Chapter

Naturally Derived Carbon Dots as Bioimaging Agents

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Abstract

The recent advances in nanoscience and technology have opened new avenues for carbon-based nanomaterials. Especially, Carbon dots (CDs) have gained significant attention due to their simple, economic and rapid green synthesis. These materials exhibit excellent water solubility, fluorescence emission, high fluorescence quantum yield, Ultraviolet (UV) to Infrared (IR) range absorbance and high bio-compatibility. Therefore, these materials are widely used for various biological applications including bio-imaging. With the integration and doping of surface passive agents and elements, respectively influenced the enhancement of fluorescence property of CDs. Also, the conjugation of receptor-based targeting ligands leads to targeted bioimaging. CDs in combination with other imaging contrast agents lead to the development of novel contrast agents for bimodal imaging and multimodal imaging techniques. The combination of diagnostic CDs with therapeutic agents resulted in the formation of theragnostic CDs for image guided therapies. In this chapter, a comprehensive view on the top-down and bottom–up green synthesis methods for naturally derived CDs discussed. Further, unique physical, chemical, optical and biological properties of CDs described. Finally, fluorescence based bimodal and multimodal imaging techniques also described.

Keywords: carbon dot, bioimaging, fluorescence, theragnostic, contrast agent

1. Introduction

The term 'imaging' is a perception that it is a type of photography; however, it is far from the biomedical domain. Bioimaging provides the anatomical visualization of cellular, subcellular structures, tissues, organs of multicellular organisms [1]. The biomedical imaging modalities utilize the various kind of energy sources such as light, magnetic resonance, positrons, ultrasound, electrons, and X-rays. The broad range of medical imaging modalities includes fluorescence (FL); X-ray computed tomography (CT), magnetic resonance (MR), ultrasound (US), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) for diagnosis of various soft tissues and hard tissue pathologies and scientific research [2]. These techniques disclose the three-dimensional molecular information of any specimen's biological structures and physiological processes.

Optical imaging, predominantly FL imaging modality received special attention from all the imaging modalities due to their simple operation process, cost-effective armamentarium, excellent resolution, incredible sensitivity and high resolution [3–5].

Therefore, a wide range of fluorescent imaging probes has been discovered for FL imaging, such as organic dyes, semiconductor quantum dots, metal nanocluster and up-conversion nanoparticles [3, 4, 6]. Nevertheless, their limitations include high toxicity, complicated synthesis mechanisms, poor water-solubility and high cost. On the other hand, single-mode fluorescence imaging techniques were limited to depth penetration and difficulty to provide tomographic information due to light attenuation and photon scattering of biological tissue. Therefore, developing FL based multimodal imaging by integrating other imaging modalities with FL imaging modality has become an essential strategy to resolve the single mode FL imaging limitations. Interestingly, FL based multimodal imaging provides several advantages including non-invasive imaging visualization with superior depth penetration, higher sensitivity and resolution [7]. Hence, it is necessary to develop a single probe by integrating other contrast agents with fluorescent materials for FL-based multimodality imaging applications.

Nanotechnology advancements in contrast agent development are progressing rapidly either by doping/incorporating or conjugation of other contrast elements into/with fluorescent nanomaterials to obtain a multimodal imaging probe [8]. Among the various types of nanomaterials, carbon dots (CDs) gained remarkable attention due to their significant physical, chemical and biological properties [9]. The integration of several unique properties in single nanosize carbon dots (CDs), make them ideal alternative material to replace the semiconductor quantum dots, metallic nanomaterials and other forms of carbon materials in various fields. Specifically, bioimaging, drug delivery, phototherapy, anti-microbial agents, sensors, solar cells, light-emitting diodes and photocatalysis [10–13]. Specifically, CDs show significant potential in fluorescence-based multimodal imaging *in vivo* and *in vitro*. For example, FL/MR dual-modal imaging was developed by integrating iron oxide nanomaterials or doping Gd^{3+} or Mn^{2+} ions into CDs. Similarly, FL/PA, FL/CT and FL/PET etc., established by incorporating respective contrast agents. This book chapter initially discusses the top-down, bottom-up and green synthesis procedures of CDs, optical properties, and elemental doping and surface functionalization. Next, the bioimaging, importance of carbon dots application in the various medical imaging techniques, and therapeutic applications of carbon dots together as theragnostic are described. Finally, the outlook of carbon dots in bioimaging is mentioned.

2. Synthesis methods of carbon dots

In 2004, during the purification process of single-walled carbon nanotubes (SWCNTs) using gel electrophoresis, the CDs were discovered [10]. Later, various procedures established for the synthesis of CDs. Broadly, the CDs synthesis methods can be classified into as "top-down" and "bottom-up" approaches (**Figure 1**).

