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Recent advances in carbon dots: synthesis and applications in bone tissue engineering

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Bone tissue engineering (BTE), based on the perfect combination of seed cells, scaffold materials and growth factors, has shown unparalleled potential in the treatment of bone defects and related diseases. As the site of cell attachment, proliferation and differentiation, scaffolds composed of biomaterials play a crucial role in BTE. Over the past years, carbon dots (CDs), a new type of carbon-based nanomaterial, have attracted extensive research attention due to their good biocompatibility, unique optical properties, and abundant functional groups. This paper reviews recent research progress in the use of CDs in the field of BTE. Firstly, different preparation methods of CDs are summarized. Then, the properties and categories of CDs applied in BTE are described in detail. Subsequently, the applications of CDs in BTE, including osteogenesis, fluorescence tracing, phototherapy and antibacterial activity, are presented. Finally, the challenges and future perspectives of CDs in BTE are briefly discussed to give a comprehensive picture of CDs. This review provides a theoretical basis and advanced design strategies for the application of CDs in BTE.

1 Introduction

Bone defects caused by infection, trauma, and tumors are a common clinical problem in the medical field. Traditional autografts are considered to be the current gold standard for bone regeneration.[1] However, autografts face some drawbacks, such as materials restrictions and donor trauma, and allografts may cause severe immune rejection. Other common bone repair techniques, such as distraction osteogenesis, are difficult to popularize due to their limited indications, complicated operations, long procedures and high cost. This huge gap needs to be bridged through bone tissue engineering (BTE). BTE uses a combination of seed cells, scaffold materials and growth factors and to promote regeneration of the target bone tissue. Thereinto, scaffolds play a key role as a template for cell interactions and the formation of bone-extracellular matrix.[2] At present, there have been a great deal of basic investigations into scaffold materials for bone regeneration.[3–5] However, the questions of how the cells are distributed after transplantation, whether they can proliferate and differentiate, and the relevant mechanisms remain to be solved, and have become the main bottleneck limiting development. Thus, finding tracer methods that are safe, non-invasive and effective in regulating osteogenic differentiation is the key to solving these problems. Most conventional scaffold materials such as common metals,[6] ceramics[7] and polymers[8] are not fluorescent in nature, meaning that cells cannot be labeled simultaneously without other fluorescent dyes and nanoprobes, which is not conducive to monitoring morphological changes in cells and tissues in real time through imaging. In recent years, carbon-based fluorescent nanomaterials, especially carbon dots (CDs), have rapidly gained unprecedented attention in the biomedical and materials science fields as a result of their remarkable physicochemical and optical properties due to their nanoscale effects.

CDs are a group of carbon-based spherical nanoparticles with a diameter of 2–10 nm, and are mainly classified as carbon quantum dots (CQDs), carbonized polymer dots (CPDs) and graphene quantum dots (GQDs), based on their formation mechanism and characteristics[9,10] (Fig. 1). CQDs are usually spherical nanoparticles with amorphous to nanocrystalline cores.[11] CPDs are a class of CDs that include a low-carbonization degree polymer dots and fluorescent nonconjugated polymer dots with a high cross-linking degree.[12] GQDs are described as nanoparticles consisting of either a single layer or a few layers of graphene.[13] Compared with other semiconductor quantum dots, CDs, which are mainly composed of

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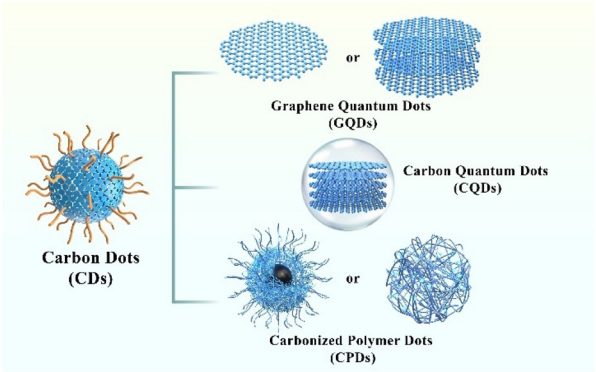

Fig. 1 Classification of CDs: graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbonized polymer dots (CPDs).

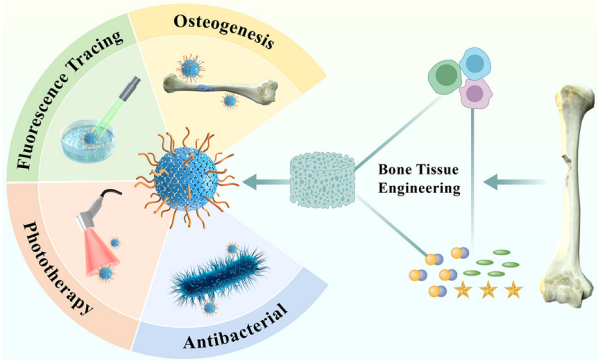

Fig. 2 Applications of CDs as scaffold materials in BTE, including osteogenesis, fluorescence tracing, phototherapy and antibacterial properties.

carbon, oxygen and hydrogen, have unique optical properties, good biocompatibility, low cytotoxicity and facile surface modification. As emerging zero-dimensional carbon nanomaterials, CDs play an important role in the biomedical field, in areas such as biological imaging,[14–18] biological sensing[19–22] and drug delivery.[23–26] Additionally, CDs offer the advantages of excellent biodegradability, induced biomineralization and antibacterial properties. In light of these merits, CDs have broad application prospects in terms of BTE.

Generally, compared to the use of other traditional materials, such as metals, ceramics and polymers, the application of CDs to BTE has several main advantages: firstly, CDs have abundant surface functional groups (*i.e.*, hydroxyl, carboxyl, amino, *etc.*) that can be easily conjugated with other components to achieve rapid bone tissue regeneration; secondly, CDs have unique optical properties that can be used to follow the action and biodegradation of scaffold materials after bone tissue regeneration; thirdly, CDs have excellent photothermal effects that can be used to kill tumor cells, especially bone cancer cells; finally, CDs have good antibacterial properties that can be used to prevent or treat common infections after bone defect repair. Based on these features, CDs are emerging as a candidate material for BTE and have been widely studied by scholars. In a recent report, dexamethasone carbon dots (DCDs) were developed *via* a facile one-pot hydrothermal method using citric acid, ammonium fluoride, and trace amounts of dexamethasone. The study demonstrated that the prepared DCDs had promising applications in the field of bone regeneration and provided a new idea for the development of novel carbon nanomaterials for repairing bone tissue defects.[27]

To date, the widely used carbon-based nanomaterials have been extensively and deeply explored in the field of bone regeneration.[28–32] However, reviews on CDs are still focused on biosensing, bioimaging and related fields;[33–37] a more focused overview of their applications in BTE is still lacking. Therefore, a systematic review of CDs with osteogenic-activity-related factors in the field of BTE may open up new research directions, which is of great significance to the selection of new biomaterials for bone regeneration. First, we will summarize the different preparation methods for CDs. Then, we will describe the properties and categories of CDs applied in BTE. Next, we will discuss existing efforts to use CDs in the field of BTE, including their osteogenesis, fluorescence tracing, phototherapy and antibacterial properties. Finally, we will discuss the shortcomings of CDs and the current efforts underway to address these concerns (Fig. 2).

2 Preparation of CDs

Originally discovered by Xu *et al.* in 2004, CDs were first separated and purified from single-walled carbon nanotubes by gel electrophoresis.[38] In 2006, Sun *et al.* prepared fluorescent carbon nanoparticles with a diameter of 3–10 nm using laser ablation and surface passivation, and gave them the name "carbon dots" for the first time.[39] Since then, research into CDs has progressed exponentially.

After several years of research, the preparation methods for CDs have become rich and varied. Based on the carbon sources used for the preparation of CDs, these methods are broadly divided into "top-down" and "bottom-up" methods. Top-down refers to the decomposition of bulk carbon structures into nanoscale carbon units through physical or chemical means, including arc discharge, laser ablation, electrochemical oxidation, and so on. In the case of bottom-up methods, small organic molecules are selected as reactants to synthesize CDs through carbonization or polymerization techniques, which mainly include hydrothermal carbonization, microwave, combustion, template, and ultrasonic methods. Several commonly used preparation methods are described briefly below.

2.1 Laser ablation method

In laser ablation, carbon nanoparticles on target materials are separated by laser irradiation under certain conditions, and then CDs are obtained by subsequent processing. Sun *et al.* prepared nanoscale carbon particles *via* laser ablation using a mixture of hot-pressed graphite powder and cement as the carbon target. They then obtained CDs with bright fluorescence using nitric acid for surface passivation.[39]

Subsequently, the researchers improved this method by combining laser ablation and surface modification in a single-step reaction, improving the method of preparing CDs.[40] Furthermore, Li *et al.* prepared CDs with visible, stable and tunable photoluminescence (PL) performance *via* laser rapid passivation of carbon nanoparticles in ordinary organic solvent. They proved that passivation by laser irradiation plays a significant role in the origin of PL.[41]

2.2 Electrochemical oxidation method

Using electrochemical cells at normal pressure and temperature, CDs can be prepared by oxidation–reduction reactions at a certain potential. In 2007, Zhou *et al.* first prepared carbon nanocrystals in a degassed acetonitrile solution with 0.1 M tetrabutylammonium perchlorate as the supporting electrolyte.[42] On this basis, Hou *et al.* used a one-step electrochemical method to prepare CDs. The solution formed by mixing sodium citrate and urea was used as the electrolyte, and two platinum sheets were employed as the positive and negative electrodes. The reaction proceeded for about 1 h at a potential of 5 V until the transparent solution turned brown. The product solution was dialyzed for 6 h to obtain the fluorescent CDs with a diameter of 1.0–3.5 nm.[43]

Although CDs prepared by this method have controllable size and high quantum yield without complicated purification and passivation processes, the method requires the preparation of compliant electrodes to provide the applied voltage, which limits its application.

