exhibit promising potentials in nanomedical applications for the low cost, scalability and simplicity.\textsuperscript{[63]} CDs doped with the nitrogen, boron, sulphur or fluorine atoms have been reported by a one-pot solvothermal/hydrothermal method. Nitrogen-doped cancer stem cell nucleus-penetrable red emissive CQDs (CSCNP-RCQDs) were synthesized via a one-pot solvothermal method, which could enter into the nuclei of cancer stem cells (Figure 2A–C). These nitrogen functional groups on the edge of CSCNP-RCQD are beneficial for their nucleus internalization. After loading the
doxorubicin (DOX), the CSCNP-RCQDs/DOX further showed a positive therapeutic effect by eliminating cancer stem cells.\textsuperscript{[64]} Furthermore, the properties of the CDs could also be optimized by multiatom doping. Nitrogen and sulphur codoped pH-responsive GQDs were synthesized by the electrolysis of graphite rods in sodium p-toluenesulfonate acetonitrile solution. Owing to the synergistic effect between the doped heteroatoms, the
as-obtained GQDs show a sharp fluorescence transition between green and blue at pH 6.8, which is a pH matching the acidic extracellular microenvironment in solid tumours.[65]

Although CDs with heteroatoms can effectively enhance their chemical activity for biomedical applications, the precise structure of the product is unclear due to the uncontrollability of the doping process, which is contrary to the original intention of biomedical product design.[60] Taking advantage of the versatile organic reagents involved, the surface modification strategy is found to be more effective for the controllable preparation of CDs with specific functional groups, because these functional groups can be selectively passivated, eliminated or converted by surface modification. Numerous biomedical applications of CDs rely on efficient modification of small molecules or polymers onto the surface of CDs. Polyethylene glycol (PEG) is widely accepted as one of the most commonly used polymers for the surface modification of CDs, which possesses high biocompatibility and resistance to nonspecific binding. For example, CDs functionalized by transferrin, 4-arm PEG and PEGI500N were synthesized and applied for cancer cells imaging.[66] In addition to the modification by polymers, a variety of CDs, which conjugate with targeted small molecules, have been prepared for bioimaging, phototherapy and drug delivery.[35] GQDs could conjugate folic acid (FA) by an EDC/NHS condensation reaction, which can specially bind to Hela cells for targeting tumours (Figure 2D).[67] FA-functionalized GQDs (GQDs-FA) exhibited high loading capacity for IR780 iodide (IR780) through strong π-π stacking interactions. It has been demonstrated that IR780/GQDs-FA with high photothermal conversion efficiency of 87.9% and enhanced tumour targeting ability were capable of killing cancer cells and eradicating tumours upon 808-nm laser irradiation.

3 CHARACTERISTICS OF CDS

Owing to the diverse structures of CDs synthesized from various precursors under different conditions, CDs usually exhibit specific properties, including high hydrophilicity, low toxicity, long-wavelength emission and two-photon (TP) fluorescence.[68–71]

Hydrophilicity is one of the most important properties of biomedical materials, which is mainly influenced by the choice of precursors and synthetic conditions.[72] Particularly, the hydrophilicity plays a vital role in determining the biocompatibility of the CDs. It has been demonstrated that CDs possess high hydrophilicity owing to the hydrophilic functional groups around their edge.[73,74] CDs with high hydrophilicity have been widely used to improve tumour theranostics efficacy. Previous reports indicated the clinical application of anticancer drugs has been usually limited by their poor hydrophilicity.[75] The CDs with high hydrophilicity can serve as efficient drug carriers to improve the therapy efficacy. For instance, IR780 is an effective theranostic agent for photothermal therapy (PTT). Nevertheless, the poor hydrophilicity of IR780 inevitably limits its biomedical applications. Improving the hydrophilicity of IR780 has been recognized as a useful method of conducting PTT for killing cancer cells. The GQDs-FA with high hydrophilicity were capable of serving as vehicles for improving the efficiency of drug delivery and offering therapeutic benefits. After loading onto GQDs-FA, the hydrophilicity of IR780 was remarkably improved by over 2400-fold.[67]