2.1 Top-down method

In the "top-down" approach, the breakdown of bulk carbon materials into nano size carbon occurs under relatively harsh conditions such as oxidative acid treatment, electrochemical exfoliation, laser ablation, and arc discharge [14]. The first described CDs were produced by the top-down process via laser ablation of graphite in the gaseous phase, subsequently acid oxidative treatment [15]. Later, numerous methods such as arc discharge, etching, electrochemical oxidation, ultrasonication, chemical exfoliation, and nitric acid/sulfuric acid oxidation developed to obtained CDs by reducing the size of bulk carbon materials [14, 16]. Mostly, graphite, graphene or graphene oxide (GO) sheets, carbon nanotubes (CNTs), carbon fibers
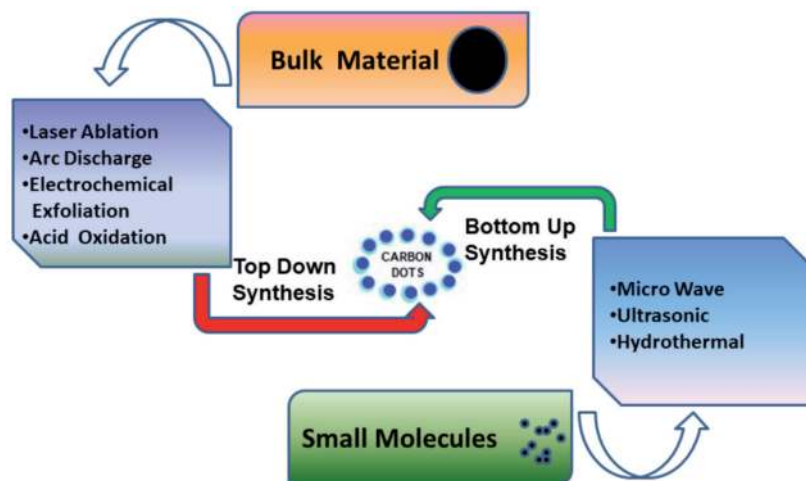
Figure 1.
Synthesis methods of carbon dots.

and carbon soot etc. used as a precursor material in these methods. Even though these methods were successfully used to prepare CDs, they are limited with harsh conditions, complicated synthesis strategies, low quantum yield, expensive, eco-unfriendly, and unsuitable for the production of CDs in industrial-scale. These methods rarely used for the preparation of CDs from natural sources.

2.1.1 Bottom-up method

In the "bottom-up" approach, the CDs are synthesized from carbon-containing small molecules in a "polymerization– carbonization" process. Several methods include combustion, hydrothermal, solvothermal, and microwave-assisted pyrolysis developed in bottom-up synthesis [16]. Typically, in these methods carbon precursor such as small organic molecules taken in a liquid or gas stage are ionized, dissociated, sublimated or evaporated and then condensed via condensation, carbonization, polymerization and passivation to form nanosize CDs. Compared with the "top-down" strategy, the "bottom-up" approach is extensively used for the green synthesis of CDs using natural renewable sources. Here, we discussed some important synthesis methods of CDs.

Acid oxidation: In this method, CDs were synthesized by exfoliation and cleaving of activated carbon, graphene oxide, carbon nanotubes, carbon fibers and soot etc. by using concentrated acids such as sulfuric acid and nitric acid [17, 18]. Typically, this method involves the decomposing of the bulk carbon into nanoparticles and simultaneously introducing hydrophilic groups on the carbon core. Generally, these raw materials are low in cost, readily available and feasible for simple operation. This method can be extended for the synthesis of hetero atom doped carbon dots. For example, the heteroatom N doped CDs prepared using activated carbon as precursor and Nitric acid as oxidizing agent [19]. However, this method limited with some disadvantages such as harsh conditions and time-consuming process to eliminate excessive acid.

Electrochemical exfoliation: This is a facile green and large-scale approach in which, CDs were prepared by avoiding excess concentrated acid, complex separation and purification process [20, 21]. In this method, high purity graphite used as anode and Pt wire used as a counter electrode. Distilled water can be used as electrolyte but the rate of reaction is very slow. In order to increase the rate of reaction, ionic liquids like 1- butyl-3 methylimidazolium tetrafluoroborate and 1-butyl

3-methylimidazolium hexafluorophosphate can be mixed with distilled water and can be used as electrolyte [22]. The electrochemical exfoliation carried by applying static potential through direct power which leads to corrosion of graphite anode and hence formation of CDs. The mechanism involves releasing carbon dots because of the electrochemical scissors OH$^-$ and O$^-$ ions from the water's anodic oxidation. Depending on the type of electrolyte nitrogen, phosphorus or boron can be doped in carbon dots.