2.3 Ultrasonic method

In this method, large-sized carbon sources are dissected into nanoscale CDs using energy generated *via* ultrasonic technology. Typically, nitrogen-doped CDs (NH_2-CDs), which have wide applications in the field of chemical probes, are prepared by a hydrothermal or reflux method under severe conditions including high temperatures and harsh chemical reactions. This makes the synthesis of NH_2-CDs quite expensive and cumbersome. Thus, Wu *et al.* synthesized NH_2-CDs through an ultrasonic method that greatly simplified their preparation and utilized them as sensing probes for cobalt(II) ions and nucleic acids.[44]

2.4 Hydrothermal method

The hydrothermal method is one of the most preferred methods for the preparation of CDs. In this method, an aqueous solution of the carbon source is placed in a reactor and undergoes hydrothermal reaction at high temperature and pressure to obtain CDs through carbonization, dehydration and other processes. Zhang *et al.* reported a one-step route to prepare water-soluble fluorescent CDs *via* a hydrothermal method using l-ascorbic acid as a carbon source for the first time. In contrast to previous methods, the preparation of CDs by this method does not require strong acid treatment or further surface modification, but their fluorescence quantum yields are only 6.79%.[45] Based on this, researchers have further improved and optimized the hydrothermal method. Yu *et al.* directly synthesized highly photoluminescent CDs with a quantum yield of 37.4% by simple hydrothermal treatment of oatmeal without using any surface passivation or oxidizing agents.[46] Further, Li *et al.* prepared blue luminescent CDs *via* the one-pot hydrothermal reaction of citric acid with poly(ethylenimine); the CDs had a high photoluminescence quantum yield of 48.3 ± 5.3%.[47]

In recent years, various types of biomass have been used as carbon sources to prepare CDs by hydrothermal methods. Biomass carbon sources are eco-friendly products with many advantages, including low cost, environmental-friendliness, accessibility and abundance.[48,49] The biomass sources used include dehydrated shiitake mushrooms,[50] sweet potato,[51] *Punica granatum*,[52] *Saccharum officinarum*,[53] bamboo leaves,[54] and soy milk,[55] among others. Furthermore, this method can achieve high-quantum-yield CDs without further oxidation or passivation. As such, it has become the most popular method for CD preparation.

2.5 Microwave method

The microwave method refers to microwave heating of a small-molecule carbon source to carbonize it and form CDs. Liu *et al.* proposed a one-step microwave-assisted polyol method to fabricate green fluorescent CDs. Using sucrose as the carbon source and diethylene glycol as a high-boiling-point reaction medium, CDs that could emit unique green fluorescence under 360 nm excitation were obtained within 1 minute under microwave irradiation. In addition, these green fluorescent CDs could be effectively taken up by C6 glioma cells, giving rise to potential applications in bioimaging.[56] Chung *et al.* synthesized fluorescent CDs from glucosamine onto a chitosan-polyethylene glycol graft copolymer using microwave irradiation. The resultant CDs demonstrated good dimensional stability and excitation-dependent fluorescence.[57] The microwave method is novel, green and efficient, but the particle size distribution is uneven and the separation and purification is difficult.

3 Properties

Owing to the excellent properties exhibited by CDs, they are widely used in the biomedical field. In this section, we detail the biocompatibility and optical properties of CDs, which make them ideal candidates for use in the medical field, especially in BTE.

3.1 Biocompatibility

The biocompatibility of CDs is an important factor affecting their biomedical applications. Compared with traditional quantum dots, CDs have higher biocompatibility and lower cytotoxicity.[58] A series of *in vitro* cytotoxicity studies showed that CDs exhibited toxic effects at high concentrations, but no significant toxic effects were seen at lower concentrations.[59] *In vivo* experiments showed that CDs can be rapidly excreted through the kidney and/or hepatobiliary system.[60] In addition,

no significant toxicity was observed in the brain, heart, lung, liver or kidneys based on blood biochemical and hematological analyses. Overall, CDs have low toxicity *in vitro* and *in vivo*, and have good prospects for use in nanomedical applications. Nevertheless, there is still an urgent need to determine the appropriate concentration of CDs in cell culture and animal models and their long-term effects on the organism before further clinical application. In addition, the biocompatibility of CDs in the context of BTE has been poorly studied. Therefore, there is a need to pay more attention to the evaluation of biocompatibility and cytotoxicity in order to more fully and accurately assess the practical effects and biosafety when applying them to BTE.

3.2 Optical properties

3.2.1 Absorption. CDs prepared from different precursors show significantly different absorption spectra.[61,62] Nevertheless, most CDs clearly exhibit one or more strong absorption peaks at 200–400 nm, with a tail extending into the visible region. Generally, absorption peaks located in the 230–280 nm region are attributed to π–π* transitions corresponding to the aromatic C=C bonds in the carbon framework; absorption peaks located in the 300–370 nm region are attributed to the n–π* transitions corresponding to the C=O bonds.[63] In particular, some CDs with red or NIR emission possess π-conjugated electrons in the sp^2 domain and the connected surface groups/polymer chains, which leads to long-wavelength absorption in the 500–800 nm range.[64] In general, the absorption properties of CDs are mainly influenced by the types and contents of surface functional groups, structure defects, and the variation of oxygen/nitrogen content.

3.2.2 Fluorescence. Compared with conventional semiconductor quantum dots, CDs usually demonstrate excitation-wavelength-dependence and strong resistance to photobleaching.[65,66] In most cases, the emission wavelength of CDs is longer than the corresponding absorption wavelength, suggesting that the emission energy is lower than the absorption energy. The exact luminescence mechanism of CDs is still under deep investigation. Currently, several possible mechanisms, including quantum confinement effects, molecular state effects, surface state effects, and edge state effects, can be used to explain the fluorescence emission of CDs. Most CDs emit blue or green fluorescence, which limits their further applications in the biomedical field, and researchers have started to try to prepare red- and NIR-emissive CDs for clinical applications. However, since the emission wavelength of CDs is closely related to the surface state, the yields of red- and NIR-emissive CDs are lower than those of ultraviolet CDs. Therefore, more feasible methods to increase the yield need to be developed for wider applications.

4 Categories

Based on their precursors and modification materials, the CDs applied to BTE can be divided into four categories: medicine-derived CDs, element-doped CDs, gene/growth factor-loaded CDs and CD-based composites.

4.1 Medicine-derived CDs

It has been widely shown that CDs prepared from medicines can retain the original pharmacological effects of the medicines.[67] During bone regeneration, dexamethasone can stimulate the upregulation of osteogenic-related genes such as alkaline phosphatase and osteocalcin to promote the differentiation of stem cells into osteoblasts. It is a widely used osteogenic medicine. Therefore, Wan *et al.* synthesized CDs from dexamethasone using a mild one-step hydrothermal method, and the obtained CDs exhibited blue fluorescence under UV light. Both *in vitro* and *in vivo* experiments demonstrated that the CDs retained the pharmacological activity of dexamethasone and had great potential for application in promoting bone regeneration.[27] Similarly, adenosine, a metabolite of ATP, could directly convert hPSCs into functional osteoblasts through the involvement of the adenosine tri-phosphate (ATP) A2b receptor (A2bR). Aspirin, a well-known nonsteroidal anti-inflammatory medicine (NSAID), has been known for decades to have immunomodulatory effects on MSCs and to prevent bone loss. As a proof of concept, Han *et al.* prepared CDs with good biocompatibility *via* a one-step hydrothermal method using adenosine and aspirin as raw materials. Cells containing the bioactive CDs exhibited more efficient osteogenic differentiation behaviour compared to cells treated with either adenosine or aspirin alone.[68]

4.2 Element-doped CDs

By doping with various elements to modify the abundant surface active groups, CDs can be endowed with different specific functions to meet specific biomedical needs. Zinc plays an essential role in bone metabolism as a trace element necessary for the maintenance of normal physiological functions of the body. Thus, Wang *et al.* prepared Zn^{2+}-passivated CDs and explored their optimum dose to promote osteogenic differentiation, providing a good theoretical basis for the application of element-doped CDs in bone regeneration.[69] Similarly, calcium and phosphorus are known to be essential elements in the maintenance of normal bone differentiation and development. They cooperate with parathyroid hormone and calcitonin to promote the expression of genes related to bone differentiation and achieve the regulation of bone cell function. In addition, both elements have an effect on bone metabolism and bone tissue morphology. Based on this, Wu and his team synthesized novel calcium- and phosphorus-conjugated CDs (Ca/P-CDs) *via* a one-pot hydrothermal method using phosphoethanolamine and calcium gluconate as precursors. The resultant Ca/P-CDs facilitated the repair of bone defects and provided a new option for osteogenic nanomaterials.[70]

4.3 Gene/growth-factor-loaded CDs

Since a single material cannot mimic the composition, structure and characteristics of natural bone, the combination of

nanomaterials with related genes or growth factors has become an emerging alternative therapy. CDs with multiple reactive groups on the surface provide a unique surface for carrying therapeutic genes. It is well known that miR-20a, miR-29b, miR-26a, miR-138, and miR-2861 are involved in the process of osteogenic differentiation. Among them, miR-2861 can target the amino-acid-coding sequences of histone deacetylase 5 (HDAC5), which is involved in the degradation of the osteogenic transcriptional factor RUNX2, thus promoting bone formation. Bu *et al.* prepared ascorbic acid-polyethylenimine CDs carrying miR-2861 *via* a microwave-assisted pyrolysis method. They demonstrated that CDs loaded with the osteogenic therapeutic gene miR-2861 have stronger bone regeneration ability *in vivo* and *in vitro*, suggesting great potential for clinical application.[71] Meng *et al.* found that recombinant adeno-associated virus (rAAV) vectors carrying candidate SOX9 or TGF-β sequences efficiently expressed transgenes in cells with the aid of CDs, promoting cell proliferation and cartilage matrix deposition (glycosaminoglycan, type II collagen) and reducing type-I and -X collagen production. These findings provide evidence of the ability of CD-assisted therapeutic rAAV gene delivery to target cartilage repair human bone marrow-derived mesenchymal stromal cells (hMSCs) in future non-invasive and safe applications for treating cartilage injury sites.[72]

4.4 CD-based composites

As a novel type of nanomaterials, CDs can be used in the field of BTE either alone or in combination with other materials to enhance their biological properties. Wang *et al.* developed a novel method to prepare carbon dots/hydroxyapatite/poly(vinyl alcohol) (CDs/HA/PVA) double-network (DN) hydrogels with high mechanical strength, degradability and fluorescence emission. CDs/HA/PVA DN hydrogels with different composite ratios were optimized to have suitable surface functional groups, good compressive strength and viscoelastic behaviour, fluorescence properties, and degradable behaviour, which facilitate their use as visualized bone substitutes in the biomedical engineering field.[73] Lu *et al.* prepared a CS/nHA/CDs scaffold by facile physical mixing and lyophilization. In a series of *in vitro* and *in vivo* studies, the synthesized scaffold showed porous structures that facilitated the adhesion of rat bone mesenchymal stem cells (rBMSCs) by promoting the gene expression of the focal adhesion pathway. Compared to the CS/nHA scaffold, the CS/nHA/CDs scaffold significantly promoted the formation of vascularized new bone tissue *in vivo*. In addition, the scaffold effectively induced tumor cell death *in vitro* due to doping with CDs. After implanting the scaffold into an osteosarcoma-bearing mouse model, this highly malignant tumor was significantly inhibited *in vivo* under NIR irradiation. Additionally, their antibacterial properties against clinical pathogenic bacteria were further enhanced both *in vitro* and *in vivo* after NIR radiation.[74]

5 Applications

To date, considerable efforts have been made to explore CDs for BTE. Table 1 summarizes the latest research and related results.

