Progress on carbon dots and hydroxyapatite based biocompatible luminescent nanomaterials for cancer theranostics

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ABSTRACT

Despite the significant advancement in cancer diagnosis and therapy, a huge burden remains. Consequently, much research has been diverted on the development of multifunctional nanomaterials for improvement in conventional diagnosis and therapy. Luminescent nanomaterials offer a versatile platform for the development of such materials as their intrinsic photoluminescence (PL) property offers convergence of diagnosis as well as therapy at the same time. However, the clinical translation of nanomaterials faces various challenges, including biocompatibility and cost-effective scale up production. Thus, luminescent materials with facile synthesis approach along with intrinsic biocompatibility and anticancerous activity hold significant importance. As a result, carbon dots (CDs) and nanohydroxyapatite (nHA) have attracted much attention for the development of optical imaging probes. CDs are the newest members of the carbonaceous nanomaterials family that possess intrinsic luminescent and therapeutic properties, making them a promising candidate for cancer theranostic. Additionally, nHA is an excellent bioactive material due to its compositional similarity to the human bone matrix. The nHA crystal can efficiently host rare-earth elements to attain luminescent property, which can further be implemented for cancer theranostic applications. Herein, the development of CDs and nHA based nanomaterials as multifunctional agents for cancer has been briefly discussed. The emphasis has been given to different synthesis strategies leading to different morphologies and tuneable PL spectra, followed by their diverse applications as biocompatible theranostic agents. Finally, the review has been summarized with the current challenges and future perspectives.

Introduction

Cancer is a major health issue and the primary cause of death around the world but unfortunately there is no particular effective treatment known to date. Due to cancer heterogeneity, personalized treatments based on the variability and medical profile of each patient holds great potential [1]. While precise detection at the disease onset plays a crucial role in the development of effective therapy, molecular imaging techniques are critical tools of medical diagnosis because they are highly specific and can provide in-vivo biological information at molecular level. They have revealed specific molecular changes that play key roles in disease progress, and have enabled earlier diagnosis and therapeutic response monitoring. Various imaging modalities, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), X-ray computed tomography (CT) and magnetic resonance imaging (MRI) are reported to have improved diagnostic ability. However, these techniques have some disadvantages like the use of high-energy radiations, radioactive tracers, high doses of contrast agents, low spatial resolution/sensitivity, long scanning times [2]. In contrast, fluorescence (FL) imaging contains outstanding real-time monitoring ability, high spatial resolution/sensitivity, and it is low cost, consequently gaining much interest in cancer diagnosis, especially in preclinical research [3].

The emergence of nanotechnology has greatly improved the narrow diagnostic and therapeutic window of conventional imaging and treatment modalities for improved diagnosis and patient survival rates. The progression of protocols for nanomaterials synthesis paved the path towards nanostructure luminescence materials also known as nanophosphors. These nanomaterials serve as excellent optical bioprobes that are significantly emerging as better alternatives for fluorophores, like organic dyes and genetically engineered fluorescent proteins [4]. Due to their nanometric size, large surface area and excellent luminescence properties, nanophosphors play a major role in the emergence of domains, including LED technology, radiation dosimetry and scintillator...
for biomedical applications [5–9]. Various luminescent materials such as quantum dots, lanthanide (Ln) doped crystals, carbon dots, and metallic nanoparticles like silver and gold are reported [10]. They possess advantages like controllable size, tunable and narrow spectra, excellent photostability and a large surface for anchoring targeting biomolecules in combination with resilience to physiological conditions. These extraordinary properties make nanophosphors suitable for diagnosis and treatment of many debilitating diseases like cancer.

Most of the conventional FL imaging probes operate in the ultraviolet-visible (UV–vis) range, a short wavelength region where most biological tissues absorb and scatter light resulting in tissue auto-fluorescence, low tissue penetration, and a low signal-to-background ratio [11]. The degree of scattering in the bio tissue is inversely proportional to the wavelength of light. Near-infrared (NIR) region from 700 nm to 1700 nm shows the lowest attenuation coefficient in the bio tissues, therefore, the rise of luminescent materials operating in the NIR region has been seen for deep tissue bioimaging [12]. Improvement in laser technology has also influenced FL bioimaging. For example, two-photon emitting laser with femtosecond pulse rate is now used for the excitation of luminescent materials [13]. Two-photon FL bioimaging has various advantages over single-photon counterpart as NIR photons for excitation can be used for deep tissue imaging along with reduced photodamage to the cells. Additionally, the excitation can be restricted to a tiny focal point in the thick sample resulting in ultrahigh-resolution images.

Besides FL bioimaging, luminescence properties of nanophosphors are also being used to improve the efficacy of therapeutic modalities like photodynamic therapy (PDT). PDT is a well-known cancer treatment strategy that uses a photosensitizer (PS) molecule to generate reactive oxygen species (ROS) to kill cancer cells. PSs are prodrugs that are activated by the irradiation of a specific wavelength of light. In the presence of oxygen, activated PSs produce ROS, which causes cancer cells toxicity. However, most of the PSs possess high absorption in UV–vis region thus limiting PDT only to superficial tumors. Therefore, a novel strategy was implemented in which a nano-scintillator (luminescent materials that can be excited with higher energy photons like X-Rays) was used as an energy mediator to convert highly penetrating X-Ray photons to UV-vis photons in the proximity of PS, to generate ROS. This strategy provides a possibility to specifically target deep-seated tumors and is known as X-Ray mediated photodynamic therapy (X-PDT) [14]. Photothermal therapy (PTT) is another photon-induced cancer treatment modality that damages cancer cells specifically by generating heat in situ, upon irradiation with specific light photons. Photothermal sensitization is an intrinsic property limited to some types of luminescent material like carbon dots (CDs), reason why CDs with variable composition have been studied for PTT against cancer [15,16].

The main reason for clinical translation of nanophosphors is the high fluorescence output with excellent biocompatibility and biodegradability. For instance, Zuo et al. reported the high efficiency fluorescent doped CDs for gene delivery. The CDs were surface modified with branched polyethyleneimine (PEI) to endow positive charge sites for gene delivery [17]. Surface modification seems a hotspot research area especially to improve the biocompatibility, however, it also enhances the complexity of the design and production of nanomaterials, which hinders the clinical applications, because minor changes in the production of nanoparticles may lead to different properties [18]. On the contrary, nanophosphors with intrinsic biocompatibility hold a great advantage.