Low toxicity is another critical property of biomedical materials. It has been demonstrated that most of the CDs are usually low toxic both in vivo and in vitro according to a large amount of researches.[76–79] Our laboratory has synthesized several types of CDs, such as GQDs-FA, CSCNP-RCQDs, nitrogen-rich CQDs (NRCQDs), pH-responsive fluorescent GQDs (pRF-GQDs) and large amino acid-mimicking (LAAM) CQDs, which were found to be low toxic in both in vivo and in vitro biomedical applications.[41,64,65,67,80] Particularly, toxicity analysis was investigated by hematoxylin and eosin (H&E) staining examination, demonstrating no obvious damages after the injection of these CDs (Figure 3A,B). In addition, CDs with a relatively small size may facilitate their direct removal by the kidneys as a result of the low in vivo toxicity.[60] Recently, a novel CD-based nanoplatfrom for the radiotherapy of tumours was established, demonstrating that CDs were accumulated in the kidneys and could be cleared away by the body through urination.[61]

CDs exhibit characteristic optical absorptions in the wavelength from 230 to 320 nm, with a tail extending in the visible range owing to the π-π* transition of C = C and n-π* transition of C = O, respectively.[82–85] The absorption characteristics of CDs are mainly modulated by the surface functional groups, composition, shape and the size of π-domains.[75] CDs with long-wavelength absorption (>650 nm) are considered good candidates for tumour theranostics.[75,86] Compared to light irradiation with higher energy, irradiation by red and near-infrared (NIR) light has been demonstrated to minimize photodamage as well as penetrate deeper into the tissue.[87] A novel class of S, N-doped CDs were prepared from dimethyl-sulfoxide, urea and citric acid through a solvothermal approach, showing intense absorption bands in the red to NIR region (centered at 720 nm). The CDs were accumulated in tumour of mice and exhibited strong photoacoustic (PA) and NIR fluorescence signals after intravenous injection. They could act as PTT agents for tumour therapy in
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![Image](WU_et_al_Figure_3.png)

**FIGURE 3** (A) Histological evaluation of tissues from the mice treated with saline and IR780/GQDs-FA. Each organ was sliced for H&E staining.[67] Copyright 2017, American Chemical Society. (B) Toxicity of pRF-GQDs, representative images of indicated tissues with H&E staining.[65] Copyright 2017, Royal Society of Chemistry. (C) In vivo imaging of supine nude mice with intravenous injection of carbonized polymer dot (CPDs) at different time points. (I: thoracic region; II: area of liver; III: area of small intestine; IV: area of large intestine; V: bladder region).[91] (D) Real-time ex vivo imaging of nude mice with intravenous injection of CPDs at different time points.[91] Copyright 2020, John Wiley and Sons. (E) TP fluorescent imaging of a fresh live rat liver tissue slice stained with NRCQDs taken at different penetration depths (0-440 μm) with 800 nm femtosecond pulse laser excitation.[41] Copyright 2018, American Chemical Society

live mice due to a high photothermal conversion efficiency of 59.2% under 655 nm laser irradiation was achieved.[88]

CDs demonstrate characteristic luminescent emission with excitation light. Owing to the quantum confinement effect, increasing the particle size of CDs generally leads to the red-shifted emission bands from blue to NIR fluorescence.[54,75,89] Multicoloured luminescent CDs have been prepared by various methods, ranging from deep-ultraviolet to NIR emission.[90] Multicolour bandgap fluorescent CQDs from blue to red with quantum yield (QY) as high as 75% were prepared using a one-step solvothermal approach.[89] As for biomedical applications, CDs with red and NIR emission are good candidates for bioimaging owing to their advantages of penetrating deeper into the tissue.[75,86,87] Deep red-emissive carbonized polymer dots with full width at half maximum of 20 nm were recently reported for in vivo imaging (Figure 3C, D).[91]

More interestingly, some CDs show strongly TP luminescent upon excitation, which are highly desirable for bioimaging in deep tissues owing to their enhanced penetration and reduced photodamage.[92] TP fluorescent CDs are urgently needed for bioimaging with a higher spatiotemporal resolution. On the basis of a donor-π-acceptor strategy, NRCQDs were prepared by a convenient and large-scale method, and then utilized for TP bioimaging (Figure 3E). The TP absorption cross-section of NRCQDs reached 61 200 Göppert-Mayer units and the QY was up to 63%, which far surpassed that of the existing TP carbon probes. The NRCQDs is found capable of imaging live liver tissues as well as Hela cells at depths of up to 440 μm.[41]