Compared with other traditional ceramics and polymers, CDs offer several advantages as BTE scaffold materials: firstly, CDs are rich in surface functional groups (*i.e.*, hydroxyl, carboxyl, amino, *etc.*) that are easily conjugated with other components (*i.e.*, BMP-2, SR-7, PS-11, IP-3, CK-23, *etc.*) to achieve rapid bone tissue regeneration;[84,85] secondly, CDs have unique optical properties that can be used to track the biodegradation of scaffold materials after bone tissue regeneration; thirdly, CDs have a good photothermal effect and can be used to kill tumor cells, especially bone cancer cells; lastly, CDs have excellent antibacterial properties and can be used to prevent or treat common infections after bone defect repair. Based on

Table 1 Selected examples of BTE applications using CDs

Carbon precursor[a]	Synthesis method	Scaffold materials[b]	*In vitro* model[c]	*In vivo* model[d]	Effects[e]	Ref.
Phosphoethanolamine, calcium gluconate	Hydrothermal	F127 hydrogel	MC3T3-E1	Wistar SD rats	C, P	70
Peel extract of sour apple	Hydrothermal	—	Osteoclast	C57BL/6J mice	O, I	75
Curcumin	Hydrothermal	3D printed scaffolds	ADSCs	—	A, C	76
Metformin hydrochloride	Hydrothermal	—	rBMSCs	Wistar rats	C, P	77
Alendronate	Hydrothermal	CDHA	HeLa cells	Rat femurs, Zebrafish	Bone imaging, bone targeting	78
Citric acid, glycine	Microwave	CS, nHA	rBMSCs	SD rats	A, C, P, tumor ablation, antibacterial properties	79
BSA	Hydrothermal	HA	MC3T3-E1	Zebrafish JBR	C, P	80
CMC-HA	Hydrothermal	—	MG63	—	A, C	81
Citric acid	Hydrothermal	—	rBMSCs	—	C, P	82
Carbon nanopowder	—	—	—	Zebrafish	Bone-specific drug delivery	83

[a] BSA: bovine serum albumin; CMC: carboxymethyl cellulose; HA: hydroxyapatite. [b] CDHA: calcium-deficient hydroxyapatite; CS: chitosan. [c] ADSCs: adipose-derived stem cells; rBMSCs: rat-bone-marrow-derived mesenchymal stem cells. [d] JBR: jawbone regeneration; SD rats: Sprague–Dawley rats. [e] A: Promote cell adhesion and proliferation; C: Promote osteogenic differentiation of cells; I: Inhibit bone resorption; O: Inhibit osteoclastogenesis; P: Promote bone regeneration.

these properties, CDs are receiving increasing attention in the field of BTE, which will be described in detail in this section.

5.1 Osteogenesis

For the past few years, it has been well documented that CDs alone promote bone tissue regeneration. Shao and his research team prepared CDs using citric acid and ethylenediamine *via* a hydrothermal method. They demonstrated that CDs could facilitate osteogenic differentiation of rat bone marrow mesenchymal stem cells (rBMSCs) by upregulating the expression of osteoblast gene markers (ALP, RUNX2, OCN, and BSP) and enhancing matrix mineralization through the ROS-mediated MAPK pathway. Noteworthily, this was the first study showing that CDs could both track and enhance the osteogenic differentiation of MSCs.[82] After that, Chen *et al.* synthesized chitosan-derived nitrogen-doped carbon dots (N-CDs) based on the hydrothermal carbonization process using chitosan and acrylamide as precursors. They revealed that N-CDs prevented RANKL-induced osteoclast precursor cells from differentiating, fusing, and mature osteoclast bone absorption *in vitro*. In terms of mechanisms, N-CDs abrogated RANKL-induced elevation in ROS generation and therefore impaired the activation of the NF-κB and MAPK pathways. In addition, *in vivo* injection of N-CDs in mice might protect against lipopolysaccharide (LPS)-induced calvarial bone destruction and breast-cancer-induced tibial bone loss by inhibiting osteoclastogenesis and bone resorption. This was the first time a nanomaterial treatment option was provided for the clinical treatment of osteolytic diseases (Fig. 3A).[86] In these studies, the ability of the CDs to promote osteogenic differentiation appears to stem from changes during their preparation, such as size and surface functional groups, as the carbon sources used to prepare these CDs have negligible effects on cellular osteogenic differentiation.

Further, researchers are investigating the role of CDs in promoting osteogenesis by altering their precursors. Studies have shown that dexamethasone is a highly stable corticosteroid, and it is clinically used to regulate the expression of inflammatory factors. In addition, during bone regeneration, dexamethasone can stimulate the upregulation of ALP, OCN and other related genes, thus promoting the differentiation of stem cells into osteoblasts. Therefore, Wan *et al.* prepared dexamethasone CDs (DCDs), which retained the pharmacological activity of dexamethasone. The prepared DCDs exhibited good biocompatibility and promoted the differentiation of rBMSCs under both normal and inflammatory conditions. Moreover,

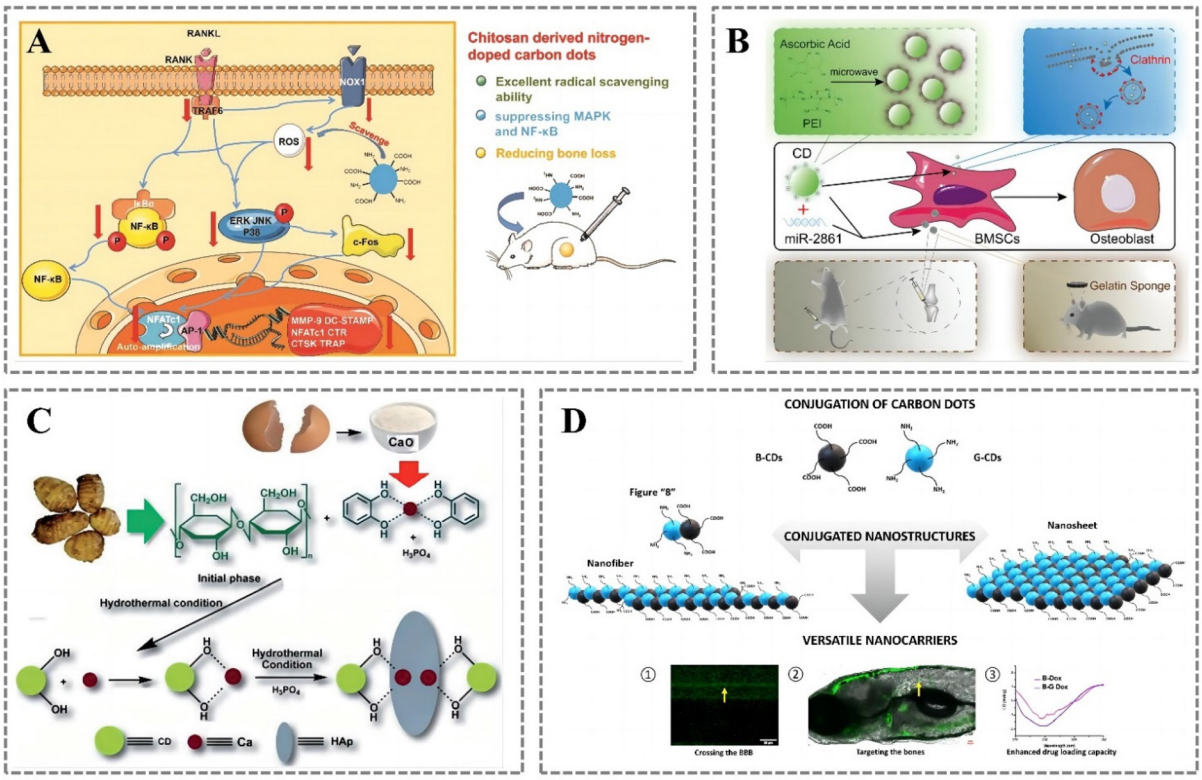

Fig. 3 Application of CDs in osteogenesis. (A) Schematic illustration of N-CDs inhibiting osteoclast formation and overactivation by downregulating ROS (This figure has been reproduced from ref. 86 with permission from Royal Society of Chemistry, copyright 2020). (B) Schematic illustration of the synergistic effect of CDs and miR2861 on osteogenic differentiation *in vitro* and new bone regeneration *in vivo* (This figure has been reproduced from ref. 71 with permission from American Chemical Society, copyright 2020). (C) Schematic illustration of the formation mechanism of CD@HA nanohybrids (This figure has been reproduced from ref. 99 with permission from Royal Society of Chemistry, copyright 2016). (D) The conjugated B–G CDs showed enhanced drug-loading capacity and exhibited BBB-crossing and bone- targeting properties (This figure has been reproduced from ref. 100 with permission from Elsevier, copyright 2020).

the DCDs had good osteoimmunomodulatory activity and could induce a bone immune microenvironment and accelerate bone regeneration.[27] As one of the essential trace elements, zinc plays an important role in human bone metabolism. It has been documented that zinc has a stimulatory effect on osteoblast proliferation through various cell divisions, promoting related enzymes and hormones that are synthesis-dependent on zinc.[87] Based on this, Wang and his group synthesized Zn^{2+}-doped carbon dots (Zn-CDs) *via* a one-step hydrothermal method. They evaluated their ability to induce osteogenic differentiation of cells *in vitro* by MTS assay, intracellular reactive oxygen species (ROS) detection, alkaline phosphatase (ALP) activity test and alizarin red staining. The results demonstrated that zinc gluconate (Zn-G) and Zn-CDs promoted the survival of BMSCs when the zinc ion concentrations were 10^{-4} mol L^{-1} (Zn-G: 45.6 μg mL^{-1}) and 10^{-5} mol L^{-1} (Zn-CDs: 300 μg mL^{-1}) or below. In terms of osteogenic capacity, the ALP activity induced by the Zn-CDs was significantly higher than that induced by Zn-G. Alizarin red staining showed a dose-dependent increase in the area of calcified nodules in the Zn-CD group. Overall, they revealed that Zn-CDs could effectively enhance bone regeneration *in vivo*.[69] Similarly, Yang *et al.* selected metal gluconate salts as raw materials to synthesize a new kind of Mg^{2+}-doped CDs (Mg-CDs) for the first time. They reported a study on the application of metal-ion-doped CDs in osteogenesis. It is well known that Mg^{2+} can significantly affect the proliferation and differentiation of osteoblast cells by stimulating hormone secretion or cytokine synthesis.[88–90] Many reports have proved that Mg and Mg-doped implants can promote osteoblastic cell adhesion and bone regeneration.[91–93] In an *in vitro* assay, the Mg-CDs exhibited low cytotoxicity to mouse embryo osteoblast precursor cells (MC3T3-E1). They promoted MC3T3-E1 osteogenesis differentiation and mineralization by increasing ALP activity and upregulating bone-related gene expression.[94]