Carbon dots (CDs) are the emerging luminescent nanomaterials having a carbon core with size mostly in the range of a few nanometers. These materials contain different surface functional groups and exhibit remarkable physiochemical properties like photoluminescence and photon mediated electron transfer. Therefore, CDs are ideally suited for various biological, environmental and energy based applications. These nanomaterials can be synthesized with various methods via bottom-up or top-down approaches and have an edge over metal based nanoparticles in terms of biocompatibility and surface functionalization. Likewise, hydroxyapatite (HA), the main constituent of bones, is a bioactive inorganic material that is widely used in clinical applications [19]. Owing to the versatile apatite structure, it exhibits exciting properties to incorporate large assortment of substitutions at PO43−, Ca2+, and OH− sites for different lanthanides (Ln) as dopants. Therefore, HA serves as an excellent host to develop fluorescent probes with excellent biocompatibility and biodegradability combined with the superior photoluminescence properties of lanthanides for biotechnological research.

Both CD and Ln-doped HA hold huge potential in clinical translational research, thus, this review focuses on the recent progress on CD and HA based nanophosphors for cancer theranostics. A deep analysis of various synthesis approaches and photoluminescence properties is covered. Moreover in clinical diagnostic imaging, modalities with highest sensitivity have relatively poor resolution while those with high resolution have relatively poor sensitivity. Multimodality imaging integrates the strength of two or more individual modalities offering higher accuracy and efficiency for diagnosis. Additionally, the use of these novel imaging techniques may avoid adding unnecessary stress to the patient by preventing the administration of multiple probes. Consequently, the applications of these nanomaterials as a multimodality platform are also discussed in detail in this review.

Luminescent materials based on carbon dots

CDs are the new addition to the family of carbon based nanomaterials. Since the accidental discovery of C-dots in 2004, they have gained exponential interest in various areas including biosensors, smart biomaterials for bio imaging, therapy, gene and drug delivery, among many others [20]. These nanomaterials demonstrate highly desirable and tunable physiochemical properties (e.g. photoluminescence, dispersibility, photostability, and biocompatibility). Moreover, they can be synthesized from various carbon precursors making the synthesis facile and cost effective. CDs possess quasi-spherical morphology and consist of a graphitic core with the coexisting aromatic sp2 and aliphatic sp3 hybridized carbon. Their size range from 1 to 60 nm while the surface groups can be varied depending on the condition of their synthesis.

Synthesis approaches

Currently, the synthesis of CDs can be categorized as top-down and bottom-up approaches. The top-down approach involves cutting and exfoliating macroscopic carbon containing graphene crystal lattice such as activated carbon, carbon nanotubes, graphite powder and carbon fibers via arc-discharge, laser ablation, or chemical oxidation [21–23]. Therefore, top-down strategy usually requires harsh reaction conditions, long reaction time and expensive equipment, but it is well suited for mass production. On the other hand, bottom-up approach utilizes carbohydrates, polymers and organic acids as a precursor to synthesize CDs by solvothermal/hydrothermal reaction, microwave synthesis, and thermal decomposition [24–28]. In bottoms-up strategy, partial dehydration and dehydrogenation are the key processes for the formation of CDs. The different factors (e.g. precursor type, solvents, reaction time and temperature) affecting the chemical constituents and size of CDs have been widely examined. Hydrothermal strategy for CDs production is most frequently employed due to its low cost and green synthesis approach. For example, graphene oxide was used as a precursor in hydrothermal process to synthesize CDs [29,30]. Waste biomass was also used to produce these nanomaterials via hydrothermal method. For instance, Mehta et al. reported the green synthesis of CDs from sugarcane (Saccharum officinarum) juice via hydrothermal route [31]. Thereafter, other biomass carbon sources were utilized for the generation of CDs through hydrothermal strategy [32–36].

In cases where one or more organic solvents are used with the aqueous medium, the synthesis is called solvothermal. Various solvents
such as N, N-dimethylformamide, ethanol, and DMSO have been utilized for the synthesis of CDs [26,37–41]. For instance, a series of CDs with tunable emission spectrum and particle size were obtained by varying the solvents in the solvothermal synthesis [42]. Moreover, the temperature and time of the reaction also determine the crystallinity and size of the CDs [43]. Shin et al. used an acid-free solvothermal approach for the synthesis of CDs from different carbon sources (graphite, multiwalled carbon nanotubes, carbon fiber, and charcoal) [41]. Similarly, other reports demonstrated the use of hydrogen peroxide, a low-cost and ecofriendly oxidant, to generate CDs form graphite via solvothermal route [38]. High temperature and pressure are important for surface passivation, however, Mitra et al. synthesized CDs for the first time at room temperature by using polyethylene glycol-200 [39]. Another rapid, green, and cost-effective method is microwave assisted synthesis where microwave irradiation provides the homogenous heating in the reaction mixture for the formation of CDs [42–45]. Pyrolysis or carbonization approach was also recently incorporated for the synthesis of CDs from macroscopic carbon structures [46–49].

Many reports have shown that CDs prepared through solvothermal/ hydrothermal synthesis present highly crystalline homogenous structure. After the synthesis, unreacted precursors and by-products are removed via filtration, dialysis, and centrifugation. Ion exchange chromatography, gel electrophoresis, and reverse-phase chromatography are also utilized [50–55]. Moreover, the properties of the synthesized CDs can be further altered by doping with different atoms like nitrogen, boron, sulphur, and selenium [56–58]. Nitrogen doped CDs have shown excitation wavelength dependent emission properties up to near-infra-red region [56,59]. Whereas, nitrogen and sulphur doping in CDs by using cysteine as carbon precursor led to the production of highly luminescent CDs with a quantum efficiency of 41.26% [57].

Another strategy to modify the resulting properties of the synthesized CDs is surface functionalization. CDs have various functional groups on their surface by the virtue of the precursors used in the reaction. These functional groups can be eliminated, modified, and introduced on the surface of CDs. For instance, CDs functionalized with aromatic molecules such as o-phenylenediamine, 2,3-diaminonaphthalene and 1,8-diaminonaphthalene showed narrowing of the band gap [31]. Furthermore, surface passivation of CDs via oligomeric PEG have shown enhancement of photoluminescence [2]. Thus, depending on bioapplication, the physicochemical properties of CDs can be tuned.

### Application of carbon dots in bioimaging

The bioapplication of CDs especially as bioimaging and therapeutic agents require careful choice of synthesis approach as the difference in physicochemical properties are seen with various methods of production. Mostly, CDs are hydrophilic in nature due to the oxygen containing functional groups on their surface, which are derivative of the carbon precursor and solvents used in the reactions. But it is possible to introduce hydrophobicity by surface functionalization with hydrophobic moieties such as octadecyl ammonium citrate and dodecylamime [60, 61]. Particularly for biomedical imaging, hydrophilic CDs have shown immense potential.

