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# Strategic engineering of carbon dots: multi-enzyme mimetics for advanced biomedical intervention in oxidative stress-related diseases

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Carbon dots (CDs), an emerging class of carbon-based nanomaterials, have garnered significant attention due to their tunable physicochemical properties, biocompatibility, and versatile enzyme-mimicking activities. This review systematically explores recent advances in the rational design of CDs for combating oxidative stress-related pathologies. Key strategies include heteroatom doping, selection of bioactive precursors, and utilization of sustainable biomaterials. Doping with Se, Fe, or N enhances reactive oxygen/nitrogen species (RONS) scavenging, peroxidase, catalase, or superoxide dismutase like activities, enabling applications in acute kidney injury, ulcerative colitis, and Parkinson's disease. CDs derived from natural compounds inherit intrinsic antioxidant and anti-inflammatory properties, facilitating gut microbiota regulation, diabetic wound healing, and periodontitis treatment. Notably, stimuli-responsive CDs dynamically modulate multi-enzyme activities, preventing RONS overproduction and enabling precise therapy. This work highlights the synergy between structural engineering and bioactivity preservation in CDs, positioning them as next generation nanotherapeutics for inflammatory, infectious, and degenerative diseases.

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## 1. Introduction

Oxidative stress, characterized by excessive accumulation of reactive oxygen and nitrogen species (RONS), underpins the pathogenesis of diverse disorders including inflammatory bowel disease, neurodegenerative conditions, sepsis, and chronic wounds. Antioxidants are currently mainly classified into two categories: natural and synthetic. Natural antioxidants (such as plant extracts) have high safety but suffer from issues like high extraction costs and poor stability.<sup>1–3</sup> Synthetic antioxidants (such as chemically synthesized compounds) are stable and inexpensive, yet their biocompatibility remains questionable. In recent years, nanomaterials have emerged as a new frontier in antioxidant research due to their unique physicochemical properties.

Among various nanomaterials, carbon dots (CDs), as a rising star of carbon-based nanomaterials, have demonstrated significant advantages. First discovered by Scrivens and colleagues in 2004,<sup>4</sup> CDs are novel carbon-based nanomaterials applied across various fields.<sup>5–8</sup> Typically, CDs are discrete spherical fluorescent nanoparticles with a diameter of less than 10 nm, primarily composed of C, O, H, and/or N. CDs possess a carbon core with unsaturated bonds and a functionalized carbon shell, which grant them unique physicochemical

properties including high water solubility, strong fluorescence, and excellent photostability.<sup>9,10</sup> These characteristics, along with their good biocompatibility, make them safer for biomedical applications. The preparation of CDs employs “top-down” and “bottom-up” approaches, each with distinct methods offering specific advantages and limitations. Early techniques such as laser ablation<sup>4</sup> and arc discharge<sup>11</sup> allow size control or large-scale production, but suffer from high cost and low quantum yield. Chemical oxidation<sup>12</sup> offers high yield and purity but poses environmental concerns. Electrochemical methods<sup>13</sup> enable size tuning but are time-consuming, while pyrolysis<sup>14</sup> is efficient yet produces polydisperse products. The template method<sup>15</sup> provides good size control but is more complex. Microwave methods<sup>16</sup> achieve rapid heating but have high energy consumption and uneven heating. Hydrothermal/solvothermal methods<sup>17</sup> are eco-friendly and cost-effective, but yield lower purity. Organic synthesis<sup>18</sup> allows precise control over size and functional groups but requires stringent conditions. Ultrasonic methods<sup>19</sup> are mild and green but have low yields and are difficult for doping.

Initially, the biomedical applications of CDs were primarily focused on fluorescence imaging, sensing, and drug delivery. It is only in recent years that attention has gradually turned to their therapeutic effects, particularly through regulating RONS to intervene in oxidative stress-related diseases. The structural versatility and tunable surface chemistry make CDs highly amenable to functionalization, which significantly enhances their antioxidant potential. Specifically, their rich surface

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defects and unpaired electrons enable them to act as proton donors and free radical scavengers.<sup>20</sup> Key factors such as surface functional groups, synthesis parameters, electron transfer capacity, heteroatom doping, surface defects, and sp<sup>2</sup>-hybridized carbon domains can optimize and regulate the performance of CDs by influencing their physicochemical properties and structural characteristics.<sup>21–24</sup>