In addition to the use of CDs alone, they have been utilized as nanocarriers to promote bone repair by loading them with therapeutic genes or growth factors. Bu *et al.* prepared CDs from ascorbic acid and polyethyleneimine *via* a microwave-assisted pyrolysis method. The results of ALP staining, alizarin red staining and reverse transcription real-time PCR (RT-QPCR) showed that the prepared CDs had a stronger effect on promoting the osteogenic differentiation of BMSCs than ascorbic acid itself. More importantly, based on the different reactive groups on the surface of CDs, they used positively charged CDs carrying the negatively charged osteogenic therapeutic gene miR-2861 to enhance the effect of osteogenic differentiation. The results showed that the CDs could be used as a good gene transfer carrier to deliver miR-2861 to BMSCs, which acted synergistically to promote osteogenic differentiation and new bone regeneration (Fig. 3B).[71] On this basis, Jin *et al.* further explored the induction mechanism of ascorbic acid CDs to promote osteogenic differentiation. In this report, they found that the CDs induced increasing intracellular calcium activated endoplasmic reticulum (ER) stress and the PERK-eIF2α-ATF4 pathway, and promoted the process of osteogenic differentiation. Combined with the activity of ALP, the number of calcium nodules and the expression levels of osteogenic differentiation genes (BSP, OCN, *etc.*) in skull defect and tibial injection models *in vivo*, it was suggested that the prepared CDs could promote pre-osteoblast differentiation *in vitro* and bone regeneration *in vivo*.[95]

In addition, CDs have been used as part of composite scaffolds for BTE to improve the interaction between materials. It has been proved that the addition of CDs could improve the mechanical strength of scaffold materials and increase cell adhesion ability as well as proliferation and differentiation.[81,96,97] As early as 2016, CDs were first added to polyvinyl alcohol (PVA) hydrogels to prepare PVA/CDs hydrogels *via* a simple freeze–thaw method to improve the mechanical properties of the hydrogels.[98] However, these composite hydrogels existed only as polymer networks based on hydrogen bonds and were far from satisfactory in terms of both mechanical strength and osteoconductivity when used in the field of BTE. Hence, Wang *et al.* prepared nanofiller and double-network (DN) structure co-enhanced carbon dots/hydroxyapatite/polyvinyl alcohol (CDs/HA/PVA) DN hydrogels using a combination of two fabrication techniques, namely, chemical copolymerization and the freeze–thaw method. The prepared $CDs_{3.0}/HA_{0.6}$/PVA DN_9 hydrogels had optimal compressive strength, prominent degradability behavior and excellent fluorescence properties, giving them the potential to be used as visualizable bone substitutes in the field of biomedical engineering.[73] Gogoi and his group synthesized CD-decorated HA nanohybrids (CD/HA) using a simple one-pot hydrothermal method for the first time and demonstrated their excellent cytocompatibility, cell proliferation and ALP activity on the MG 63 osteoblast cells. They then fabricated CD@HA *in situ* from tannic-acid-based waterborne hyperbranched polyurethane. Studies showed that these CD-based nanocomposites had good mechanical properties and osteogenic activity (Fig. 3C).[99] Further, Khajuria *et al.* prepared NCDs-HA nanoparticles by conjugating nitrogen-doped carbon dots (NCDs) with hydroxyapatite (HA) nanoparticles and evaluated the effects of the NCDs-HA nanoparticles on the proliferation, differentiation and mineralization of murine osteoblast cell line MC3T3-E1 *in vitro*, while detecting the osteogenic ability of NCDs-HA nanoparticles using the zebrafish (ZF) jawbone regeneration (JBR) model. The results showed that the NCDs-HA nanoparticles significantly enhanced ALP activity, mineralization and expression of osteogenic genes in osteoblast cells, and also increased ZF bone regeneration and bone density compared with HA nanoparticles, suggesting that the NCDs-HA nanoparticles could have therapeutic potential as bone regenerative drugs for the treatment of bone defects.[80] Recently, Wu and his team successfully prepared Ca/P-CDs with osteogenic ability by introducing calcium and phosphorus through one-pot hydrothermal carbonization. Further, in order to achieve administration *in situ*, Ca/P-CDs were loaded onto an F127 thermally sensitive hydrogel. The results showed that the Ca/P-CDs loaded F127 hydrogel effectively promoted the repair of bone defects *in vivo* compared with the unloaded F127 hydrogel.[70]

Interestingly, Zhou and their group proposed the unprecedented idea that CDs could be used as customizable Lego-like building blocks and be directly conjugated in a similar way to organic molecules. Specifically, they prepared black CDs (B-CDs) and gel-like CDs (G-CDs) from carbon nanopowder and citric acid, and then synthesized black-gel CDs (B–G CDs) through conjugation at a mass ratio of 5 : 3 (B-CDs to G-CDs) and a two-step purification process. In addition to increasing the drug loading capacity, the synthesized B–G CDs exhibited bone-targeting and blood–brain barrier (BBB)-crossing properties, which were properties of the B-CDs and G-CDs, respectively (Fig. 3D).[100]

5.2 Fluorescence tracing

Scaffold materials have been widely studied to promote bone regeneration. However, developing methods to conveniently track the cells after implantation and monitor their dynamic regenerative capacities remains a key obstacle for their application in BTE. Thus, finding a non-invasive, real-time tracer method that can also effectively regulate osteogenic differentiation is the key to break this bottleneck. Common fluorescent nanomaterials such as rare-metal-based nanomaterials, polymeric nanoprobes, and semiconductor quantum dots may have cytotoxicity and can adversely affect osteogenic differentiation. For example, Hsieh *et al.* delivered CdSe/ZnS quantum dots (QDs) into hBMSCs *via* liposome-mediated transfection with high efficiency and found that the QD-containing-hBMSCs exhibited lower ALP activity as compared to those without QDs, and reverse transcriptase polymerase chain reaction further confirmed that the expression of both osteogenic markers, osteopontin and osteocalcin, was significantly inhibited, indicating that the presence of CdSe/ZnS QDs prevented osteogenic differentiation of BMSCs.[101] Therefore, the development of fluorescent nanomaterials with good biocompatibility for both cell labeling and osteogenic differentiation is of great importance in the field of BTE. In addition, researchers have found that CDs have high affinity and specificity with calcified bones *in vivo*, which could be used for targeted imaging, diagnosis and treatment of bone and related diseases.[102–104] Based on these factors, researchers have proposed the possibility of using CDs for cellular tracing in BTE.

Cai *et al.* successfully prepared bifunctional CDs *via* one-pot hydrothermal synthesis using d-glucosamine hydrochloride (GA·HCl) and sodium *p*-styrenesulfonate (NaSS) as the reactants (Fig. 4A). The results showed that the prepared CDs were cytoplasmically distributed after being ingested by rBMSCs, enabling effective cellular imaging. Moreover, the addition of CDs to the osteogenic and chondrogenic induction media effectively promoted the differentiation of rBMSCs toward osteogenesis and chondrogenesis, respectively (Fig. 4B).[105] Similarly, Shao *et al.* synthesized citric-based CDs *via* a hydrothermal method using citric acid and ethylenediamine as raw materials, and found that the prepared CDs enhanced matrix mineralization and promoted the osteogenic

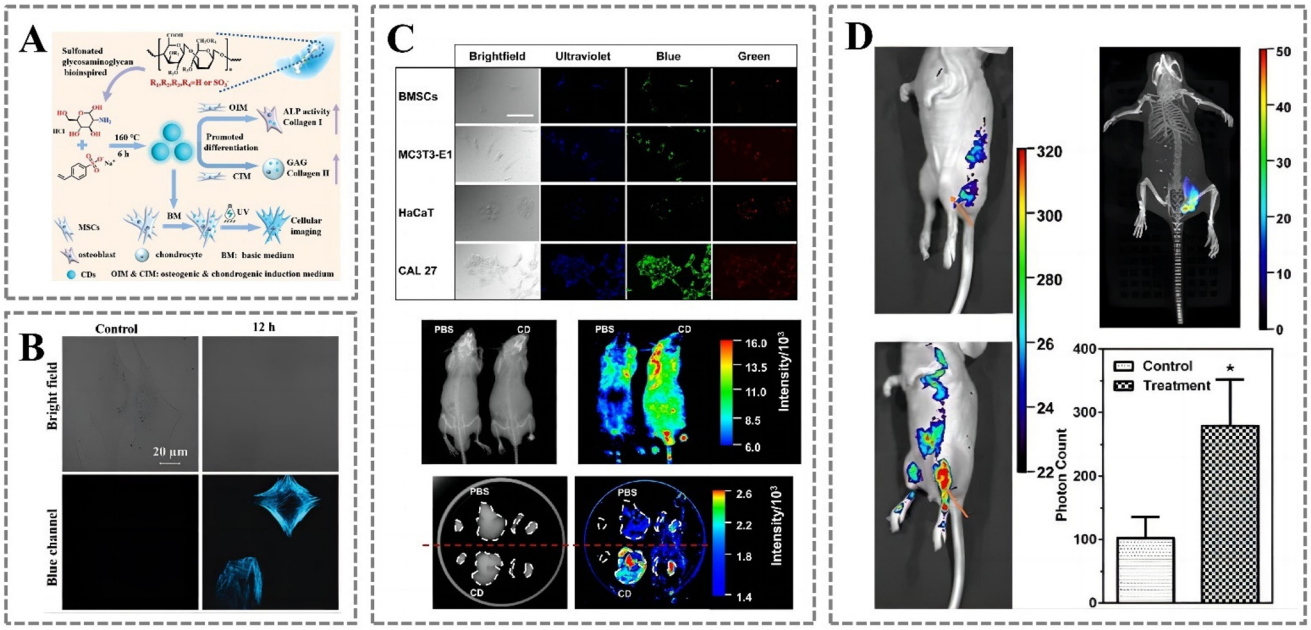

Fig. 4 Application of CDs in fluorescence tracing. (A) Synthesis of sulfonated glycosaminoglycan bioinduced CDs and their applications in effective cell labeling and the promotion of differentiation of mesenchymal stem cells (MSCs) in different culture media (This figure has been reproduced from ref. 105 with permission from Royal Society of Chemistry, copyright 2020). (B) Confocal laser scanning microscopy images of rBMSCs cultured without or with CDs for 12 h under the blue channel (This figure has been reproduced from ref. 105 with permission from Royal Society of Chemistry, copyright 2020). (C) Fluorescence images of cells or mouse tissues treated with CDs *in vitro* and *in vivo* (This figure has been reproduced from ref. 71 with permission from American Chemical Society, copyright 2020). (D) *In vivo* fluorescence of monophosphonated CDs in the tibia with distinct site enhancement in the signal-to-background intensity (This figure has been reproduced from ref. 108 with permission from American Chemical Society, copyright 2018).