Despite many efforts, exact mechanisms of CDs absorption and emission properties are still not uncovered. However, some probable mechanisms suggest that these properties are related to two mechanisms: (i) due to the bandgap transitions of the conjugated π domains, (ii) due to the structural and surface defects [62]. Apart from understanding the exact principle for photoluminescence property in CDs, significant efforts are made to alter them to suit the potential application in bio imaging. Generally, CDs present excitation dependent photoluminescence with intense emission in the blue region of visible spectra, which gradually decays towards the red region. However, blue region from ultraviolet and visible light has limited penetration in the human tissue because of scattering from pigments and collagen networks and absorption by intrinsic proteins like melanin and hemoglobin. Therefore, biological optical window for deep tissue imaging is considered in the 650-950 nm (NIR I) wavelength range [63].

Consequently, enhanced red shift in the emission of CDs towards the biological optical window is vital for their fate as a bioimaging probe. Recently, high red emitting CDs have been prepared by changing the size and surface chemistry. For example, by controlling the HNO₃ concentration during the reaction, the CDs average size was increased from 3.67 nm to 5.74 nm [64]. The size increment resulted in the narrowing of band gap enhancing the emission of CDs in the red region. Likewise, surface conjugation with polyaromatic structures results in narrowing of the band gap of the CDs, thereby enhancing the red shift emission [62]. On the other hand, red shift emission can also be enhanced by doping of nitrogen, sulphur and fluorine in the CD crystal. In 2014, Ge et al. reported the hydrothermal synthesis of nitrogen and sulphur doped CD using polythiophene as a precursor [65]. The CDs presented broad absorption from UV and visible region with an intense deep red emission centered at 680 nm. Afterwards, various polythiophene derivatives were used to prepare a series of CDs with tunable emission ranging from blue to near infra-red (NIR) excitable at 400 nm [66]. Until now, various hetero atoms have been introduced with different synthesis methods to find CDs emitting in the red/NIR region for bioimaging. Some of these examples are summarized in Table 1. Moreover, the doping of CDs with lanthanide ions like ytterbium and neodymium was also reported by Wu et al. via hydrothermal approach [67]. Synthesized CDs not only demonstrated the excitation dependent photoluminescence behavior with emission from 443 to 552 nm, but also NIR emission of Yb³⁺ and Nd³⁺ centered at 998 and 1068 nm respectively. Among various red and far-red emitting CDs, the highest quantum efficiency of 86% is seen in the one prepared by using N, N-dipropyl-p-phenylenediamine as carbon precursor via solvothermal synthesis [68]. However, these CDs were proposed for application in white light- emitting diode. Considering its highest quantum yield this CD can prove to be a very efficient bioimaging probe. Various strategies used to prepare CDs with NIR red shift emission are summarized in Fig. 1.

In comparison to visible/ NIR-I emitting imaging probes, NIR-II (1100-1600 nm) exhibits significantly low tissue autofluorescence and scattering which enables imaging in deep tissue with high spatial

### Table 1

| Carbon precursor                  | Method          | Emission wavelength (nm) | Quantum yield (%) | Reference |
|----------------------------------|-----------------|--------------------------|-------------------|-----------|
| Formamide, citric acid           | Microwave       | 640                      | 22.9              | [71]      |
| Lemon juice (without pulp)       | Solvothermal    | 631                      | 28                | [72]      |
| Citric acid, ethanediamine, formamide | Solvothermal    | 627                      | 53                | [73]      |
| N,N-dimethyl, N, N-diethyl-, and N, N-dipropyl-p-phenylenediamine | DMF | 637, 642, 645 | 86 | [68] |
| Citric acid, urea, sodium fluoride | Microwave       | 600                      | 1.2               | [74]      |
| Polythiophene derivatives        | Hydrothermal    | 680                      | 5.4               | [65]      |
| Spinach                          | Solvothermal    | 680                      | 15.34             | [75]      |
| Citric acid + ethylenediamine + (Ytterbium, neodymium) | Hydrothermal | 998 and 1068 | 52.32 | [67] |
| Citric acid, urea                | Solvothermal DMSO | 720                      | 0.2               | [76]      |
| Glutathione formamide            | Microwave       | 683                      | 16.8              | [77]      |
**HETERO-ATOM DOPING**

**BY USING VARIOUS CARBON PRECURSOR**

(a) Chemical reaction and resulting carbonized polymer dots.

(b) Optical spectra showing intensity and wavelength.

(c) Schematic of watermelon and juice production.

(d) Absorption and emission spectra with wavelength.

(e) Chemical structures and molecular diagrams.

(f) Photoluminescence intensity vs. wavelength graph.

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**VARYING THE SIZE OF CDs**

(g) Reaction pathways for PT1 and PT2.

(h) Normalized fluorescence spectra.

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**LANTHANIDE HYBRIDIZATION**

(i) Chemical structures and reaction conditions.

(j) Emission intensity versus wavelength.

(k) Excitation and emission spectra for lanthanide complexes.

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*(caption on next page)*
resolution [78]. Hence, an increase in the development of luminescent materials displaying emission in NIR-II region is seen [79–81]. However, reports related to NIR II emitting CDs as bioimaging probes are quite a few. The first report was shown by Hao and coworkers where the CDs were synthesized hydrothermally by watermelon juice and analyzed for NIR-II in-vivo bioimaging in mice [69]. The synthesized CDs presented an emission in the range of 1000-1400 nm when triggered by 808 nm light with QY of 0.4%. Wang’s group also presented a CD based NIR-II fluorescence agent doped with nitrogen and boron using 3-aminoephenylnboryonic acid monohydrate as carbon precursor via solvothermal approach [70]. The doped CDs displayed the maximum emission at 1000 nm upon excitation with 808 nm light. Finally, the study revealed successful in-vivo NIR-II imaging of the internal organs and blood vessels.

Two-photon bioimaging (TPB) is another approach that was recently explored to attain imaging with minimized tissue autofluorescence, deep penetration, and reduced photodamage to the tissue [13,82]. TPB commonly utilizes NIR laser with femtosecond pulse rate, which allows the excitation of luminescent materials with two photons on lower energy to generate emission corresponding to single photon excitation. Presently, various CDs have been fabricated as two-photon excitable bioprobes. In 2007, Cao et al. were the first to report the use of CDs for multiphoton bioimaging on human breast cancer cell line [83]. Subsequently, Zhu et al. demonstrated the two photon excitability of CDs with the emission in the range of 400–500 nm inside the mouse osteoblastic cell line upon illuminating with 808 nm laser [84]. Nitrogen doped CDs were synthesized by Liu et al. using dimethylformamide as nitrogen precursor exhibiting absorption cross section of 48,000 GM which is the highest value for any carbon based luminescent material [85]. Interestingly, imaging penetration depth of 1800 μm was achieved in phantom tissue, which is beyond the fundamental imaging depth limit for TPB.