A particularly effective strategy for tailoring the electronic properties and bioactivities of CDs is heteroatom doping, which significantly enhances their RONS-scavenging capabilities and confers specific enzyme-mimetic functions.<sup>25</sup> For example, Fe/N co-doping enhances peroxidase (POD)-like activity for antibacterial applications, while Se doping enables glutathione peroxidase (GP<sub>x</sub>) and superoxide dismutase (SOD)-mimetic cascades used in rheumatoid arthritis (RA) therapy. Moreover, CDs derived from natural biomolecules or pharmaceuticals often retain or even amplify the bioactivity of their precursor molecules, enabling synergistic therapeutic outcomes. Recent advances have also led to the development of stimuli-responsive CDs whose enzyme-like activities (*e.g.*, POD *vs.* catalase) can be reversibly switched by light or pH, allowing spatiotemporal control over RONS generation and elimination within pathological microenvironments.

To date, no comprehensive review has systematically summarized the research progress of CDs-based RONS regulation, which leaves a significant gap in understanding their potential for oxidative stress management in biomedicine. Given the escalating demand for effective RONS-modulating nanotherapeutics in diseases ranging from inflammation to cancer, addressing this knowledge gap is critical for advancing CDs-based precision medicine. This review comprehensively examines the design principles, mechanistic insights, and therapeutic efficacy of engineered CDs in managing oxidative stress-driven diseases. We categorize strategies into heteroatom

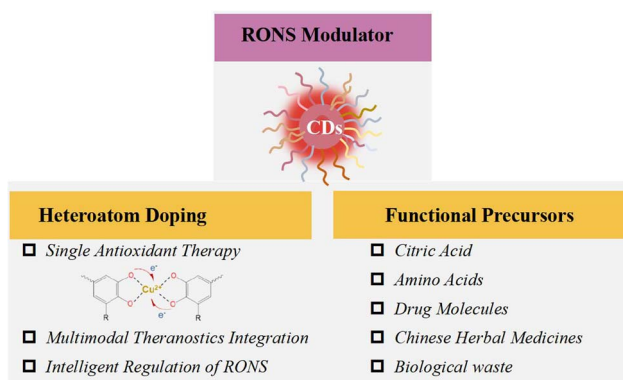
doping, precursor selection and biomaterial utilization, emphasizing structure–activity relationships. By critically analyzing advances and challenges, we aim to guide the rational development of CD-based nanoplatforams for precision nanomedicine (Scheme 1).

## 2. Heteroatom doping

Aiming at the limitations of traditional antioxidants and the complexity of RONS imbalance in diseases, heteroatom doping engineering is leading the evolution of CDs from single “scavengers” to intelligent “regulators”. By precisely introducing elements such as Se, Fe, N, P, O, Cu, and Ce, the electronic structure, surface chemistry, and catalytic activity of CDs are profoundly reshaped. This not only endows them with efficient, targeted, and even broad-spectrum RONS scavenging capabilities but also enables dynamic, switchable regulation of RONS metabolism and theranostic integration. The rational selection of dopants is not arbitrary but is fundamentally guided by the intended therapeutic mechanism and the specific electronic properties each element confers. For instance, the choice of Se is often driven by its singular capacity to mimic GP<sub>x</sub> activity, making it ideal for diseases characterized by excessive peroxides, such as RA. In contrast, Fe doping is strategically employed to impart POD-like activity for antibacterial applications or chemodynamic therapy. N doping, which enhances electron-donating capacity and creates metal-chelating sites, is particularly suited for neural applications involving metal ion sequestration and enhanced blood–brain barrier penetration. Therefore, the design of doped CDs must transition from empirical discovery to a rational paradigm where the dopant is selected based on a precise understanding of its physicochemical role in the targeted pathological process. Such atomic-level “activity programming” strategies break through the instability and cost bottlenecks of natural enzymes, providing revolutionary nanoplatforams for on-demand customized antioxidant interventions and precise treatment of inflammation, neurodegenerative diseases, and drug-resistant infections. This marks the entry of disease antioxidant therapy into a new era of intelligence, efficiency, and multifunctional synergy.