differentiation efficiency of rBMSCs by upregulating the expression of osteogenic-related gene markers (ALP, Runx2, OCN and BSP). Additionally, cells labeled with the CDs were observed to still emit blue fluorescence signals after 21 days, demonstrating the potential of CDs as dual tools for tracing and promoting osteogenic differentiation.[82]

Although a large number of studies have demonstrated the ability of CDs to be used for cellular tracing, most of these CDs emit blue-green fluorescence under UV light, which can induce spontaneous fluorescence of tissue, severely interfere with the signal of the CDs and cause damage to cells and tissues.[106] Therefore, it is more meaningful to study CDs in the long-wavelength and near-infrared regions for cellular tracing. Based on this, Huang *et al.* reported the first endoplasmic-reticulum-targeted near-infrared CDs for the detection of Cu^{2+} in biosystems. The prepared CDs exhibited minimal cytotoxicity, high biocompatibility and endoplasmic-reticulum-targeting ability, which further confirmed their promising application in biomedical fields.[107]

5.3 Phototherapy

Bone tumors, which are one of the most common tumors, have a low long-term survival rate and seriously threaten the life and health of human beings. Commonly used treatments are generally based on surgery, supplemented by radiotherapy and chemotherapy. However, bone tumors are not sensitive to radiotherapy and are prone to drug resistance. Thus, researchers have begun to explore alternative treatments. In this context, phototherapy, including photothermal therapy (PTT) and photodynamic therapy (PDT), has received widespread attention as an emerging minimally invasive treatment. PTT converts irradiated light into reactive oxygen species or heat with the help of photosensitizers, thereby inducing local apoptosis of tumor cells. Nevertheless, postoperative bone defects and local recurrence remain the most important reasons for the low long-term survival rate of patients with bone tumors, which are mostly treated clinically by simple means, such as repairing bone tissue by implanting biological scaffolding materials or killing residual tumor cells in the body by hyperthermia. However, combining these needs into a simple material is the focus of current research.

Studies have suggested that CDs with red/near-infrared (NIR) light emission have a photothermal effect and can be used to convert light energy into heat, thereby killing cancer cells.[109] Ge *et al.* prepared CDs with red light emission from polythiophene phenylpropionic acid (PPA) and verified the PTT efficacy by monitoring the survival rates and tumor growth rates of HeLa-tumor-bearing nude mice, demonstrating that only mice injected with CDs and irradiated with a 671 nm laser showed substantial empyrosis and exhibited significant suppression of tumor growth. After 16 days, the dark burn scabs were removed from the skin and the mice recovered completely. They demonstrated that red-emitting CDs can act as both fluorescent and PTT materials for cancer diagnosis and treatment in living mice for the first time, thereby greatly broadening the biomedical applications of CDs.[110] Lu *et al.* prepared a novel CD-doped chitosan/nanohydroxyapatite (CS/nHA/CDs) scaffold using a freeze-drying method. Compared with pure CS/nHA scaffolds, the CS/nHA/CDs scaffolds promoted cell adhesion and osteogenic differentiation of rBMSCs by up-regulating the expression of local adhesion and osteogenic genes. Inspired by the photothermal effect of CDs, the scaffolds were used for the PTT of tumors. The scaffolds exhibited an excellent photothermal effect under NIR irradiation (808 nm), and effectively induced apoptosis of tumor cells *in vitro* due to the doping with CDs. Under 1 W cm^{-2} NIR irradiation, the cell viability in the CS/nHA/CDs group was significantly lower than that of the other treatment groups, which was also confirmed by live/dead staining and CCK-8 evaluation. Subsequently, after implanting the scaffolds under the tumor in osteosarcoma-bearing mice, the highly malignant tumors were significantly inhibited *in vivo* under NIR light irradiation. As shown in the figure, the tumors were huge in the CS/nHA/CDs, CS/nHA + NIR, and CS/nHA groups, while there were only small scars on the backs of mice in the CS/nHA/CDs + NIR group (Fig. 5A).[74] All these studies demon-

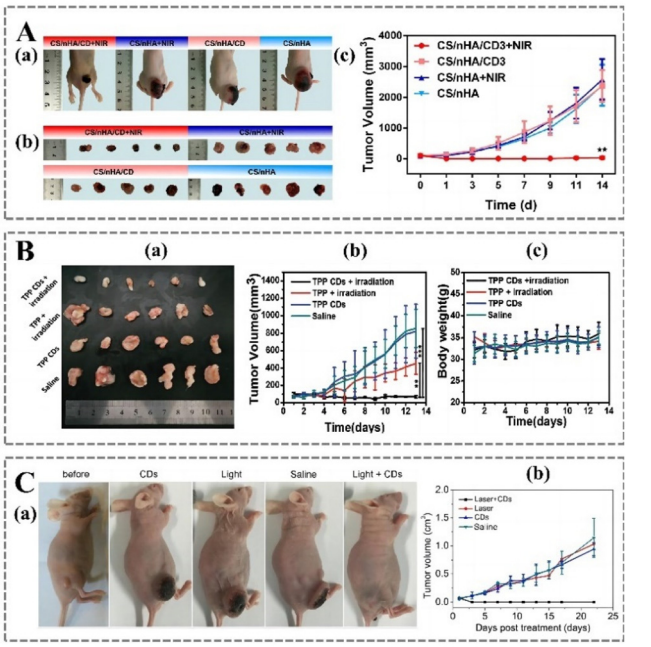

Fig. 5 Application of CDs in phototherapy. (A) *In vivo* PTT with different scaffolds. (a) Representative photographs of tumor-bearing nude mice in the different groups 14 days after treatment; (b) tumors collected from the different groups of mice 14 days after treatment; (c) tumor volume growth curves of the different treatment groups over a period of 14 days (This figure has been reproduced from ref. 92 with permission from American Chemical Society, copyright 2018). (B) *In vivo* PDT with different materials. (a) Photographs of excised tumors on day 13; (b) tumor volumes of the mice as a function of time; (c) body weight of the H22 cancer-bearing mice as a function of time (This figure has been reproduced from ref. 113 with permission from John Wiley and Sons, copyright 2016). (C) (a) Photographs of mice before and after the different treatments for 22 days; (b) time-dependent tumor volumes after different treatments (This figure has been reproduced from ref. 115 with permission from John Wiley and Sons, copyright 2018).

strate the potential application of CDs in the treatment of bone tumors due to their osteogenic properties as well as their excellent photothermal effects.

In 2012, Huang *et al.* designed and developed a novel theranostic platform based on photosensitizer-conjugated carbon dots. They confirmed that the prepared C-dots-Ce6 had remarkable photodynamic efficacy upon irradiation and showed significant promise for NIR-fluorescence-imaging-monitored PDT treatment.[111] Since then, the applications of CDs in PDT have attracted extensive attention. It has been documented that CDs can be used as photosensitizers in PDT and produce moderate amounts of reactive oxygen species.[112] Li *et al.* prepared porphyrin-based CDs (TPP CDs) using monohydroxyphenyl triphenylporphyrin (TPP) and chitosan as precursors through a one-pot hydrothermal method. The prepared TPP CDs exhibited good water solubility, photostability, cellular uptake, and the ability to generate cytotoxic singlet oxygen. The *in vitro* PDT efficacy of TPP CDs on HepG2 cells was confirmed by methyl thiazolyl tetrazolium (MTT) assay. It was further demonstrated through *in vivo* experiments that TPP CDs could effectively suppress the growth of solid tumors without side effects, showing their potential to enhance PDT in tumor treatment (Fig. 5B).[113] Similarly, Elsherbiny *et al.* prepared CDs with excellent water solubility and optical properties using broccoli as a carbon source, proving that they could effectively generate $^1O_2$ under 660 nm light. Next, they chose non-parasitic nematode *Caenorhabditis elegans* (*C. elegans*) as an animal model and confirmed that the CDs could lead to PDT effects by causing DNA damage in worms. Interestingly, this work not only provided a new photodynamic agent, but also introduced *C. elegans* as a simple, high-throughput model for rapid evaluation of the efficiency of PDT.[114]

Furthermore, researchers have integrated PTT and PDT to expand the applicability of this treatment and improve its therapeutic efficiency. Lan *et al.* prepared CDs with a fluorescence yield of 49% *via* a hydrothermal method using 1,3,6-trinitropyrene and Na_2SO_3 as precursors. The prepared CDs could simultaneously emit intense fluorescence and generate $^1O_2$ through a two-photon excitation mechanism, and they also exhibited excellent photothermal conversion capability under the irradiation of an 800 nm femtosecond pulsed laser. The biocompatibility and phototoxicity of CDs on HeLa cells were evaluated by detecting the metabolic activity of HeLa cells using the MTT assay. The results showed that the cell viability was close to 100% after incubation of the HeLa cells with CDs at concentrations ranging from 12.5 to 100 μg mL^{-1} for 24 h under dark conditions. When the concentration of CDs was increased to 200 μg mL^{-1}, the cell viability remained above 80%. However, under the irradiation of the 800 nm laser, the cell viability was only about 10%, which proved that the prepared CDs had good biocompatibility and high phototoxicity. Next, the *in vivo* phototherapy performance of the CDs was evaluated using the 800 nm laser as the illumination source and Balb/c nude mice bearing subcutaneous 4T1 murine breast cancer cells as experimental animals. The results showed that the tumor growth was effectively inhibited in the CDs + Laser group compared with the control group, and no tumor recurrence was observed during the experimental period. In contrast, the tumors in the control group grew significantly, demonstrating that neither laser irradiation nor CD injection alone could inhibit the tumor growth. These results suggested that CDs could be used as an effective phototherapeutic agent to kill tumor cells under NIR laser irradiation. Their combined *in vitro* and *in vivo* experiments showed that the CDs had good biocompatibility and could be used for photothermal/photodynamic synergistic cancer therapy (Fig. 5C).[115]

Based on the aforementioned studies, the excellent phototherapy ability of CDs make them a promising BTE candidate for multimodal treatment of bone tumors.

5.4 Antibacterial properties

Infected bone defects have long been a problematic issue in orthopedics, as infection can significantly impair local tissue and bone regeneration, making the defect difficult to heal.[116] Commonly used treatments include removal of necrotic bone, use of antibiotics and reconstruction of bone defects. However, these treatments are time-consuming and ineffective. Therefore, the development of bone replacement materials with dual antibacterial and osteogenesis functions for the one-stage treatment of infected bone defects without subsequent surgery would be a therapeutic strategy of important clinical significance. Considering the advantages of CDs, their application to BTE scaffolds is expected to promote bone repair and suppress early inflammation and excessive immune response induced by material implantation, thus creating an ideal microenvironment for bone regeneration.