Laser ablation method: The term ablation refers to the removal of surface atoms. Laser ablation method involves the absorption of highly energetic laser pulse by the carbon precursors and stripping of electrons from the atoms through a process like photoelectric effect generating a high electric field. Production of CDs takes place due to the repulsive force generated between positive ions and solid material [23, 24]. The size of the CDs can be controlled by a laser furnace. The precursors for laser ablation method are toluene, bulk graphite, graphene oxide and graphite powder etc. Laser ablation method provide high quality product with great velocity depending on the purity of the target and ambient media (gas or liquid). The size and other properties of carbon dots were controlled by irradiation time and laser fluence. The limitations of the method are requirement of high input energy and sophisticated equipment.

Ultrasonic treatment: In this green synthetic method, carbon materials can be broken down by the action of very high energy of ultrasonic waves [25, 26]. Ultrasonic waves create high pressure and low-pressure waves in liquid medium resulting in the formation, growth and violent collapse of small vacuum bubbles. The collapse of the bubbles lead to local high temperature and pressure up to 5000 K and 1000 atm respectively, producing the CDs. The precursors used for making CDs in this method are crab shell powder, glucose, active carbon, polyethylene glycol, citric acid, tri-ammonium citrate, and arginine. N, S, and P elements doped CDs can also be prepared by this method.

Microwave synthesis: This method involves the irradiation of electromagnetic radiations within a range of 1 mm to 1 m through the carbon precursor containing reaction mixture, which results from rapid and uniform heating. The microwaves absorbed by the solvent and precursor leading to the activation of molecules directly and its leads to formation of CDs [27, 28]. So, reaction volumes as such as 200 µl to >100 ml can be used without difficulty. The advantages of microwave irradiation are fast, higher efficiency and require less purification. The microwave irradiation can be controlled instantaneously so the risk of overheating is also minimized. However, the main drawback of this method is that solvents with lower boiling point cannot be used. This method widely used to convert bio-waste and natural sources such as plant materials, sea food waste and kitchen waste into CDs [29–31].

Thermal decomposition: In ordinary thermal decomposition, a carbon containing compound or substance decomposes chemically by action of heat and converted into CDs [32]. In general, CDs were synthesized from the variety of precursors like citric acid and L-cysteine etc. by simple heating under pyrolytic condition and controlled pressure using ionic liquid like 1-butyl 3-methyl imazonium bromide [33, 34]. The advantages of ionic liquid are high thermal stability, chemical stability; low melting point and low vapor pressure. At very high temperature, an irreversible thermal decomposition of organic matter takes place in inert mixture. Low cost, easy to operate, less time consuming and large-scale production are the advantages of the thermal decomposition method.

Pyrolysis: Pyrolysis is an irreversible thermal decomposition reaction in which decomposition of organic materials take place in inert atmosphere and at high pressure. Pyrolysis of the carbonaceous material is a simple, clean and inexpensive route for synthesizing CDs because no need of additives, acids or bases [35, 36].

In this method, solid residues with high carbon content were formed from organic materials by prolonged pyrolysis in an inert mixture. During pyrolysis, dehydration and fragmentation occurs. The natural precursors used for producing CDs in this method are cheap biowaste materials like rice husk, coffee grounds, watermelon peel, sago waste, peanut shells and wool etc.

Hydrothermal or solvothermal synthesis: In hydrothermal synthesis, carbon precursors undergoes "polymerization–carbonization" and leads to formation of CDs in water media taken in a sealed container under high temperature and pressure [37]. In solvothermal synthesis, organic solvents like methanol, ethanol, n-butanol and N, N- dimethylformamide etc. can be used as the solvent instead of water [38, 39]. This method was proved to be a cheap and eco-friendly route to the synthesis of carbon dots. However, solvothermal reactions can lead to an explosion in a few cases because the temperature rises rapidly in limited space. This can be avoided by taking a small quantity of solvent and reactant.

2.1.2 Natural materials as a green precursor for preparation of CDs

The green synthesis of CDs relies on natural precursors such as plant materials, protein products and waste materials [40]. Compared with bulk carbon materials (Graphene, Graphene Oxide and carbon tubes etc.) and toxic organic compounds including aromatic molecules, natural materials are renewable, economical, eco-friendly, safer and easier to get industrial-scale production. Mostly, "bottom-up" methods adopted for the synthesis of CDs due to the existing small organic molecules in natural sources can be carbonized into CDs at a specific temperature. A few top-down methods developed for the waste bulk carbon materials or bio-waste are broken down/cut into small-sized CDs. Among the various techniques, hydrothermal and microwave approaches are extensively used to prepare CDs from multiple natural materials. Most natural sources or biomass materials are made with small organic molecules, converted into CDs by carbonization and pyrolysis.