Two photon triggerable NIR-emitting CDs with an emission peak at 683 nm and QY of 16.8% was first reported by Lin’s group. Two photon excited emission spectra analysis demonstrated excitation wavelength independent emission ranging from 800 nm to 1000 nm. The optimal wavelength of excitation was reported to be ~ 850 nm. In vitro imaging analysis in MCF-7 cells showed the deep red light from the internalized CDs upon two-photon excitation. Also, sulphur and selenium codoped CDs prepared via hydrothermal method presented NIR emission in the range of 600-800 nm with a peak maxima at 731 nm independent of excitation wavelengths [16]. Synthesized CDs were also able to be efficiently excited by two photons of 880 nm and fluorescence was also seen in the HeLa cells internalized with CDs.

Different imaging modalities have their advantages and disadvantages. For instance, fluorescence imaging is highly sensitive, however it lacks spatial resolution due to poor penetration of photons in the tissue. Apart from being applied as a fluorescence imaging probe, CDs can also be used as a photoacoustic (PA) imaging probe [56,87]. PA imaging works on the principle of generation of acoustic waves upon absorption of light, therefore, it provides high resolution and contrast. On the other hand, metal doping of Gd, Yb, Mn, and Fe in the CD crystal is used to introduce contrast for magnetic resonance imaging [88–90].

Application of carbon dots in cancer treatment

Photodynamic therapy

Initially, CDs were seen as impressive fluorescent imaging probes with functionalizable surface for targeting and delivering drugs. CDs were also found to possess intrinsic therapeutic properties by the virtue of their band structure and interactions at the interface that can be implemented in cancer therapy. Photosensitization is one such property of CDs that has been widely explored in recent years. One of the applications of photosensitization is photodynamic therapy (PDT). It utilizes a photosensitizer (PS), which is capable of transferring energy to oxygen molecules in its vicinity under excitation of a specific wavelength of light and generates reactive oxygen species (ROS). The highly reactive species like $O_2^-$, $O_2^*$, $^*O_H$, and $H_2O_2$ can kill cancer cells or induce cell apoptosis specifically at the site of illumination. One of the early reports related to CDs for PDT demonstrated the blue light activated ROS production by the CDs whose surface was passivated with polyethylene glycol [91]. Later, Markovic and coworkers showed the killing of U251 human glioma cells by ROS generation upon photoexcitation of CDs [92]. These findings generated great interest in the scientific community to explore the potential CDs as a PS for PDT against cancer. Thereafter, Han and coworkers studied the CDs mediated PDT via white light of 400-800 nm irradiation [65]. In-vivo PDT examination on mice showed tumor suppression in mice without regrowth Fig. 1 (a-d). However, CD based PS has low therapeutic potential for deep-seated tumors because of the absorption in visible spectra of light. Therefore, the same group designed CD nanosphere by the self-assembly of individual CD as building block, producing NIR-light responsive PDT [93]. This assembly of CDs led to the ~50 nm red shift in the absorption and even enhanced the NIR emission at 730 nm.

The photosensitization property of CDs is highly dependent on the selected carbon precursor and synthesis. Nevertheless, intrinsic photosensitization properties can also be appended in the CDs by using porphyrin based compounds during the carbonization process. Various porphyrin derivatives have been used such as metals or metal-free tetra (meso-aminophenyl) porphyrin [94], triphenylporphyrin [95], and phoephyrin [96]. Natural carbon sources can also be used to prepare CDs for PDT, like Mobin and coworkers synthesized nitrogen doped CDs using Vigna radiata sprouts as carbon precursor and ethylenediamine as nitrogen source [97]. These examples highlight the potential of CDs to be applied as theranostic agents for cancer.

Photothermal therapy

Another photon induced cancer treatment approach is photothermal therapy (PTT) where photonic energy is transduced into heat energy. This leads to the increase in temperature at the area of illumination thereby killing the cells. In recent years, various studies have found CDs to have high efficiency for photothermal conversion. For example, Ge et al. hydrothermally prepared CDs with polyliphene phenylpropionic acid (PPA) which exhibited high photothermal effect with 38.5% efficiency rate [98]. Additionally, the prepared CDs could also be used for fluorescence and photo acoustic imaging of tumors in mice under 671 nm irradiation. The temperature of the tumor site injected with CDs reached 60 °C upon irradiation. Another study developed red emitting CDs with critic acid and formamide using microwave assisted heating
The CDs presented a respectable quantum yield of 22% with a photothermal conversion efficiency of 43.9%. Separately, another group reported N-S doped CDs with high photothermal conversion efficiency of 59.2% at 655 nm [76]. Also, the CDs showed minimal toxicity in-vivo after intravenous admistration, with intense NIR fluorescence and PA signals (Fig. 1e-g). The in-vivo analysis revealed renal clearance and CDs accumulation in tumor.

Fig. 2.

NIR activated CDs showing photothermal properties for deep tissue penetration were also reported in recent years. In one study, Kim et al. produced N-doped CDs using citric acid and nitric acid demonstrating a photothermal effect as well as photoacoustic imaging after the irradiation of 808 nm laser [99]. Additionally, NIR-I emitting CDs made from watermelon juice as mentioned before also demonstrated excellent photothermal efficiency of 30.6% under 808 nm excitation [69]. The highest photothermal conversion efficiency of 42.3% was reported by Huang’s group by using freeze-dried phenolic resin via in situ solid state transformation. The synthesized CDs were highly crystalline and possessed full color emission along with intense photoacoustic signals. Two photon excitation was also used in one of the reports to attain a photothermal effect. It was achieved by sulphur and selenium doping in CDs prepared using polythiophene derivatives and diphenyl diselenide [100]. The CDs were able to simultaneously perform two photon excited fluorescence imaging and PTT of cancer. Hence, the above mentioned photoinduced therapeutic ability of CDs could be applied to form the basis of excellent theranostic agents.