The most commonly used medical hard tissue replacement materials, titanium-based alloys, have excellent corrosion resistance, biocompatibility and mechanical properties.[117] However, bacteria-induced related infections are still unavoidable.[118] Currently, the misuse of antibiotics has led to an increase in bacterial resistance, which has led to the emergence of drug-resistant bacteria. To solve these problems, He *et al.* introduced CDs into a titanium (Ti) implant by using a hydrothermal method to prepare CD-doped titanium dioxide (TiO_2) nanorod arrays (C-TiO_2 NRs) to enhance the photocatalytic and photothermal capabilities for effective bacteria killing under 660 nm visible light (VL) and 808 nm near-infrared (NIR) light irradiation. Results showed that C-TiO_2 NR had a good antibacterial effect against *Staphylococcus aureus* (*S. aureus*) *in vitro* and *in vivo* due to the combined effect of hyperthermia, ROS and the nanorod structure. In addition, C-TiO_2 NRs could also promote the adhesion and diffusion of BMSCs (Fig. 6A).[119]

Although CDs have been shown to have antibacterial properties, most of them have antibacterial activities against both Gram-positive bacteria and Gram-negative bacteria, lacking selectivity. Liu *et al.* first synthesized bifunctional CDs with selective antibacterial activity and PL *via* a hydrothermal method using metronidazole as a carbon source without

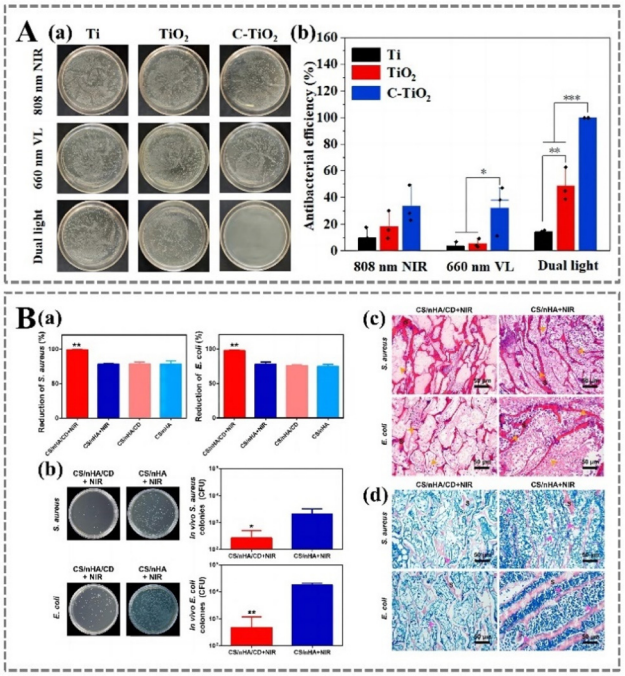

Fig. 6 Application of CDs for antibacterial properties. (A) (a) Pictures of *S. aureus* colonies on the surface of Ti, TiO_2 and C-TiO_2 NRs; (b) antibacterial efficiency of Ti, TiO_2 and C-TiO_2 NRs (This figure has been reproduced from ref. 119 with permission from Elsevier, copyright 2022). (B) Antibacterial properties of different scaffolds. (a) *In vitro* antibacterial rate of the different groups against clinically relevant *S. aureus* (left) and *E. coli* (right); (b) number of clinically relevant *S. aureus* (top) and *E. coli* (bottom) bacterial colonies after bacteria from the harvested samples were cultured for 24 h after 1 week treatments *in vivo*; HE staining (c) and Giemsa staining (d) of the samples harvested from the different groups (This figure has been reproduced from ref. 92 with permission from American Chemical Society, copyright 2018).

further functionalization. Biological experimental data demonstrated that the prepared CDs could only directly inhibit the growth of obligate anaerobes, such as *Porphyromonas gingivalis* (*P. gingivalis*), but had no inhibitory effect on the growth of facultative anaerobes, obligate aerobes or microaerophilic bacteria. Therefore, the prepared CDs may provide a valuable way to treat related diseases caused by *P. gingivalis* and explore the pathogenic mechanism through real-time tracking.[120]

In addition to promoting bone tissue formation and ablating osteosarcoma, the CD-doped chitosan/nanohydroxyapatite (CS/nHA/CDs) scaffolds prepared by Lu *et al.* have been proven to have significant antibacterial properties against clinically collected *S. aureus* and *E. coli*. Their antibacterial activities were further strengthened both *in vitro* and *in vivo* under NIR irradiation. Chitosan (CS) is a biocompatible and biodegradable natural polymer with unique characteristics.[121] It kills bacteria, especially Gram-negative bacteria, by inhibiting DNA transcription or blocking nutrient transport.[122] For this reason, to further verify the antibacterial role of CDs, they evaluated the *in vivo* antibacterial effect using the CS/nHA/CDs and CS/nHA scaffolds, respectively. The results showed that after 1 week of treatment, there were still a great number of *S. aureus* and *E. coli* in the CS/nHA + NIR group, while the number of bacteria decreased significantly in the CS/nHA/CDs + NIR group. HE staining showed many lobulated neutrophils in the CS/nHA + NIR group due to inflammation, while the CS/nHA/CDs + NIR group showed only slight inflammation, which proved the enhanced antibacterial effect after NIR irradiation. In addition, Giemsa staining showed the presence of bacteria in the CS/nHA + NIR group, while no obvious bacteria were found in the CS/nHA/CDs + NIR group, indicating that hyperthermia was also beneficial in inhibiting bacterial growth and reproduction. Given the above, NIR-irradiated CS/nHA/CDs scaffolds exhibited enhanced antibacterial properties and could effectively eliminate clinically relevant bacterial infections (Fig. 6B).[74]

6 Conclusions and future directions

In summary, this review mainly summarizes recent progress in the use of CDs for BTE, including osteogenesis, fluorescence tracing, phototherapy and antibacterial activity. The unique properties of CDs (*e.g.*, biocompatibility, low cytotoxicity, facile surface modification, induced biomineralization, antibacterial properties, *etc.*) make them attractive candidates for BTE. Despite the above progress, there are still several critical issues that must be addressed for successful translation of CDs for clinical application.

First, it is difficult to ensure the reproducibility and consistency of the synthesized CDs because their synthesis mechanism and luminescence mechanism are still unclear, despite the abundance of synthesis strategies. Moreover, it has been shown that the prepared CDs are actually complex mixtures that exhibit different spectral and biological properties after fine separation.[123] For this purpose, it is necessary to systematically explore the selection of carbon precursors and the influence of reaction conditions such as temperature, time and pH on CD performance, and apply modern separation techniques (*e.g.*, high performance liquid chromatography) to separate the prepared CDs, so as to achieve their controlled preparation and commercial mass production. Second, until now, the absorption wavelength and emission wavelength of CDs have been mainly concentrated in the ultraviolet/visible region, while the development of CDs with red/NIR light is still rare.[124] This makes it difficult to achieve deep tissue imaging, limiting their further study in the biomedical field. In the future, the preparation of CDs with deep red/NIR absorption and emission properties should be further investigated to expand their application in BTE. The current studies mainly focus on the construction and application of CDs and scaffold materials, while the mechanism of action behind their biological effects has been little studied. Systematic studies that focus on the relationship between various characterizations of CDs and the biological mechanism of their action will provide a theoretical basis for the rational design of BTE scaffolds.

As such, future efforts should be directed toward finding solutions for these issues. With the development of production

technology and characterization, we hope that the preparation, structure, properties, applications and mechanisms of CDs can be explored in more depth for the mass production and further biomedical applications, especially in BTE.

Author contributions

R. Z. designed the structure and content, assembled the literature and wrote this review. Y. H., L. S., X. L., Y. Z., Q. Z., Y. Z., L. W., R. L., C. W., X. W. and B. L. provided suggestions and revised this review.

Conflicts of interest

There are no conflicts to declare.

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References

1 S. Bhumiratana, J. C. Bernhard, D. M. Alfi, K. Yeager, R. E. Eton, J. Bova, F. Shah, J. M. Gimble, M. J. Lopez, S. B. Eisig and G. Vunjak-Novakovic, *Sci. Transl. Med.*, 2016, **8**, 343–383.
2 V. Karageorgiou and D. Kaplan, *Biomaterials*, 2005, **26**, 5474–5491.
3 H. D. Kim, S. Amirthalingam, S. L. Kim, S. S. Lee, J. Rangasamy and N. S. Hwang, *Adv. Healthcare Mater.*, 2017, **6**, 1700612.
4 S. Yin, W. Zhang, Z. Zhang and X. Jiang, *Adv. Healthcare Mater.*, 2019, **8**, e1801433.
5 L. Zhang, G. Yang, B. N. Johnson and X. Jia, *Acta Biomater.*, 2019, **84**, 16–33.
6 R. Eivazzadeh-Keihan, E. B. Noruzi, K. K. Chenab, A. Jafari, F. Radinekiyan, S. M. Hashemi, F. Ahmadpour, A. Behboudi, J. Mosafer, A. Mokhtarzadeh, A. Maleki and M. R. Hamblin, *J. Tissue Eng. Regener. Med.*, 2020, **14**, 1687–1714.
7 K. Bavya Devi, V. Lalzawmliana, M. Saidivya, V. Kumar, M. Roy and S. Kumar Nandi, *Chem. Rec.*, 2022, e202200136.
8 L. Guo, Z. Liang, L. Yang, W. Du, T. Yu, H. Tang, C. Li and H. Qiu, *J. Controlled Release*, 2021, **338**, 571–582.
9 S. Zhu, Y. Song, J. Shao, X. Zhao and B. Yang, *Angew. Chem., Int. Ed.*, 2015, **54**, 14626–14637.
10 J. Liu, R. Li and B. Yang, *ACS Cent. Sci.*, 2020, **6**, 2179–2195.
11 D. L. Zhao and T. S. Chung, *Water Res.*, 2018, **147**, 43–49.
12 J. H. Liu, Y. Li, J. H. He, D. Yuan, R. S. Li, S. J. Zhen, Y. F. Li and C. Z. Huang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 4815–4820.
13 S. Chung, R. A. Revia and M. Zhang, *Adv. Mater.*, 2021, **33**, e1904362.
14 X. W. Hua, Y. W. Bao, Z. Chen and F. G. Wu, *Nanoscale*, 2017, **9**, 10948–10960.
15 Y. Su, S. Liu, Y. Guan, Z. Xie, M. Zheng and X. Jing, *Biomaterials*, 2020, **255**, 120110.
16 L. Qiao, T. Sun, X. Zheng, M. Zheng and Z. Xie, *Mater. Sci. Eng., C*, 2018, **85**, 1–6.
17 M. Zheng, S. Ruan, S. Liu, T. Sun, D. Qu, H. Zhao, Z. Xie, H. Gao, X. Jing and Z. Sun, *ACS Nano*, 2015, **9**, 11455–11461.
18 J. Liu, D. Li, K. Zhang, M. Yang, H. Sun and B. Yang, *Small*, 2018, **14**, e1703919.
19 Q. Y. Cai, J. Li, J. Ge, L. Zhang, Y. L. Hu, Z. H. Li and L. B. Qu, *Biosens. Bioelectron.*, 2015, **72**, 31–36.
20 A. Kundu, S. Nandi, P. Das and A. K. Nandi, *J. Colloid Interface Sci.*, 2016, **468**, 276–283.
21 M. L. Liu, B. B. Chen, C. M. Li and C. Z. Huang, *Green Chem.*, 2019, **21**, 449–471.
22 X. Sun and Y. Lei, *TrAC, Trends Anal. Chem.*, 2017, **89**, 163–180.
23 Q. Zeng, D. Shao, X. He, Z. Ren, W. Ji, C. Shan, S. Qu, J. Li, L. Chen and Q. Li, *J. Mater. Chem. B*, 2016, **4**, 5119–5126.
24 T. Kong, L. Hao, Y. Wei, X. Cai and B. Zhu, *Cell Proliferation*, 2018, **51**, e12488.
25 P. Gao, S. Liu, Y. Su, M. Zheng and Z. Xie, *Bioconjugate Chem.*, 2020, **31**, 646–655.
26 X. W. Hua, Y. W. Bao and F. G. Wu, *ACS Appl. Mater. Interfaces*, 2018, **10**, 10664–10677.
27 C. Wan, M. Y. Hu, X. Peng, N. N. Lei, H. M. Ding, Y. H. Luo and X. X. Yu, *Biomater. Sci.*, 2022, **10**, 6291–6306.
28 B. Pei, W. Wang, N. Dunne and X. Li, *Nanomaterials*, 2019, **9**, 1501.
29 D. Zhang, X. Wu, J. Chen and K. Lin, *Bioact. Mater.*, 2018, **3**, 129–138.
30 I. Erezuma, T. Eufrasio-da-Silva, N. Golafshan, K. Deo, Y. K. Mishra, M. Castilho, A. K. Gaharwar, S. Leeuwenburgh, A. Dolatshahi-Pirouz and G. Orive, *Adv. Healthcare Mater.*, 2021, **10**, e2100217.
31 L. Daneshmandi, M. Barajaa, A. T. Rad, S. A. Sydlik and C. T. Laurencin, *Adv. Healthcare Mater.*, 2021, **10**, e2001414.
32 Y. Zheng, X. Hong, J. Wang, L. Feng, T. Fan, R. Guo and H. Zhang, *Adv. Healthcare Mater.*, 2021, **10**, e2001743.
33 J. Hu, Y. Sun, A. A. Aryee, L. Qu, K. Zhang and Z. Li, *Anal. Chim. Acta*, 2022, **1209**, 338885.
34 M. E. Khan, A. Mohammad and T. Yoon, *Chemosphere*, 2022, **302**, 134815.
35 C. L. Shen, H. R. Liu, Q. Lou, F. Wang, K. K. Liu, L. Dong and C. X. Shan, *Theranostics*, 2022, **12**, 2860–2893.