### 2.1 Single antioxidant therapy

Breaking through the limitations of traditional drug delivery and efficacy, Fe-doped CDs have demonstrated exceptional therapeutic potential in complex intestinal environments. Kong *et al.*<sup>27</sup> synthesized green-emissive CDs (MML-CDs) *via* a hydrothermal method using magnetite and medicated leaven as precursors. MML-CDs, with an Fe-doped surface structure, demonstrate remarkable capabilities in scavenging reactive oxygen species (ROS) and alleviating oxidative stress in Caco-2 cells (*in vitro*). Furthermore, MML-CDs exhibited promising therapeutic effects against dextran sulfate sodium (DSS)-induced ulcerative colitis (UC) in mice (*in vivo*). Subsequent experiments revealed that MML-CDs ameliorate UC by accelerating hemostasis, regulating inflammatory responses and oxidative stress, and repairing colonic barrier damage (Fig. 1a).



**Scheme 1** The design of CDs as RONS modulators. It categorizes engineering strategies into heteroatom doping (enabling single-antioxidant therapy, multimodal theranostics integration, and intelligent RONS regulation) and functional precursors (including citric acid, amino acids, drug molecules, Chinese herbal medicines, and biological waste), highlighting pathways to tailor CDs for precision oxidative stress management. For instance, in heteroatom doping, the electron cloud density of the Cu single atom in the Cu–N<sub>4</sub> structure changes, enabling it to serve as the catalytic center for redox regulation.<sup>26</sup>