**4 | TUMOUR DIAGNOSIS OF CDS**

Tumour microenvironment (TME) is the cellular environment surrounding malignant cells in tumours. Compared to normal tissues, some characteristic features of the TME were reviewed, such as the overexpressed receptors, abnormal physiological parameters (e.g., mild acidity and hypoxia), high glutathione and H2O2.[93] Increasing evidences indicate that TME has close relationship to tumour development, such as releasing biological factors to promote tumour angiogenesis, growth, progression and metastasis.[94] However, the TME also shows positive effects in the field of tumour diagnosis. These characteristics can offer good chances to design stimulus-responsive diagnostic and therapeutic platforms to achieve efficient tumour treatment.[95] For instance, pH-responsive CDs were prepared on the basis of the significance of intratumoural acidosis. Our group reported novel pRF-GQDs with a sharp fluorescence transition at pH 6.8. Apart from fluorescence, the upconversion photoluminescence (UCPL) property of pRF-GQDs was investigated as well. It was confirmed that solid tumours of different origins can be detected by combining UCPL and fluorescence switch at an early developmental stage. Therefore, pRF-GQDs can serve as universal probe for cancer diagnosis.[65] In subsequent study, red fluorescent CQDs (R-CQDs) were further developed, and a block copolymer (MeO-PEG-PDPA) was modified on the surface of R-CQDs, which then obtained pRF-R-CQDs. The products got a response at a pH of around 6.8. In normal tissue (pH > 6.8), the amine groups of the pRF-R-CQDs deprotonation led to fluorescence...
quenching. In contrast, protonation of the amine groups would lead to a dramatic increase in fluorescence emission. The in vivo imaging revealed that the pRF-R-CQDs can distinguish tumours from normal tissues, indicating the great potential in TME diagnosis (Figure 4).\cite{96}

In addition to the diagnosis of TME, the early diagnosis of solid tumours remains an important issue. In recent years, the fluorescence imaging effect of CDs is also estimated on basis of their internalization by cancer cells.\cite{75} CDs with long-wavelength emission, especially with NIR emission, are critically needed for developing fluorescence imaging probes, because they can cause little photodamage to the cells and have low autofluorescence interference to biological samples.\cite{97-100} Water-soluble red fluorescent GQDs (RF-GQDs) were synthesized with low cytotoxicity via exfoliation of graphite in K$_2$S$_2$O$_8$ solution. The as-obtained products can simultaneously label the cell membrane and cytoplasm of Hela cells, demonstrating such CQDs could be successfully applied to cell labelling.\cite{86} Particularly, some CDs are also able to penetrate the nucleus.\cite{58,80} For instance, CSCNP-RCQDs with long-wavelength emission could effectively penetrate the nucleus of cancer stem cells, which were used to image the nucleus.\cite{64} Gradually, as the attention paid to diagnosis grows, CDs have been applied to in vivo diagnosis. Fluorescence imaging technique is in widespread use for various clinical studies, offering a probability of surveying the pathogenic organization and tracing the distribution and metabolism of drugs in therapy. Although high sensitivity can be achieved, the spatial resolution is still poor due to a limited penetration depth.\cite{101} As a hybrid imaging modality, PA imaging combines the merits of optical imaging and ultrasound imaging, emerging as a new noninvasive bioimaging technique with deep-tissue penetration and high spatial resolution.\cite{102} Nitrogen-doped CDs (N-CDs) were recently developed by using citric acid and nitric acid as carbon and nitrogen sources, respectively. The as-prepared N-CDs with strong NIR absorption exhibited corresponding PA signals for in vivo imaging. Whole-body PA imaging was carried out to confirm the physical distribution and clearance of N-CDs. The PA signal in the bladder increased after 100 minutes postinjection of N-CDs, and gradually strengthened up to 450 minutes. These results demonstrated the efficient removal of N-CDs from the body by renal clearance after subcutaneous injection.\cite{103} Our laboratory recently has developed the LAAM TC-CQDs by hydrothermally treating 1,4,5,8-tetraminoanthraquinone (TAAQ) and citric acid in H$_2$O solution.\cite{80} The as-obtained LAAM TC-CQDs, which were found to exhibit characteristic NIR fluorescence (the emission peak at 700 nm), were applied to in vivo fluorescence tumour imaging and PA tumour imaging by intravenous injection (Figure 5).