Luminescent materials based on hydroxyapatite

Hydroxyapatite (HA molecular formula Ca_{10}(PO_4)_6(OH)_2) is the primary inorganic constituent of bones and teeth. Due to its superior biocompatibility and osteoconductivity, it is widely implemented for bone and teeth reconstruction. The nanometric form of HA can also be synthesized by various methods and it is referred to as nano-hydroxyapatite (nHA). Owing its nanometric size range, nHA are nanocrystals with a large surface area, which make them great drug-carriers to deliver anti-tumor medication. Additionally, HA crystal lattice allows the substitution of plethora of cationic species, leading to the modification of physical and chemical properties of HA. Luminescent properties can be introduced in nHA by replacing calcium (Ca^{2+}) ions in the crystal lattice with various luminescent rare-earth elements. HA has a hexagonal crystal structure and it is mainly consisting of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) with a Ca:P ratio of 1.67 [101]. Due to its unmatched biocompatibility, various rare-earth doped luminescent nHA have been studied for their potential application in biomedicine [102]. Luminescent nHA can be synthesized with different approaches such as Sol-gel, combustion, hydrothermal and co-precipitation. In addition, continuous efforts have been made to control the morphology, size, shape, porosity, and degree of agglomeration by studying new synthesis routes or by modifying the preexisting ones. Recently, environment friendly synthesis techniques have also been developed such as bio-mimetic synthesis, hydrothermal synthesis, molten-salt synthesis, and template synthesis. Each approach gives rise to varying morphology and luminescent properties as can be seen in Table 2. Moreover, nHA can be hybridized with other biocompatible materials and polymers to produce versatile drug delivery platforms [103-106]. However, to synthesize luminescent material based on nHA the most widely used synthesis techniques are precipitation and hydrothermal. Wang et al. studied the effect of synthesis conditions on the morphology of nHA produced by precipitation route [107]. Various morphologies of nHA such as sphere, rod, needle, and wire were produced by changing the pH and the temperature of the reaction. Similarly, Yang et al. were able to obtain nanorods, microsphere, hexagonal prism and hollow flower on nHA by adjusting reaction parameters (concentration of reagents and reaction time) of the hydrothermal synthesis [108].

Fig. 2. Photoinduced therapy by CDs. In-vivo imaging and PDT (a) Bright-field image and (b) red-fluorescence image after subcutaneous injection of GQDs in different areas. The excitation wavelength was 502-540 nm, and the collected fluorescence channel was 695-775 nm. (c) Photographs of mice after various treatments on the 1st, 9th, 17th and 25th day. (PDT: GQDs+ light irradiation; C1: GQDs only; C2: light irradiation only.) (d) Time-dependent tumor growth curves (n = 5) after different treatments. P < 0.05 for each group. Reprinted with permission [65]. Photothermal therapy via intravenous injection based on CDs e) NIR fluorescence images of mice’s bodies after intravenous injection of CDs (0.2 mL, 1000 µg mL⁻¹) at various time points; f) Photographs that document H22 tumor development on several days in live mice under various treatment conditions; g) Tumor growth curves of H22 tumors in mice and survival rates of the groups after therapy. Reprinted with permission [76].
Nanohydroxyapatite (nHA) is widely used for cancer detection and treatments due to its outstanding properties. Firstly, upon dispersion in water, hydroxyl group on the surface dissolves exposing the positively charged rich Ca\(^{2+}\) sites. This behavior allows the absorption of acidic protein such as human serum albumin [131]. Secondly, HA is the main component of the bone matrix and it is a bioactive material which affects the hexagonal structure. Therefore, Ca\(^{2+}\) can easily form chemical bonds with oxygen of any biomolecule. Besides, HA as a host allows substitution of varieties of cation and anions without significantly affecting the hexagonal structure. Therefore, Ca\(^{2+}\) can not only be replaced by isovalent ions like Sr\(^{2+}\) but also with multivalent ions like Eu\(^{3+}\), Tb\(^{3+}\), Gd\(^{3+}\), Sm\(^{2+}\), Nd\(^{3+}\) [132]. Various studies have doped lanthanide ions in hydroxyapatite crystal to study the luminescence behavior and to apply the attained luminescence property for biomedical imaging [109,110,115,133–137].

The development of europium doped mesoporous nHA for drug delivery was reported [138]. In this study, the luminescent nHA was loaded with ibuprofen (IBU) and the drug release was analyzed. Interestingly, it was found that the luminescence of Eu\(^{3+}\) varied with the release of IBU from the pores and the drug release could be easily monitored. Later in 2010, Zhang et al. reported the synthesis of mesoporous luminescent strontium doped nHA nanorods [139]. These nanorods demonstrated intense blue emission under UV excitation along with the loading and controlled release properties for IBU, showing the potential application of luminescent nHA in the fields of drug delivery and disease therapy. In another research, nanoplates of Gd\(^{3+}\) and Eu\(^{3+}\) co-doped nHA were studied for the luminescent behavior and potential applications for in vitro and in-vivo imaging [140]. The study revealed that nHA host led to the luminescence enhancement with the increase in Gd doping due to the efficient energy transfer from Gd\(^{3+}\) to Eu\(^{3+}\). Moreover, the nanocomposite depicted great biocompatibility and biodegradability with 65% degradation after 72 h and exhibited successful cell labeling and in-vivo imaging (Fig. 2a-e). Gadolinium doping in nHA was also performed by Liu et al. to produce nHA nanorods for T1 contrast imaging and drug delivery [141]. In-vivo experiments showed that Gd-nHA is an excellent MR contrast agent (Fig. SF-i). Doxorubicin (DOX) loaded Gd nHA showed better tumor treatment than free DOX and no significant damage to the major organ was observed. The above mentioned studies show that nHA is a great host material to develop luminescent material with enhanced emission. Apart from that nHA based material could be structurally modified to different morphology with pores possessing in-vivo biocompatibility.

One of the limitations of the luminescent material with excitation and emission falling in the UV–visible light region (300–800 nm) is failing to penetrate deep in the tissue due to scattering and autofluorescence issues discussed in the previous section. Therefore, for high tissue penetration, biological optical window in the range of 800–1300 nm is optimum [78]. Thus, new generation of nHA that can be excited and emit in highly penetrating near infra-red region are developed. Neodymium doping was performed in nHA to attain the emission at 880 nm, 1060 nm, and 1334 nm upon excitation with 808 nm light [118]. Recently, co-doping of Nd and Yb was performed on nHA and studied for the luminescence property [111]. Photoluminescence analysis showed that the characteristic Yb emission at 980 nm was enhanced in the presence of Nd ions due to efficient energy transfer. Erbium doped nHA was also prepared and was able to produce the emission at 1540 nm. Victor et al. reported Nd doped nHA as a therapeutic platform where a model drug 4-Acetyl salicylic acid (4ASA) was loaded. This theranostic nanoplat form was tested in vitro on colorectal cancer cells and showed the drug release in the pH > 7 [142]. Importantly, the internalized nanoparticles were able to show NIR emission. In another study, a facile synthesis of Nd doped needle-like hydroxyapatite (HA) nanoparticle complexes containing cyclodextrin was reported [143]. The in vitro studies demonstrated that the nanoparticles were able to successfully load and deliver doxorubicin and presented bright NIR emission. Even though this new generation of NIR emitting nHA displayed remarkable results in vitro exhibiting great potential for deep tissue imaging and phototherapy, they still need to be studied in animal models to get better insights.