In recent years, various kind of plant material such as coriander leaves, ginger, garlic, grass, coffee beans, lemon, orange juice etc. due to the existence of various carbon containing organic molecules including carbohydrates, cellulose and phenolic compounds [41]. Compared with commercial precursors, the plant material derived CDs showed enhanced fluorescence emission with high quantum yield due to heteroatoms such as nitrogen, sulfur and phosphorus. Hence, the optical and structural properties of CDs are mainly relying on the selection of natural precursors. Besides this, with the growing concern about environmental pollution and sustainability, variety of waste materials including different kind of agriculture, kitchen, fruit peel and seafood waste etc. used as a starting material for the preparation of CDs [30, 42, 43]. These waste resources, also containing organic molecules, can be polymerized and followed by carbonized to form CDs.

2.2 General properties

The general properties of CDs illustrated in **Figure 2**. Structurally, CDs belongs to the quasi-spherical zero-dimension carbon nanomaterials class with a size of less than 10 nm [44, 45]. They are amorphous or nanocrystalline cores with a typical sp^2 carbon hybridization. The absorption band of CDs exhibits UV–Visible region to the NIR region and contains various functional groups. The electrifying properties of CDs are their excitation wavelength-dependent emission spectrum, high photostability and resisting to photobleaching, which permits CDs for multicolour and long-term imaging applications, respectively [45]. The cytotoxicity and pre-clinical biocompatibility of CDs on various models such as cell lines, zebrafish, mice
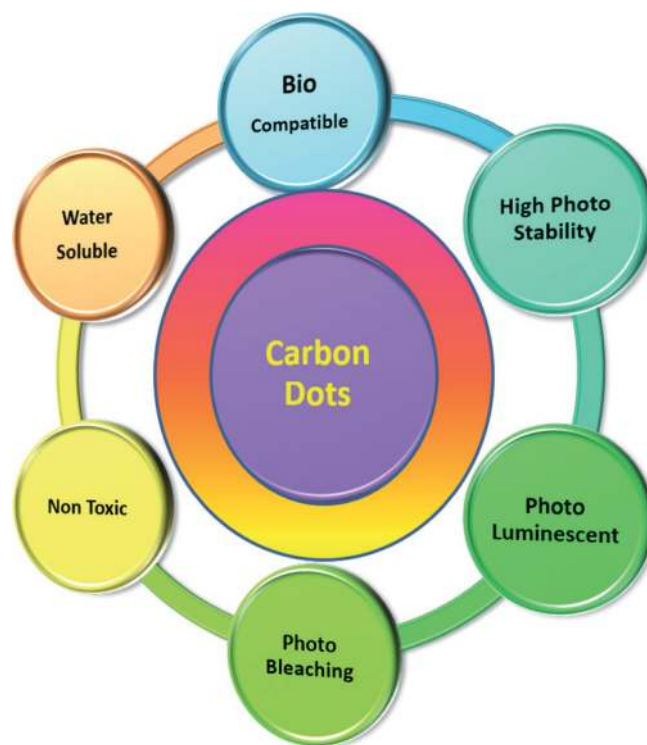
Figure 2.
Properties of carbon dots.

displayed CDs have no apparent toxic effects. Extensive studies are to be done on the toxicological and biocompatibility properties to translate from preclinical to clinical application. For targeted bio-imaging, the surface functional groups such as hydroxyl, amine and carboxylic groups allow conjugation with targeting agents.

2.2.1 Optical properties

Among all the CDs' properties, optical properties such as absorbance and fluorescence are vital for bio-imaging applications [45, 46]. Usually, CDs exhibited a strong absorption band at UV region with a falling intensity absorption tail increased to the visible light region. Usually, absorption peak around 230–340 nm was typically ascribed due to π-π^* transition of the C=C bonds of the carbon core. Similarly, the absorption band of 350–550 nm is ascribed to the surface functional groups on the carbon core. Besides this, one exciting feature of CDs is their excitation wavelength-dependent emission spectrum by varying excitation light wavelength, which is commonly observed in most CDs, which allows for multicolour imaging applications. CDs' exact PL mechanism is currently debatable due to the various methods available for the preparation of CDs and the lack of consistency in CDs' PL behavior. Nevertheless, three main mechanisms have been proposed to explain the PL of CDs: (1) The intrinsic band gap arising from the quantum confinement effect or the conjugated π domains, determined by CDs carbon core. (2) The creation of trap states (such as surface defects) in the band gap due to the surface functionalization and CDs doping. (3) The presence of individual fluorescent molecules (fluorophores) on or within the CDs. According to these theories, the wide tunable emissions of CDs have been attributed to their broad size distribution, variable surface chemistry, and the uncontrolled preparation conditions. Another fascinating PL property about CDs is that they generally exhibit high photostability, resisting photobleaching, which is very important for long-term imaging applications.