36 C. Ji, Y. Zhou, R. M. Leblanc and Z. Peng, *ACS Sens.*, 2020, **5**, 2724–2741.
37 M. Maruthapandi, A. Saravanan, P. Das, J. H. T. Luong and A. Gedanken, *Biotechnol. Adv.*, 2021, **53**, 107843.
38 X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker and W. A. Scrivens, *J. Am. Chem. Soc.*, 2004, **126**, 12736–12737.
39 Y.-P. Sun, B. Zhou, Y. Lin, W. Wang, K. A. S. Fernando, P. Pathak, M. J. Meziani, B. A. Harruff, X. Wang, H. Wang, P. G. Luo, H. Yang, M. E. Kose, B. Chen, L. M. Veca and S.-Y. Xie, *J. Am. Chem. Soc.*, 2006, **128**, 7756–7757.
40 S. L. Hu, K. Y. Niu, J. Sun, J. Yang, N. Q. Zhao and X. W. Du, *J. Mater. Chem.*, 2009, **19**, 484–488.
41 X. Li, H. Wang, Y. Shimizu, A. Pyatenko, K. Kawaguchi and N. Koshizaki, *Chem. Commun.*, 2011, **47**, 932–934.
42 J. Zhou, C. Booker, R. Li, X. Zhou, T. K. Sham, X. Sun and Z. Ding, *J. Am. Chem. Soc.*, 2007, **129**, 744–745.
43 Y. Hou, Q. Lu, J. Deng, H. Li and Y. Zhang, *Anal. Chim. Acta*, 2015, **866**, 69–74.
44 Y. Wu, Y. Liu, J. Yin, H. Li and J. Huang, *Talanta*, 2019, **205**, 120121.
45 B. Zhang, C. Y. Liu and Y. Liu, *Eur. J. Inorg. Chem.*, 2010, 4411–4414, DOI: 10.1002/ejic.201000622.
46 C. Y. Yu, T. T. Xuan, Y. W. Chen, Z. J. Zhao, Z. Sun and H. L. Li, *J. Mater. Chem. C*, 2015, **3**, 9514–9518.
47 J. Y. Li, Y. Liu, Q. W. Shu, J. M. Liang, F. Zhang, X. P. Chen, X. Y. Deng, M. T. Swihart and K. J. Tan, *Langmuir*, 2017, **33**, 1043–1050.
48 W. Meng, X. Bai, B. Wang, Z. Liu, S. Lu and B. Yang, *Energy Environ. Mater.*, 2019, **2**, 172–192.
49 R. Wang, K. Q. Lu, Z. R. Tang and Y. J. Xu, *J. Mater. Chem. A*, 2017, **5**, 3717.
50 W.-J. Wang, J.-M. Xia, J. Feng, M.-Q. He, M.-L. Chen and J.-H. Wang, *J. Mater. Chem. B*, 2016, **4**, 7130–7137.
51 J. Shen, S. Shang, X. Chen, D. Wang and Y. Cai, *Mater. Sci. Eng., C*, 2017, **76**, 856–864.
52 B. S. B. Kasibabu, S. L. D'Souza, S. Jha, R. K. Singhal, H. Basu and S. K. Kailasa, *Anal. Methods*, 2015, **7**, 2373–2378.
53 V. N. Mehta, S. Jha and S. K. Kailasa, *Mater. Sci. Eng., C*, 2014, **38**, 20–27.
54 Y. S. Liu, Y. A. Zhao and Y. Y. Zhang, *Sens. Actuators, B*, 2014, **196**, 647–652.
55 C. Zhu, J. Zhai and S. Dong, *Chem. Commun.*, 2012, **48**, 9367–9369.
56 Y. Liu, N. Xiao, N. Q. Gong, H. Wang, X. Shi, W. Gu and L. Ye, *Carbon*, 2014, **68**, 258–264.
57 S. Chung and M. Zhang, *Front. Bioeng. Biotechnol.*, 2021, **9**, 711534.
58 J. Liu, Y. Geng, D. Li, H. Yao, Z. Huo, Y. Li, K. Zhang, S. Zhu, H. Wei, W. Xu, J. Jiang and B. Yang, *Adv. Mater.*, 2020, **32**, 1906641.
59 M. Havrdova, K. Hola, J. Skopalik, K. Tomankova, M. Petr, K. Cepe, K. Polakova, J. Tucek, A. B. Bourlinos and R. Zboril, *Carbon*, 2016, **99**, 238–248.
60 X. Huang, F. Zhang, L. Zhu, K. Y. Choi, N. Guo, J. Guo, K. Tackett, P. Anilkumar, G. Liu, Q. Quan, H. S. Choi, G. Niu, Y. P. Sun, S. Lee and X. Y. Chen, *ACS Nano*, 2013, **7**, 5684–5693.
61 R. Wang, K. Q. Lu, F. Zhang, Z. R. Tang and Y. J. Xu, *Appl. Catal., B*, 2018, **233**, 11–18.
62 K. Q. Lu, Q. Quan, N. Zhang and Y. J. Xu, *J. Energy Chem.*, 2016, **25**, 927–935.
63 R. Atchudan, T. N. J. I. Edison, S. Perumal, N. Muthuchamy and Y. R. Lee, *Fuel*, 2020, **275**, 117821.
64 J. J. Liu, Y. J. Geng, D. W. Li, H. Yao, Z. P. Huo, Y. F. Li, K. Zhang, S. J. Zhu, H. T. Wei, W. Q. Xu, J. L. Jiang and B. Yang, *Adv. Mater.*, 2020, **32**, 1906641.
65 S. H. Li, M. Y. Qi, Y. Y. Fan, Y. Yang, M. Anpo, Y. M. A. Yamada, Z. R. Tang and Y. J. Xu, *Appl. Catal., B*, 2021, **292**, 120157.
66 C. Han, Y. H. Li, M. Y. Qi, F. Zhang, Z. R. Tang and Y. J. Xu, *Sol. RRL*, 2020, **4**, 1900577.
67 R. Sun and S. C. Liu, *Artif. Cells, Nanomed., Biotechnol.*, 2019, **47**, 1321–1325.
68 Y. Han, F. Zhang, J. Zhang, D. Shao, Y. N. Wang, S. Li, S. Lv, G. F. Chi, M. Zhang, L. Chen and J. G. Liu, *Colloids Surf., B*, 2019, **179**, 1–8.
69 B. Wang, M. Yang, L. Liu, G. Yan, H. Yan, J. Feng, Z. Li, D. Li, H. Sun and B. Yang, *Biomater. Sci.*, 2019, **7**, 5414–5423.
70 L. Wu, Y. C. Wu, J. J. Liu, S. W. Liu, Q. Z. Li, M. Y. Yang, M. M. Zhang, Q. Ji, F. M. Zhang and F. Y. Du, *Biomed. Mater.*, 2022, **17**, 055007.
71 W. Bu, X. Xu, Z. Wang, N. Jin, L. Liu, J. Liu, S. Zhu, K. Zhang, R. Jelinek, D. Zhou, H. Sun and B. Yang, *ACS Appl. Mater. Interfaces*, 2020, **12**, 50287–50302.
72 W. K. Meng, A. Rey-Rico, M. Claudel, G. Schmitt, S. Speicher-Mentges, F. Pons, L. Lebeau, J. K. Venkatesan and M. Cucchiarini, *Nanomaterials*, 2020, **10**, 855.
73 Y. Wang, Y. Xue, J. Wang, Y. Zhu, Y. Zhu, X. Zhang, J. Liao, X. Li, X. Wu, Y. X. Qin and W. Chen, *Polymers*, 2019, **11**, 1112.
74 Y. Lu, L. Li, M. Li, Z. Lin, L. Wang, Y. Zhang, Q. Yin, H. Xia and G. Han, *Bioconjugate Chem.*, 2018, **29**, 2982–2993.
75 L. Wu, Y. Wu, L. Jingjin, S. Liu, Q. Li, M. Yang, M. Zhang, Q. Ji, F. Zhang and F. Du, *Biomed. Mater.*, 2022, **17**, 5007.
76 X. Li, Y. Lu, J. Li, S. Zhou, Y. Wang, L. Li and F. Zhao, *J. Nanobiotechnol.*, 2022, **20**, 301.
77 A. Nasrin, M. Hassan, M. M. Mirabet, N. Windhab and V. G. Gomes, *J. Biomed. Mater. Res., Part A*, 2022, **110**, 916–927.
78 C. Ren, X. Hao, L. Wang, Y. Hu, L. Meng, S. Zheng, F. Ren, W. Bu, H. Wang, D. Li, K. Zhang and H. Sun, *Adv. Healthcare Mater.*, 2021, **10**, e2100196.
79 K. K. Lee, J. G. Lee, C. S. Park, S. H. Lee, N. Raja, H. S. Yun, J. S. Lee and C. S. Lee, *RSC Adv.*, 2019, **9**, 2708–2717.
80 D. K. Khajuria, V. B. Kumar, D. Gigi, A. Gedanken and D. Karasik, *ACS Appl. Mater. Interfaces*, 2018, **10**, 19373–19385.