Apart from doping, luminescence in nHA can also be introduced through conjugation or absorption with fluorescent dyes [116,144]. However, chemical modifications for conjugation of fluorescent dyes are tedious and the dyes also tend to lose fluorescence after consecutive excitations. On the other hand, luminescent Eu-complex with organic
chelators is also used for surface functionalization on nHA [145]. Luminescent nHA could also be formed by hybridizing luminescent nanoparticles with nHA to form a composite material. For instance, Liu et al. fabricated a composite of up conversion magneto-luminescent Na(Y/Gd)F$_4$:Yb$^{3+}$,Er$^{3+}$, and porous HA fiber [146]. This composite was proposed for multimodal imaging (MRI and luminescence) along with cargo carrying ability. More examples of luminescent hydroxyapatite for biomedical application are summarized in Table 3.

Although, nHA do not possess intrinsic cancer treatment photosensitization properties like CDs, the release of calcium ions due to degradation of nHA at physiological pH has a positive influence on cancer treatment. Intercellular calcium ions concentration plays an important role in homeostasis of the cell. But under specific conditions overload of intercellular calcium ions can trigger pathways that release apoptotic factors leading to cell death [147]. For example, Xiaoyo et al. studied the therapeutic effect of doxorubicin loaded nHA against gastric adenocarcinoma [148]. The observations showed the simultaneous release of drug and calcium ions inside the treated cells with the enhanced therapeutic effect. Besides, the level of sodium ions is also crucial to maintain stability of the cell and cancer cells contain more sodium ions compared to normal cells. Each molecule of HA can scavenge three sodium ions that disrupt the homeostasis in the cancer cell leading to triggered apoptosis. This phenomenon was seen when lithium doped nHA to treat liver cancer cells (HepG2). An increased rate of apoptosis in the treated liver cancer cells (HepG2) was observed with the decrease in the intercellular sodium level [149].

Cancer targeting strategies

The uptake of nanoparticles can be selectively enhanced in cancer cells by the use of targeting strategies. It allows the desired therapeutic response only at the targeted site at lowered drug doses minimizing the drug associated side effects in normal cells. Cancerous cells grow abnormally at faster rates and require high nutrition. This induces angiogenesis forming wide and leaky blood vessels around them. The abnormalities in the formation of basement membranes in the blood vessels are responsible for the leaky vasculature, facilitating the efflux of nanoparticles around the tumor through passive diffusion. Furthermore, due to the lack of well-defined lymphatic network in the tumor, the localized nanoparticles have higher retention time compared to the normal cells. This phenomena is termed as enhanced permeability and retention effect (EPR) which improves the nanoparticle accumulation in tumors. The EPR effect is highly dependent on the blood circulation half-life of nanoparticles that is governed by their physicochemical properties including size, shape, and surface charge.

Alternatively, the nanoparticles can be actively targeted to specific

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Fig. 3. In-vivo fluorescent imaging of noncalcined HAP:Eu/Gd (2:1.5) nanocrystals in BALB/c-nu mice (a) with no injection, (b) with injection in enteroceola, (c) 5 min after vein injection, and (d) 1.5 h after vein injection. e) Tissue biodistribution of noncalcined HAP:Eu/Gd (2:1.5) nanocrystals in BALB/c-nu mice via vein injection for about 3 h. Reprinted with permission [140]. f) MRI (above) and the color-mapped images (below) of mice postinjection and 15 min. after injection of NHA:Gd-DOX at 10 mg/kg. Scale bar, 5 mm. g) Tumor growth of mice intratumorally injected with saline, free DOX and NHA:Gd-DOX, for 28 days. (**P < 0.001). h) H&E staining of major organs 28 days after intravenous injection of saline, NHA:Gd-PEI, free DOX, and NHA:Gd-DOX. All scale bars, 50 μm. Reprinted with permission [141].
Similarly, other cellular receptors such as transferrin, integrin, folic acid functionalized nanoparticles show improved tumor uptake. Demands, the folate receptors are overexpressed on cancer cells, thus, towards the folate receptors of the cells. Due to the increased nutritional nanoparticles is functionalized with a targeting moiety by chemical conjugation techniques. For example, folic acid shows high specificity conjugated on CDs by Ghosh et al. to target the triple negative breast cancer peptide for surface modification of nanoparticles [152, 153]. Combining both active and passive targeting that facilitates the delivery would facilitate tumor uptake in vivo due to the biological barriers. Noticeably, uptake via EPR effect allows ~3% cellular internalization, asialoglycoprotien have been utilized to attain active cancer targeting.