2.2.2 Elemental doping and surface functionalization

Usually, most of the bare CDs showed comparatively weak FL ability than traditional semiconductor quantum dots or organic dyes. In this line, CDs' structure altered by incorporating elements or surface functionalization strategies to improve fluorescence properties which are essential for fluorescence-based bio-imaging applications. So far, a variety of element doping is adopted to obtain CDs with charming FL properties. At present, heteroatoms such as N, S, Si, P, B, Ga, halogen (Cl, Br, I), Se, Ge, Mg, Cu, Zn, Tb, Ru and Mn incorporated into CDs during the synthesis process [47, 48]. Besides this, large functional groups such as carboxylic acid, amine, hydroxyl and amide groups presence on the surface of CDs facilitate the opportunity to conjugate with various passivate agents [38, 49]. Therefore, several groups focused on improving the fluorescence efficiency through conjugation with variety of passivating agents such as Polyethylene glycol, polyethyleneimine, poly (propionyl ethyleneimine co-ethyleneimine), 4,7,10-trioxa-1,13-tridecane-diamine, 1-hexadecylamine, poly(ethylenimide)-b-poly(ethyleneglycol)-b-poly (ethylene imide) and amino acids etc. Generally, these passivating conjugate with CDs via electrostatic interactions, covalent bonds, and hydrogen bonds. More interestingly hetero element doped or surface modified CDs also improved water solubility, photostability, biocompatibility, NIR absorbance and multicolour fluorescence emission, which are the vital parameters for bioimaging. Moreover, unique surface functional CDs have been prepared based on individual cell membrane lipids, proteins, targeting ligands and biomarkers of different cells to develop impactful imaging applications. Furthermore, variety of targeting moieties including peptides (transferrin), aptamers, antibodies and small molecules (folic acid) which have been selected to integrate on the surface of CD through N-hydroxy succinimide (NHS) or 1- ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) chemistry [50]. These targeting moieties offer the internalization of CDs into cells or tissues via a ligand-receptor interaction. Remarkable specific cell targeting bioimaging besides adequate circulation of CDs avoid the side effects originating from the nonspecific interactions. The targeting moiety linked to CDs improves the specificity of bioimaging. Moreover, various cancer therapeutic drugs, anti-microbial agents and photosensitizers were conjugated with the CDs' surface for image guided therapeutic applications.

3. Bio-imaging

Bioimaging is an emerging field of biomedical science that comprises of the development and application of various materials for imaging technologies [51, 52]. The bioimaging techniques principles are mainly based on optics, magnetic resonance, nuclear medicine, radiation, ultrasonics, photonics and spectroscopy. The anatomical and physiological quantification of clinical parameters is measured with the image processing and analysis. With the recent development of biomedical science and emerging newer technologies, the *in vivo* or *ex vivo* biological tissue characterization of imaging properties aids in discerning its structure and function through visualization at several resolutions, extending from organ and cellular to molecular level. FL, near IR CT, PET, MRI and ultrasound images are commonly used for clinical diagnosis and research (**Figure 3**). The characteristic 'energy-matter' interaction is utilized by most bioimaging techniques to provide precise particulars of the biological processes. The imaging modality's uniqueness defines in terms of anatomical and molecular details, spatial and temporal resolution, depth of imaging and properties of contrast agents of augmented imaging. In many clinical
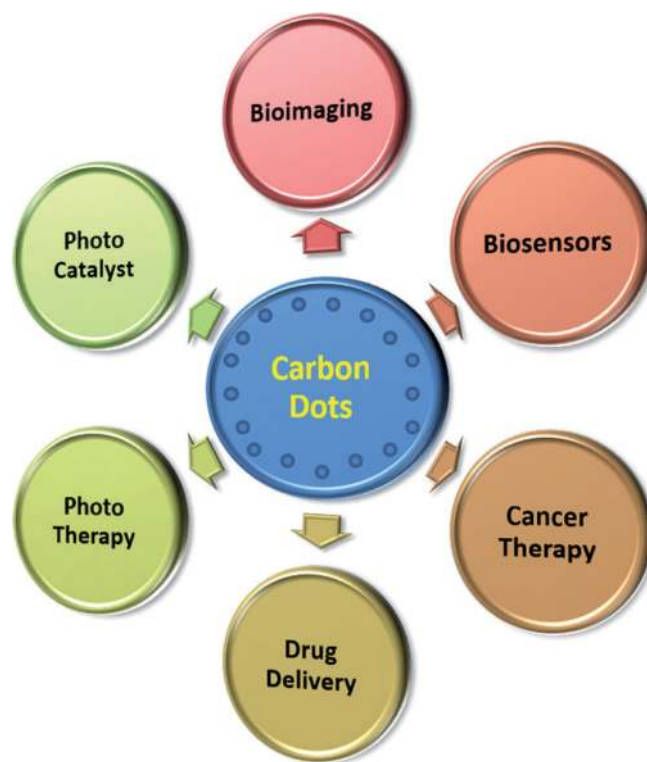
Figure 3.
Applications of carbon dots.

scenarios, the application of multimodal imaging techniques is advantageous for a simultaneous, faster and more accurate diagnosis. Exogenous contrast agents which are used in many imaging modalities enhances the signal to noise ratio. So, the development of multimodal contrast agents is essential to achieve better efficacy and accuracy in diagnosis and therapeutics.