81 C. Sarkar, A. R. Chowdhuri, A. Kumar, D. Laha, S. Garai, J. Chakraborty and S. K. Sahu, *Carbohydr. Polym.*, 2018, **181**, 710–718.
82 D. Shao, M. Lu, D. Xu, X. Zheng, Y. Pan, Y. Song, J. Xu, M. Li, M. Zhang, J. Li, G. Chi, L. Chen and B. Yang, *Biomater. Sci.*, 2017, **5**, 1820–1827.
83 R. DuMez, E. H. Miyanji, L. Corado-Santiago, B. Barrameda, Y. Zhou, S. D. Hettiarachchi, R. M. Leblanc and I. Skromne, *Drug Delivery*, 2021, **28**, 1281–1289.
84 S. H. Li, B. Weng, K. Q. Lu and Y. J. Xu, *Acta Phys. – Chim. Sin.*, 2018, **34**, 708–718.
85 S. Gogoi, S. Maji, D. Mishra, K. S. Devi, T. K. Maiti and N. Karak, *Macromol. Biosci.*, 2017, **17**, 1600271.
86 R. Chen, G. Liu, X. Sun, X. Cao, W. He, X. Lin, Q. Liu, J. Zhao, Y. Pang, B. Li and A. Qin, *Nanoscale*, 2020, **12**, 16229–16244.
87 Q. Ding, X. Zhang, Y. Huang, Y. Yan and X. Pang, *J. Mater. Sci.*, 2015, **50**, 189–202.
88 S. Erem, A. Atfi and M. S. Razzaque, *J. Steroid Biochem. Mol. Biol.*, 2019, **193**, 105400.
89 G. Liu, J. Miao, S. Sun, Z. Luo, Z. Xia, B. Guo, F. Liu, Y. Chen, D. Ying, B. Miao and Y. Cao, *Food Funct.*, 2017, **8**, 4487–4495.
90 M. M. Belluci, R. S. de Molon, C. Rossa Jr., S. Tetradis, G. Giro, P. S. Cerri, E. Marcantonio Jr. and S. R. P. Orrico, *J. Nutr. Biochem.*, 2020, **77**, 108301.
91 J. Wu, F. Liu, Z. Wang, Y. Liu, X. Zhao, C. Fang, F. Leung, K. W. K. Yeung and T. M. Wong, *Front. Bioeng. Biotechnol.*, 2022, **10**, 887252.
92 F. Kaiser, L. Schröter, S. Stein, B. Krüger, J. Weichhold, P. Stahlhut, A. Ignatius and U. Gbureck, *Acta Biomater.*, 2022, **145**, 358–371.
93 B. Yuan, H. Chen, R. Zhao, X. Deng, G. Chen, X. Yang, Z. Xiao, A. Aurora, B. A. Iulia, K. Zhang, X. Zhu, A. V. Iulian, S. Hai and X. Zhang, *Bioact. Mater.*, 2022, **18**, 354–367.
94 M. X. Yang, Y. Meng, J. J. Liu, W. X. Yu and B. Yang, *Part. Part. Syst. Charact.*, 2019, **36**, 1800315.
95 N. Jin, N. Jin, Z. Wang, L. Liu, L. Meng, D. Li, X. Li, D. Zhou, J. Liu, W. Bu, H. Sun and B. Yang, *Biomater. Sci.*, 2020, **8**, 2840–2852.
96 S. Samadian, A. Karbalaei, M. Pourmadadi, F. Yazdian, H. Rashedi, M. Omidi and S. Malmir, *Int. J. Polym. Mater. Polym. Biomater.*, 2022, **71**, 395–402.
97 S. Shafiei, M. Omidi, F. Nasehi, H. Golzar, D. Mohammadrezaei, M. R. Rad and A. Khojasteh, *Mater. Sci. Eng., C*, 2019, **100**, 564–575.
98 M. Hu, X. Gu, Y. Hu, Y. Deng and C. Wang, *Macromol. Mater. Eng.*, 2016, **301**, 1352–1362.
99 S. Gogoi, M. Kumar, B. B. Mandal and N. Karak, *RSC Adv.*, 2016, **6**, 26066–26076.
100 Y. Zhou, K. J. Mintz, L. Cheng, J. Chen, B. Ferreira, S. D. Hettiarachchi, P. Y. Liyanage, E. S. Seven, N. Miloserdov, R. R. Pandey, B. Quiroga, P. L. Blackwelder, C. C. Chusuei, S. Li, Z. Peng and R. M. Leblanc, *J. Colloid Interface Sci.*, 2020, **576**, 412–425.
101 S. C. Hsieh, F. F. Wang, C. S. Lin, Y. J. Chen, S. C. Hung and Y. J. Wang, *Biomaterials*, 2006, **27**, 1656–1664.
102 Z. Peng, E. H. Miyanji, Y. Zhou, J. Pardo, S. D. Hettiarachchi, S. Li, P. L. Blackwelder, I. Skromne and R. M. Leblanc, *Nanoscale*, 2017, **9**, 17533–17543.
103 K. K. Lee, J.-G. Lee, C. S. Park, S. H. Lee, N. Raja, H.-s. Yun, J.-S. Lee and C.-S. Lee, *RSC Adv.*, 2019, **9**, 2708–2717.
104 S. Li, I. Skromne, Z. Peng, J. Dallman, A. O. Al-Youbi, A. S. Bashammakh, M. S. El-Shahawi and R. M. Leblanc, *J. Mater. Chem. B*, 2016, **4**, 7398–7405.
105 H. Cai, J. Ma, X. Xu, H. Chu, D. Zhang and J. Li, *J. Mater. Chem. B*, 2020, **8**, 5655–5666.
106 H. Ding, J. S. Wei, N. Zhong, Q. Y. Gao and H. M. Xiong, *Langmuir*, 2017, **33**, 12635–12642.
107 H. Huang, S. Li, B. Y. Chen, Y. Wang, Z. F. Shen, M. Qiu, H. Pan, W. K. Wang, Y. G. Wang and X. Li, *J. Colloid Interface Sci.*, 2022, **627**, 705–715.
108 F. Ostadhossein, L. Benig, I. Tripathi, S. K. Misra and D. Pan, *ACS Appl. Mater. Interfaces*, 2018, **10**, 19408–19415.
109 B. Li, S. Zhao, L. Huang, Q. Wang, J. Xiao and M. Lan, *Chem. Eng. J.*, 2021, **408**, 127245.
110 J. Ge, Q. Jia, W. Liu, L. Guo, Q. Liu, M. Lan, H. Zhang, X. Meng and P. Wang, *Adv. Mater.*, 2015, **27**, 4169–4177.
111 P. Huang, J. Lin, X. Wang, Z. Wang, C. Zhang, M. He, K. Wang, F. Chen, Z. Li, G. Shen, D. Cui and X. Chen, *Adv. Mater.*, 2012, **24**, 5104–5110.
112 Z. Lu, S. Liu, Y. Le, Z. Qin, M. He, F. Xu, Y. Zhu, J. Zhao, C. Mao and L. Zheng, *Biomaterials*, 2019, **218**, 119190.
113 Y. Li, X. Zheng, X. Zhang, S. Liu, Q. Pei, M. Zheng and Z. Xie, *Adv. Healthcare Mater.*, 2017, **6**, 1600924.
114 S. M. Elsherbiny, C. S. Shao, A. Acheampong, M. A. Khalifa, C. Liu and Q. Huang, *Biomater. Sci.*, 2022, **10**, 2857–2864.
115 M. Lan, L. Guo, S. Zhao, Z. Zhang, Q. Jia, L. Yan, J. Xia, H. Zhang, P. Wang and W. Zhang, *Adv. Ther.*, 2018, **1**, 1800077.
116 Y. Cui, H. Liu, Y. Tian, Y. Fan, S. Li, G. Wang, Y. Wang, C. Peng and D. Wu, *Mater. Today Bio*, 2022, **16**, 100409.
117 L. Mishnaevsky, E. Levashov, R. Z. Valiev, J. Segurado, I. Sabirov, N. Enikeev, S. Prokoshkin, A. V. Solov'yov, A. Korotitskiy, E. Gutmanas, I. Gotman, E. Rabkin, S. Psakh'e, L. Dluhoš, M. Seefeldt and A. Smolin, *Mater. Sci. Eng., R*, 2014, **81**, 1–19.
118 B. H. Kapadia, R. A. Berg, J. A. Daley, J. Fritz, A. Bhave and M. A. Mont, *Lancet*, 2016, **387**, 386–394.
119 D. He, X. Zhang, X. Yao and Y. Yang, *Colloids Surf., B*, 2022, **211**, 112318.
120 J. Liu, S. Lu, Q. Tang, K. Zhang, W. Yu, H. Sun and B. Yang, *Nanoscale*, 2017, **9**, 7135–7142.
121 A. Z. Hameed, S. A. Raj, J. Kandasamy, M. A. Baghdadi and M. A. Shahzad, *Polymers*, 2022, **14**, 2335.
122 R. C. Cheung, T. B. Ng, J. H. Wong and W. Y. Chan, *Mar. Drugs*, 2015, **13**, 5156–5186.
123 J. C. Vinci, I. M. Ferrer, S. J. Seedhouse, A. K. Bourdon, J. M. Reynard, B. A. Foster, F. V. Bright and L. A. Colón, *J. Phys. Chem. Lett.*, 2013, **4**, 239–243.
124 F. Wu, H. Su, X. Zhu, K. Wang, Z. Zhang and W.-K. Wong, *J. Mater. Chem. B*, 2016, **4**, 6366–6372.