Cancer targeting using carbon dots

Carbon dots (CDs) can be targeted to the cancerous cells via passive and/or active mechanism. Jia et al. prepared manganese doped CDs (Mn-CDs) for bimodal imaging and enhanced photodynamic effect in solid tumors [86]. The Mn-CDs (~4 nm) were aggregated via self-assembly with DSPE-PEG of ~426 nm size to enhance the water solubility and biocompatibility. The aggregated Mn-CDs presented high accumulation at the tumor site after 6 h of intravenous administration in the 4T1-tumor-bearing mouse. In another work, nitrogen and boron doped CDs (~4.7 nm) successfully demonstrated the photothermal therapy in C6 rat glioma cell xenografted nude mice [70]. The biodistribution of CDs was realized in normal mice showing the high accumulation in liver and kidney after 120 min post-injection. NIR-II imaging was also performed in tumor-bearing mice after 24 h intravenous injection. Moreover, their study also showed that a single systemic injection of CDs under NIR light for 5 min completely suppressed the tumor growth. The study highlighted the use of CDs in image guided cancer therapy and treatment monitoring. Similarly, high tumor accumulation was observed in the HeLa and 4T1 tumor-bearing mice after intravenous administration of red and NIR emissive CDs, respectively, through EPR effect that allowed the efficient photothermal treatment of the tumor [76, 98]. This data shows the ability of CDs to passively accumulate at tumor site. Conversely, some recent studies have analysed the CDs conjugated with receptor specific ligand to demonstrate active tumor targeting. Karakoçak et al. covalently conjugated hyaluronan to CDs, which is specific to CD44 receptors. The actively targeted CDs were intravenously administered in the breast cancer bearing mice expressing High CD44 receptors. The actively targeted CDs were intravenously administered in the breast cancer bearing mice expressing High CD44 receptors. In vivo fluorescence imaging showed profound accumulation of targeted CDs at tumor site with high signal to noise ratio [154]. Also, folic acid was functionalized on CDs by Ghosh et al. to target the triple negative breast cancer systemically, through EPR effect that allowed the efficient photothermal treatment of the tumor [76, 98]. This data shows the ability of CDs to passively accumulate at tumor site. Conversely, some recent studies have analysed the CDs conjugated with receptor specific ligand to demonstrate active tumor targeting. Karakoçak et al. covalently conjugated hyaluronan to CDs, which is specific to CD44 receptors. The actively targeted CDs were intravenously administered in the breast cancer bearing mice expressing High CD44 receptors. In vivo fluorescence imaging showed profound accumulation of targeted CDs at tumor site with high signal to noise ratio [154]. Also, folic acid was functionalized on CDs by Ghosh et al. to target the triple negative breast cancer systemically, through EPR effect that allowed the efficient photothermal treatment of the tumor [76, 98]. This data shows the ability of CDs to passively accumulate at tumor site. Conversely, some recent studies have analysed the CDs conjugated with receptor specific ligand to demonstrate active tumor targeting. Karakoçak et al. covalently conjugated hyaluronan to CDs, which is specific to CD44 receptors.
normal animals. Although there are reports showing nHA as drug delivery vehicle, we could not collect much information on lanthanide doped nHA nanoparticles in cancer targeting. Liu et al. analyzed the DOX loaded and Gd-doped nHA nanoparticles in breast tumor models. As the intratumoral injection was given, no biodistribution data was collected. Nevertheless, the nanoparticles showed enhanced toxicity in cancer cells as well as in tumor bearing mice as compared to the free drug [141]. Various reports have shown the intrinsic capability of nHA as antitumor agent. It selectively inhibits the proliferation and induces apoptosis in various cancer cells including glioma cells [157], breast cancer cells [158], osteosarcoma cells [159], liver cancer cells [160], and colon cancer cells [161]. Recently, Zhang et al. analyzed the translational value of nHA for bone regeneration and proliferation suppressive effect against cancer cells in vivo [162]. In the study n-HA–releasing scaffold was able to suppress tumor growth and osteolytic lesion along with the promotion of bone regeneration. This data reveals the intrinsic anti-cancerous activity of nHA which can offer an added advantage to toxic or trigger immunological responses when in contact with tissue or biological fluids. When the nanomaterials enter the bloodstream their biodistribution mainly depends on their surface chemistry, interaction with different types of cells, and macromolecules such as proteins, carbohydrates, DNA. These biological interactions eventually lead to the accumulation of nanomaterials in the non-targeted organs causing serious damage. The size, shape, charge, surface chemistry, and hydrophilicity influence the affinity to the different biological proteins leading to opsonization. These opsonized nanomaterials are usually removed from blood circulation via the mononuclear phagocyte system. Therefore, optimization of nanomaterials is done to reduce the protein interaction. The organ accumulation or the excretion pathway of any nanomaterial can be examined in real time by imaging techniques. Alternatively, the organs of mice are collected at different times post-injection. Later, the hematoxylin and eosin (H&E) staining of the tissue sections of the collected organs is studied and compared to the non-treated mice to identify the damage or inflammation.

Several studies reported the real time organ bio-distribution of CDs after intravenous injection in mice. For example, CDs prepared from watermelon juice successfully showed high NIR-II fluorescence signals in the kidney just after a minute of intravenous CDs administration (Fig. 4a). After 6 h of injection, the fluorescence signals in the kidney started lowering, showing an effective renal clearance (Fig. 4b and c). In other work, nitrogen and boron doped CDs were tracked for their circulation in real-time using the NIR emission up to 120 min post-injection. After 120 min the fluorescence signals became higher in the liver and kidneys compared to the other organs however, signals decreased with time (Fig. 4d) [70]. Another study also reported high accumulation of CDs in the liver and kidney after intravenous administration. Moreover, the fluorescence signals at 30 min were found to be much stronger than those from other organs [76]. Similarly, strong NIR fluorescence was also observed in the urine samples collected from the mice at 30 min and 1 h post injection demonstrating the quick renal clearance of the tested CDs. Unlike the semiconductor based luminescent materials, CDs identification in the body by inductively coupled plasma mass spectrometry (ICP-AES) is limited. Additionally, fluorescence may provide false positive/negative signals as high CDs concentration decreases the fluorescence output. Other than that fluorescence can also be masked by biological fluids. Very few reports have used both fluorescence imaging and ICP-AES techniques to track the biodistribution of the CDs. For example, Mn-doped CDs were monitored by fluorescence imaging as well as by analyzing the Mn concentration ICP-AES in various organs post injection [86]. The Mn-CDs exhibited higher uptake in the liver and lungs which eventually decreases over time. A similar biodistribution trend was observed by the evaluation of Mn-CDs uptake by ICP-AES. It is worth mentioning that in most of the reports the histological analysis of tissue sections of various organs obtained from CDs treated mice did not show any significant tissue damage or inflammation (Fig. 4e) [70,86,87,98,163]. Thus, CDs present

Biocompatibility and biodistribution of CDs and nHA

Biocompatibility of nanomaterials is a very important factor to estimate its complete therapeutic potential, which eventually paves the way toward clinical translation. Biocompatible material do not induce toxicity or trigger immunological responses when in contact with tissue or biological fluids. When the nanomaterials enter the bloodstream their biodistribution mainly depends on their surface chemistry, interaction with different types of cells, and macromolecules such as proteins, carbohydrates, DNA. These biological interactions eventually lead to the accumulation of nanomaterials in the non-targeted organs causing serious damage. The size, shape, charge, surface chemistry, and hydrophilicity influence the affinity to the different biological proteins leading to opsonization. These opsonized nanomaterials are usually removed from blood circulation via the mononuclear phagocyte system. Therefore, optimization of nanomaterials is done to reduce the protein interaction. The organ accumulation or the excretion pathway of any nanomaterial can be examined in real time by imaging techniques. Alternatively, the organs of mice are collected at different times post-injection. Later, the hematoxylin and eosin (H&E) staining of the tissue sections of the collected organs is studied and compared to the non-treated mice to identify the damage or inflammation.