4. Importance of carbon dot in bio-imaging

For clinical imaging application, good biocompatibility and low cytotoxicity of imaging probes are essential. The traditional quantum dots (QDs) such as CdSe, CdTe, CdS, and PbS were applied in *invitro* and *in vivo* optical bioimaging procedures [53]. But their bioimaging application is restricted due to heavy toxic metals which may cause toxicological and pathological problems for health and the environment. The silicon quantum dots (Si QDs) can be considered alternatives to heavy metal QDs in bioimaging [54]. But they undergo oxidative biodegradation in biological systems and are slightly toxic. The gold and silver nanoclusters were deemed to be alternative, non-toxic, photo luminescent nanomaterials, but suffering with poor water solubility and photostability [55]. So, there is a requirement for biocompatible imaging probes with low toxicity for bioimaging. Some CDs such as low toxicity, good biocompatibility, excellent photoluminescence and high photo stability makes them a novel nanoprobe for bioimaging.

4.1 Fluorescence imaging

FL imaging is a promising technique for observing and assessing various cells, tissues and organisms, by the high fluorescence emission with biocompatible

fluorescent agents [56, 57]. Currently, fluorescent CDs are considered as a significant alternative ideal contrast agent for fluorescence imaging. Several reports revealed that cells incubated with CDs emitted fluorescence mainly due to accumulation of CDs in the cells. Generally, unmodified/bare CDs are majorly identified in the cell membrane and cytoplasmic region without reaching the nuclei. To resolve this issue, excessive effort have been devoted to surface modify the CDs with targeting agents including antibodies, peptides and other biomolecules to enhance the specific targeted bioimaging. These target ligands modified CDs showed a significant capacity to bind to the overexpressed reprehensive receptor/biomarker on cells. In addition, to resolve the issues related to deep tissue fluorescence imaging, the NIR receptive CDs were designed with longer excitation/emission wavelengths that enhanced fluorescence imaging ability [58] for *in vivo* applications. Under suitable excitation wavelength the NIR emitting CDs can be well differentiated from the auto-fluorescence background (green) with good optical contrast.

4.2 Multimodal imaging

Every imaging technique has its unique advantages in consort with integral limitations such as insufficient sensitivity or spatial resolution (**Figure 4**) [59]. Even the fluorescence imaging provides high sensitivity but lacks of sufficient resolution. To compensate this drawback, the combination of fluorescence imaging techniques with other modalities, such as FL/MRI has gained attention to enhance the currently used imaging techniques for diagnosis [58]. Multimodal imaging is a combination of two or more imaging techniques to overcome individual limitations. The development of multi-modality imaging with the FL imaging is

Figure 4.
Advantages and disadvantages of various imaging techniques.

to achieve non-invasive imaging at greater depths of penetration, sensitivity and higher resolution required for an accurate diagnosis. Thus, optical imaging assisted multi-modalities has emerged as potent tools, which can improve the detection sensitivity, precise identification and provide more detailed anatomical or biological information of the pathology. Each imaging technique uses different contrast agents with distinctive functional, chemical compositions and sizes. In designing and developing a multi modal contrast agent, the researchers should judiciously forbid the overlay of pros and somewhat counterbalance each modality's limitations to enhance the synergistic effect. Thus, the FL imaging modality with high sensitivity is frequently combined with other imaging modalities with a high spatial resolution modality such as MR, CT, and PA etc. (**Figure 5**). Hence, multimodal contrast agents' development and application are clinically significant for enhanced imagery from desirable imaging modality. Multimodal imaging agents based on fluorescent CDs are the recent cutting-edge technologies where CDs' advantages are maximized. CDs based multimodal imaging agents are prepared by conjugating or incorporating one or more imaging agents into CDs. Here we have discussed various kinds of fluorescent CDs' based multi-modality imaging approaches such as FL/PA, FL/MR and FL/CT imaging.