Because fluorescence imaging and PA imaging have the advantages of safety, cost- and time-effectiveness, they are extensively applied to in vivo imaging of tumour load in small animals. Unfortunately, they are unpractical for deeper tissues or larger living objectives. These limitations can be conquered by using nuclear imaging technology that can improve tissue penetration of imaging, and enable a clearer visualization of the tumour and its borders. As expected, they can provide quantitative information for a biological event in vivo with ultrahigh sensitivity.\cite{104} Positron emission tomography and single-photon emission computed tomography (SPECT) are considered important parts of nuclear imaging technology, which have been devoted to fundamental biological research and clinical diagnostics. Currently, the nuclear-targeted imaging by CDs with target-specific agents represents one of the most significant approaches. GQDs functionalized by PEG and FA were synthesized and labelled with $^{131}$I for biological behaviour evaluation, showing low cytotoxicity and high biocompatibility.\cite{105} Tumour-bearing mice were intravenously injected via the tail, and by using SPECT imaging, the uptake of $^{131}$I-GQDs-PEG-FA at tumour tissues can be undoubtedly detected. This result suggests that $^{131}$I-GQDs-PEG-FA can be used as a probe for clinical application. It can be concluded that bioimaging techniques of CDs play a fundamental role in developing clinical diagnosis and realizing surveillance of tumour response to treatment.

**Figure 4** Representative confocal microscopy images of indicated tumours in red fluorescence channel and white field (left two panels) and muscles in red fluorescence channel and white field (right two panels). Scale bars: 24 μm.\cite{96} Copyright 2019, Acta Physico-Chimica Sinica
The long-term studies have been carried out for anticancer drugs’ selective delivery, such as EPR-based tumour therapy, but they are not suitable for the clinical application owing to the inability of drugs to selectively deliver therapeutics to tumours. \cite{2-4,109,110} Besides, receptor-mediated therapy strategies of CDs have been recently developed. Tumour-targeted CDs can specifically bind to receptors that overexpressed on cancer cell membrane after surface-functionalizing with targeted ligands. \cite{4,111} The receptor-based modification of CDs is very important for drug delivery. Some researches have been carried out and exhibited higher cellular uptake of the CDs in vivo. Hyaluronic acid (HA) is a targeted ligand, which specifically binds to CD44 overexpressed on various tumour cells. HA modified carbon dots (HA-CDs), which were obtained by hydrothermal treatment with branch-poly (ethylene mine) and citric acid, have been demonstrated to accumulate in tumour tissue after intravenous injection (Figure 6). After loading with DOX, antitumour efficacy of HA-CDs was observed in two different kinds of tumour models, which give it a good application prospect for targeted cancer therapy. \cite{112}

Although previous studies focusing on receptor-based tumour therapy also made progress, accumulating evidences suggests that this strategy has several major
limitations. Receptors, only upregulation on membrane of cancer cells, are very few since most of them are present in both cancer and normal cells. Different types of cancer cells may not be targeted using the same ligand. One promising approach to tumour-specific theranostics leverages specific carrier transporters that are differentially highly expressed in cancer cells. Example transporters typically include LAT1, serine, alanine, cysteine-prefering transporter 2 and glucose transporters. LAT1, which mainly mediates the transport of large neutral amino acids, has been drawing great attention in cancer research because it has been shown to be overexpressed in various tumours.

LAT1 is mainly responsible for delivering neutral amino acids into cells, which is composed of 12 transmembrane domains that form the permeation pathway. They associate with the heavy glycoprotein subunit of the LAT1 on the plasma membrane. In normal tissue, the distribution of LAT1 is restricted to only a few organs, including the placenta, BBB, spleen, testis, and colon. Four LATs have so far been identified. LAT2 was separated according to the sequence similarity to LAT1. However, it is expressed in normal body. LAT3 and LAT4 appear to act as important transporters in some specific cancers. For example, LAT3 is highly expressed in prostate cancer patients. LAT4 is found to be expressed in the basolateral membrane of the small intestine, thick ascending limb epithelial cells and kidney proximal tubule. Among LATs, LAT1 is the most widely studied transporter. In previous studies, LAT1 has been used as a target for cancer treatment using LAT1 inhibitors, such as 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH). However, the use of BCH in chemotherapy has failed because of its lack of potency and specificity. All four LATs can be inhibited by BCH.

Our laboratory has recently developed the LAAM TC-CQDs by hydrothermally treating TAAQ and citric acid in H2O solution. Characterization analyses suggest the formation of polyaromatic structures and the existence of free carboxyl and amino groups at the edge of LAAM TC-CQDs. It has been revealed that there are about eight amino acid groups of each LAAM TC-CQD, which is more like to be a large amino acid. LAT1 happened to principally mediate the transport of large neutral amino acids. The LAAM TC-CQDs were mimicked as large amino acids and internalized through LAT1-dependent, clathrin-mediated endocytosis.