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Fig. 4. CDs based bioimaging and biocompatibility (a) Time-dependent bioimaging of mouse treated with CDs (20 μg/g) through the tail vein. (b) Digital camera photographs and corresponding in vitro phantom imaging of the urine samples collected at different periods. (c) CDs cumulative urine washout curve. Reprinted with permission [89]. d) Biodistribution of N-B-GQDs in various organs/tissues of nude mice receiving N-B-GQD injection, determined at various time points post-injection. (e) H&E stained tissue sections of mouse heart, kidneys, liver, lungs, and spleen obtained from non-injected animals (control, top row) and those injected with N-B-GQDs (1 mg/mL, bottom row). The scale bar represents 50 μm. Statistical analysis was performed using the Student’s two-tailed t-test (**p < 0.01, ***p < 0.001). Reprinted with permission [70].
impressive traits of biocompatibility. Nevertheless, the small size of CDs suffers from short blood circulation half-life due to the high renal clearance. This issue could be address by inducing CDs aggregation using different self-assembly of polymer/proteins or making nano-composites using different nanoparticles.

Hydroxyapatite is a biomaterial that promotes cellular conductivity and acts as a good framework for fibrin network. Thus, it is used as a main ingredient to prepare bone grafts for clinical use. While, nano-hydroxyapatite (nHA) possess 30 to 50% more surface area than micrometric counterpart, which increases the wettability, thus improving the bone formation with higher number of blood cells. Moreover, nHA also attained great attention in dentistry [164]. Various studies also demonstrated excellent in vivo biocompatibility of the nHA alone or in the form of a composite for application in drug delivery [165–167]. Therefore, the biocompatibility of nHA is very well known and tested in different cell lines under different conditions. However, there are very few reports about the biocompatibility of lanthanide doped nHA. Xie et al. demonstrated the biocompatibility of Eu and Gd doped HA nanocrystals for in vivo imaging. The nanocrystal showed less than 5% hemolysis rates at different concentrations in blood while cytotoxicity evaluation in L02 cells at different concentrations (0.0125–0.2 mg/mL) did not affect the cell proliferation rate up to 3 days. These results demonstrated the high biosafety and biocompatibility of nanocrystals. Moreover, intracellular degradation experiments depicted the dissolution of nanoparticles in the acidic microenvironment of cells. A cumulative degradation of ~65% after 72 h of cell treatment was achieved highlighting the high biodegradability of nanocrystals. In-vivo imaging in normal mice displayed the presence in blood circulation for at least 3 h after intravenous injection while maximum uptake was found in the liver. The accumulation in reticuloendothelial system could be lowered to improve blood circulation half-life by providing the stealth property after surface modification with biopolymers [140]. Liu et al. also performed the in-vivo toxicity analysis of Gd-doped HA nanorods that showed no obvious damage to major organs in nanorods treated mice [168]. Similarly, Li et al. found good in vivo biocompatibility of HA doped with Yb/Ho possessing upconversion emission under NIR excitation. Overall, the studies demonstrate highly biocompatible and biodegradable nature of Ln-doped nHA with minimal systemic toxicity in vivo.

Concluding remarks and future perspectives

CDs and nHA stand out as promising nanomaterials for the design of new luminescent nanomaterials for biomedical application. Both the materials are inherently biocompatible and biodegradable along with intrinsic anticancerous activity, which lacks in many other inorganic and synthetic nanoparticles. Besides their synthesis is facile and cost effective due to the easy availability of raw materials. Rather some reports show their synthesis using biowaste further reducing the cost of production. Moreover, both the nanostructures allow the tuning of photoluminescence properties in the NIR region, beneficial for obtaining high signal to noise ratio in bioimaging. With no doubt these materials great potential for clinical translation. Thus, this literature review summarizes the recent progress towards the development of CDs and nHA based biocompatible and biodegradable materials as nano-phosphors for cancer diagnosis and therapy. First, the different synthesis methods and their effect on physicochemical properties (size, morphology, surface chemistry, optical properties, along with others) are covered. Then, the emphasis is made on the application of CDs and nHA as innovative tools for fluorescence imaging as well as therapy (drug/gene delivery, PDT, PTT). In particular, the design of these materials and tuning of the emission range to operate in the biological window for deep tissue penetration is discussed. Finally, tumor targeting strategies, biocompatibility and biodistribution of these materials is summarized. Although these materials are studied extensively, there remain various challenges. For instance, in case of CDs the design, controlling, and quantifying the chemical structures, the structure-property relationships, the molecular mechanism as well as the control of the photoluminescence property are still unknown. However, CDs offer cost effective synthesis with remarkable properties as fluorophore and are a better alternative to heavy metal based quantum dots. Meanwhile, there still exist room for the design of new CD assemblies with bioactive and biomimetic cells, organelles, self-assembling proteins/peptides to regulate their size and combat the existing challenges like active targeting, tumor uptake, short blood circulation half-life and instability in complex bio-environment. Further emphasis could be made towards the assembly of CDs with therapeutic enzymes as their structure could provide a good medium for substrate channeling as is reported for other carbon based nanomaterials like carbon nanotubes and graphene oxide [169,170]. Moreover, most therapeutic enzymes are responsive to tumor microenvironment (TME) thus, it would be interesting to observe the effect of photoluminescence properties in the close vicinity of different enzymatic reactions, which could lead to the design of TME responsive CD based materials. The intrinsic photosensitization property (used in PDT and PTT) of CDs could also be applied towards the design of smart theranostics agents.

Like CDs, nHA does not possess intrinsic luminescence properties, however, considering its excellent biocompatibility, biodegradability, non-immunogenic properties and most importantly, the unique apatite structure to accommodate various lanthanide doping, it has successfully been applied as biocompatible luminescent probe for fluorescence imaging of cancer cells. Advancement in luminescent nHA as multimodal/multifunctional agent is still in infancy and requires further attention. Also, the applicability of nHA as smart biomaterial for triggered drug release requires more work in the future. Recently, the hybrid materials of CDs and nHA were also reported endorsing the high load bearing ability and strong bioactivity for bone tissue engineering with excellent biocompatibility [171]. Mesoporous nHA shows great potential for high drug loading owing to larger surface area, thus, amalgam of CDs and nHA as nanohybrids could be of potential use for development of delivery vehicle with luminescent property without the need of heavy metal ions. Moreover, the issue of small size CDs could also be resolved. Furthermore, the use of nHA as core for TPI and PDT applications are still limited, which could also be studied in the future.

Overall both CDs and nHA serve as excellent biomaterials to construct various luminescent nanomaterials with varied applications. More focus is required in the CDs and nHA based materials to work in NIR region with high quantum yield. As discussed above, the future requires more exploration of these nanomaterials as theranostics and multifunctional agents for cancer. Moreover, the synthesis and design of such nanomaterials should be facile and reproducible for bulk production, one of the major requirements for clinical translation.

CRediT authorship contribution statement

Prakhar Sengar: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. Kanchan Chauhan: Data curation, Formal analysis, Investigation, Writing – original draft. Gustavo A. Hirata: Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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