4.2.1 Fluorescence/photoacoustic imaging

Photoacoustic (PA) imaging is a non-invasive, hybrid, optical and ultrasound imaging modality. The PA imaging is performed at varying depths with high depth to resolution ratio with rich optical contrast beyond the optical detection limit. The CDs have shown significant PA imaging application because of the NIR absorption, high extinction coefficient, and non-radiative heat generation. Wang, *et al.* synthesized dual-mode FL/PA imaging agent based on CDs [60]. Within the NIR spectrum, the imaging agent exhibited a maximum optical absorption at 710 nm approximately. In *in vivo* PA imaging of mice tumor model, enhancement of signal and clear demarcation of the tumor was observed due to long circulation time and increased tumor accumulation of CDs. Hence, the combination of fluorescence and photoacoustic imaging into a single probe based on CDs enabled deeper tissue penetration for tumor identification. Compared with single optical imaging, the
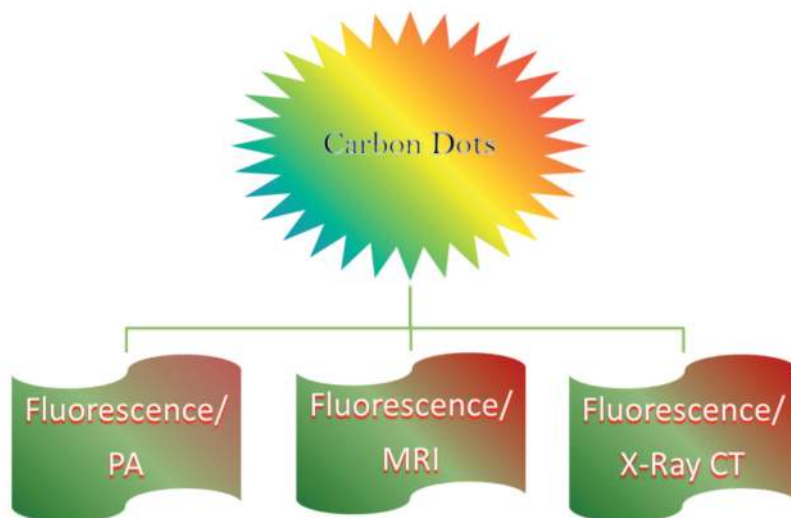

Figure 5.
Carbon dots integrated multi-modal bio imaging.

dual-mode FL/PA imaging upholds the sensitivity and provides higher-resolution anatomical images. Porphyrin implanted CDs developed by selective pyrolysis with good aqueous dispersibility displayed a strong PA signal at 686 nm in a slightly acidic or neutral environment and somewhat alkaline conditions pH 7–8 the signal was weak [61]. In breast cancer, the sentinel lymph nodes detection, the application of photo acoustic visualization of CDs was reported by Wu, *et al.* [62]. After injection the CDs exhibited rapid signal enhancement and relatively fast clearance from the lymph nodes.

4.2.2 Fluorescence/magnetic resonance imaging

Magnetic resonance imaging (MRI), is a radiation-free and non-invasive imaging technique widely used to detect various diseases including clinical cancer diagnosis and therapeutic response assessment [63]. With an external radiofrequency pulse magnetic field applied on the body, it simultaneously obtains anatomical and physiological information of regions of interests with a high spatial resolution by manipulating magnetic nucleus's resonance (^{1}H). Further, the integration of FL imaging with MR imaging considered as most effective non-invasive imaging tool in diagnosis and clinical research due its excellent spatial resolution, high temporal and sensitivity. Hence, the CDs assisted FL/MR dual modality potentiality can take benefits of the spatial resolution, outstanding soft-tissue contrast with MR imaging as well as superior sensitivity and the rapid data acquiring with FL imaging. This dual imaging modality is facilitated precise diagnosis with effective treatment based on corresponding imaging evidence. The CDs-relayed FL/MR dual-mode imaging modality probe can be obtained by the doping/ conjugation of magnetic elements. Specifically, extremely paramagnetic ions including Gd^{3+}, Mn^{2+}, and Fe^{3+} employed as dopant for the preparation of FL/MR bimodal contrast agent such as Gd or Mn elements into CDs. For example, Gd^{3+} doped CDs were synthesized from Gd^{3+} containing precursors and sucrose as carbon precursors via microwave assisted method polyol by Gong Ningqiang, *et al.* The attained Gd-CDs showed green fluorescence emission, low cytotoxicity and optically label cells. Meanwhile, the r_1 relaxivity of Gd-CDs was measured to be 11.356 $mM^{-1} s^{-1}$. This high r_1 value together with the r_2/r_1 ratio approximately 1. These results indicating that Gd-CDs is not only significant fluorescent imaging agent but also remarkable T_1 contrast agent for MR imaging [64].