LAAM TC-CQDs act as carriers and enable drug delivery to tumour tissue, regardless of their origins and locations. The LAAM TC-CQDs were successfully applied for multiple tumour chemotherapies by intravenous injection. Unlike traditional CQDs, LAAM TC-CQDs are capable of selectively delivering therapeutic drugs to tumours. After loading with topotecan (TPTC), the LAAM TC-CQDs showed the capacity of selectively delivering TPTC to Hela and A549 tumours, which showed improved chemotherapy efficacy.

More importantly, LAT1 is expressed in the BBB, which has been a major hurdle for drug delivery to brain tumours. Up till now, in order to treat brain cancer, finding drugs or drug carriers with the ability of crossing

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FIGURE 6 (A) In vivo image of 4T1 tumour-bearing mice after intravenous injection of free ICG and HA-CD@p-CBA-DOX@ICG. (B) The images of ex vivo tumours of mice at predetermine time point after injection. (C) The average fluorescence intensity of ex vivo tumours of mice at predetermine time point after injection ($n = 3$, mean ± SD). **$P < .01$ and ***$P < .001$, respectively. Copyright 2020, Elsevier BV
FIGURE 7  The use of LAAM TC-CQDs for brain cancer imaging and treatment. (A) NIR fluorescence images of a representative U87 tumour-bearing mouse that received intravenous injection of LAAM TC-CQDs at the indicated time points. All the images are set to the same scale. (B) Ex vivo NIR fluorescence imaging of the indicated organs and tumour 8 hours after injection of LAAM TC-CQDs. (C) 3D reconstruction of the distribution of LAAM TC-CQDs in the mouse 8 hours after injection. (D) Quantification of LAAM TC-CQDs BBB penetrability without and with treatment of Leu in an in vitro BBB model. (E) Semi-quantification of LAAM TC-CQDs in mice bearing intracranial U87 tumours with or without pretreatment of Leu. (F) The viability of U87 cells after treatment with LAAM TC-CQDs, TPTC or TPTC-LAAM TC-CQDs. Data are mean ± SD (n = 3). (G) Changes in serum concentration of TPTC, when delivered in the form of free drug or with TPTC-AAM TC-CQDs over time in mice bearing intracranial U87 tumours. (H) Kaplan-Meier survival analysis revealed that treatment with TPTC-LAAM TC-CQDs significantly enhanced the survival of mice bearing intracranial U87 tumours; n = 10; P < .001; statistical analysis was performed using the log-rank Mantel-Cox test. In (A) and (B), the colour scale indicates intensities in units of radiance (photons/s/cm²/sr). In (D), (E) and (G), data are mean ± SD. Statistical analysis was performed using two-tailed unpaired Student’s t-tests.[80] Copyright 2020, Springer Nature

the BBB has remained a major challenge. Drug delivery to the brain by traditional ligand-based targeted therapy has been far from success. Fortunately, LAAM TC-CQDs can penetrate the BBB and interact with brain tumours. It has been demonstrated that LAAM TC-CQDs have the capacity to achieve bioimaging and delivering TPTC to U87 tumours. LAAM TC-CQDs may successfully treat brain tumours through their interaction with LAT1, which is a target overexpressed in both the BBB and in tumour cells (Figure 7).

6 CONCLUSIONS AND PERSPECTIVES

In this review, we discuss the recent research development of CDs about the preparation, hydrophilicity, biotoxicity, optical properties and their advanced applications in tumour diagnosis and therapy. We also highlight LAAM TC-CQDs-based tumour theranostics through LAT1-mediated targeted strategy. With the rapid growth of CDs-based tumour theranostics, future research still needs to confront challenges in terms of being applied to clinical assessment.

First of all, the low-cost synthetic approach and facile purification process is urgently needed to achieve the mass production of CDs. Second, developing CDs with high hydrophilicity will pave the way for anticancer drug carriers with improved delivery and tumour therapy efficacy. Third, the tumour gene therapy is accomplished through either chemical or physical techniques by delivering genetic materials to tumour cells for cancer therapy. With extraordinary ability to target cancer cells, CDs as promising carriers are capable of delivering the genetic materials into cancer cells. Fourth, cancer immunotherapy relies on the patient’s own immune system to trigger or enhance the antitumour response, which has shown unique advantages for cancer treatment. With the various merits and rapid development of CDs in biomedical applications, its use as an extraordinary vaccine adjuvant for tumour inhibition deserves to be explored for immunotherapy. Once the above issues are well resolved in the near future, we believe that CDs could hold the key for precision
medicine and bring more targeted therapies for cancer treatment.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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