Further, Jia Qingyan, *et al.* demonstrated the magneto-fluorescent Mn-doped CDs for bimodal FL/MR imaging in a single probe. The study reported the development of ultrafine Mn-doped CDs with a concurrent bimodal imaging ability through the solvothermal procedure of the precursor manganese (II) phthalocyanine [65]. The Mn-doped CDs showed strong T_1-weighted MRI signals and low cytotoxicity. The MRI signal intensities increased with the concentration, exhibiting a clear difference in brightness with a measured relaxation (r_1) value of $\approx$6.97 $mM^{-1} s^{-1}$. Furthermore, the *in vivo* T1-weighted results fortified the high retention rate of the Mn-doped CDs in tumors. The MRI signal intensity at the tumor site increased quantitatively by $\approx$320%, after 6 hrs injection while the MRI signal remained nearly unchanged for the analogous CDs without manganese (II) doping. CDs doped with dysprosium for a magneto-fluorescent bimodal imaging agent showed strong blue-green fluorescence at 452 nm. The excellent transverse relaxivity r_2 makes them also suitable for T_2 weighted imaging of live cells [66].

4.2.3 Fluorescence/X-ray computed tomography

X-ray computed tomography (CT) is a non-invasive medical imaging technique for disease diagnosis. The CT has intrinsic advantages such as high spatial

resolution and good density; it still has inherent drawbacks of low sensitivity. On the other hand, the fluorescence imaging has high sensitivity, facile operation and low cost, but its application was hampered due to the low spatial resolution and limited penetration depth. To improve clinical diagnostic accuracy and sensitivity, the FL and CT imaging are combined for a synergistic effect. The CDs doped with Hafnium (Hf) was used for diagnostic imaging of the orthotopic liver cancer preclinical model [67]. Rapid imaging was achieved with Hafnium doped CDs due to preferential tumor accumulation within 1 min. These imaging nanoprobes with efficient renal clearance offers good biocompatibility. Iodine doped CDs conjugated with a chemotherapeutic agent like cetuximab simultaneously rendered the cancer diagnosis and targeted anti-cancer therapeutic potential in lung cancer cells [68].

5. Imaging guided therapeutic application

Nanotechnology provides the possibility of developing non-toxic CDs nanoprobes with enhanced sensitivity, accuracy and advanced functionalities for imaging-guided synergistic therapy [69, 70]. The unique advantages of CDs include high relaxivity, prolonged blood circulation time, multiple functionalities for accurate accumulation in the target site, good biocompatibility and renal clearance. The inherent radio resistance of tumors and inaccurate positioning of the radiotherapeutic equipment leads to decreased radiotherapy effectiveness. Du Fengyi, *et al.* reported the theragnostic Gd-CDs with stable photoluminescence at the visible region, relatively long circulation time, efficient passive tumor targeting ability and renal clearance for MRI-guided radiotherapy a tumor [71]. Changhong Zhao *et al.* developed red-emitting wavelength multifunctional CDs for cancer theragnostic with *in vivo* bimodal imaging of tumor tissues and anti-cancer chemo-dynamic treatment (CDT). The functionalization of red CDs was done with Ethylene di amine tetra-acetic acid, Fe^{2+} and Gd^{3+} exhibited strong T1 weighted MR imaging and excellent bright and stable fluorescence. The anticancer CDT effect was based on Fenton reaction, by releasing Fe^{2+} into the tumor both *invitro* and *in vivo* [72].

6. Conclusions and outlook

Naturally, renewable sources derived CDs are kind of newly born luminescent carbon-based nanomaterials in this decade. They gained great potential in bio-imaging not only because of their cost-effective and eco-friendly green synthetic approaches but also their physical, chemical and biological properties. We have elaborately discussed various synthesis methods, significant properties. Furthermore, the recent development of CD in multimodal bio-imaging. Their strong fluorescence emission, high fluorescent quantum yield, and good absorbance are widely used for fluorescence imaging. Specific CDs also allow for multicolour bioimaging due to their multicolour emission capability. Further, numerous surface functional groups provide an opportunity to conjugate with targeting moieties such folic acid for targeting imaging. Accordingly, CDs conjugation with targeted moieties can precisely transport imaging contrast agents to internal organelles or cell membranes to attain the goal of targeted bio-imaging. In the meantime, the large surface area of CDs permits them to have a more quantity of hetero atom loading ability, consequently showing remarkable multimodal imaging ability. Finally, the nano size of CDs (typically >10 nm) facilitates their navigation in tissues, endocytosis, and intracellular trafficking. Even though significant efforts have devoted to improving the multimodal imaging effect of CDs, several limitations hinder the

application of CDs in bio-imaging. Primarily, the emission from most of the natural sources derived CDs showed blue or green, thus developing the methods and finding suitable natural precursor for yellow or red emissive CDs is highly desired. CDs exhibit excellent biocompatibility; however, majority studies are confined to cellular and preclinical experiments, but translation into clinical investigations is still unclear. In summary, more research still needs to be made for the effective and real-time clinical application of CDs in multimodal imaging.

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