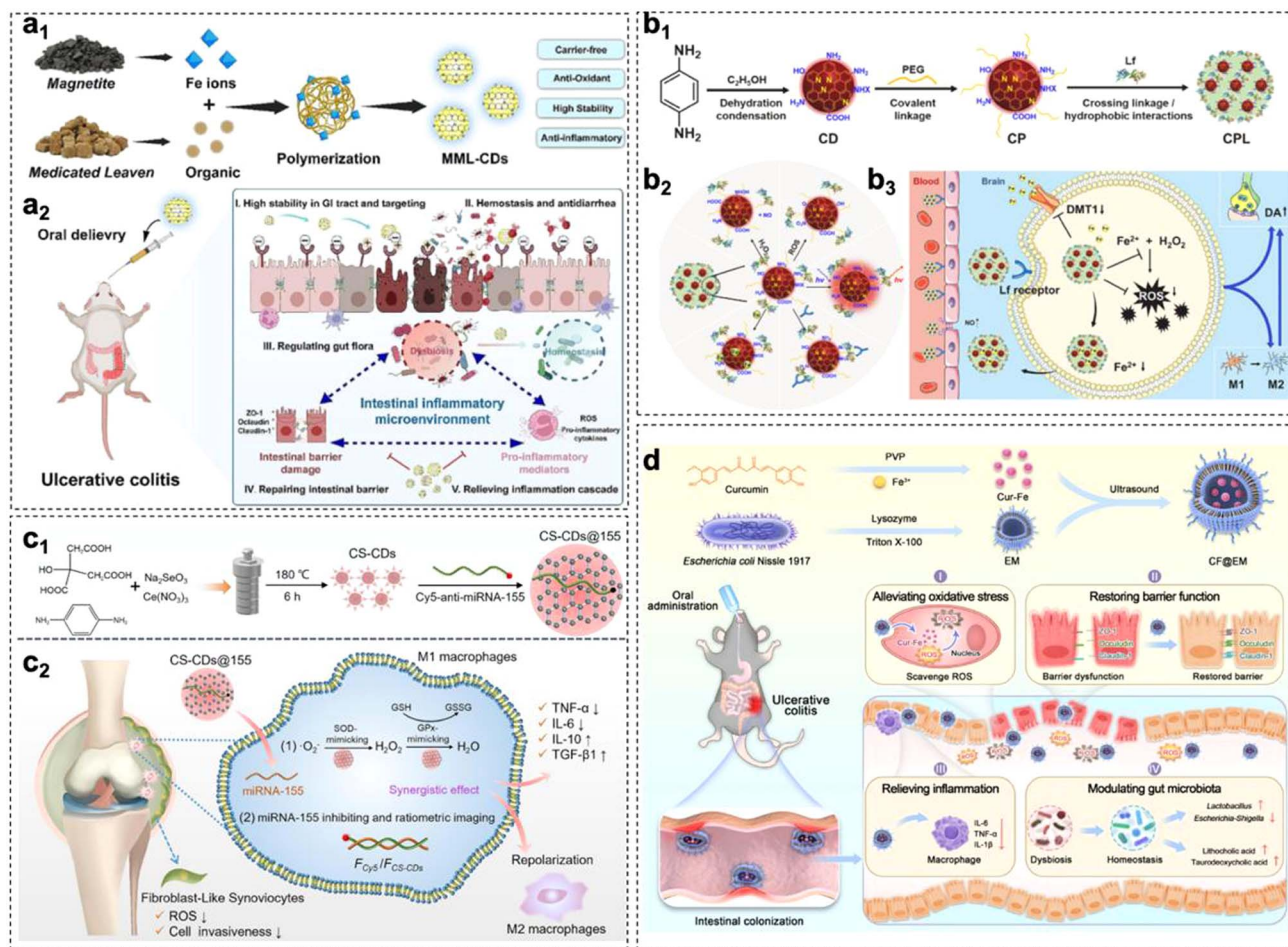


Fig. 1 (a) Schematic of MML-CDs synthesis and their oral delivery for modulating colonic microenvironment to treat ulcerative colitis in mice. Reproduced with permission.<sup>27</sup> Copyright 2024, BioMed Central. Schematic of CD-based nano-formulations: (b<sub>1</sub>) synthesis of diverse nano-formulations; (b<sub>2</sub>) multifunctional properties of CPL; (b<sub>3</sub>) Fe-chelating, antioxidative CD-based nano-formulation with NO release for Parkinson's disease therapy. Reproduced with permission.<sup>28</sup> Copyright 2024, Elsevier BV. Schematic of CS-CDs/Cy5-anti-miRNA-155 (CS-CDs@155) fabrication and application: (c<sub>1</sub>) hydrothermal synthesis of CS-CDs and  $\pi$ - $\pi$  stacking-mediated adsorption of Cy5-anti-miRNA-155 (quenching Cy5 fluorescence); (c<sub>2</sub>) miRNA-155-responsive fluorescence recovery in M1 macrophages for ROS scavenging, inflammation regulation, and RA progression monitoring. Reproduced with permission.<sup>30</sup> Copyright 2024, Elsevier. (d) Schematic of antibacterial CDs synthesis and their application in treating wound bacterial infections. Reproduced with permission.<sup>32</sup> Copyright 2024, Elsevier.

## 2.2 Multimodal theranostics integration

Introducing the concept of precision therapy into the field of neurodegenerative diseases, N-doped CDs have enabled targeted theranostics across the blood-brain barrier (BBB). Guo *et al.*<sup>28</sup> synthesized red-emissive N-doped CDs *via* a solvothermal reaction of *p*-phenylenediamine. These CDs possess a high reduction potential due to abundant electron-rich amino groups conjugated on the sp<sup>2</sup>-hybridized  $\pi$ -system of the carbon core, allowing them to scavenge ROS and generate nitric oxide (NO) under oxidative stress (*in vitro*). The electron-localizing effect induced by pyridinic N-doping enables chelation of free Fe ions, inhibiting Fe-catalyzed cycles and hydroxyl radical production to block Fenton reactions. After modification with lactoferrin (Lf), the resulting CPL nanosystem achieves non-invasive *trans*-BBB delivery through Lf receptor-mediated transport and NO-induced reversible BBB opening. Meanwhile, leveraging Lf receptor targeting, CPL accumulates in

dopaminergic neurons, where the Fe-chelating and ROS-scavenging functions of CDs-synergized with Lf to prevent Fe ion reflux-enable fluorescent imaging of Parkinson's disease (PD), inhibition of brain oxidative stress and inflammation, and improvement of behavioral performance in MPTP-model mice (*in vivo*) (Fig. 1b).

Beyond single therapeutic functions, N/Fe-co doped CDs ingeniously integrate highly sensitive biosensing and cytoprotective roles. Geng *et al.*<sup>29</sup> synthesized multifunctional N,Fe-doped CDs (N,Fe-CDs) *via* a one-step hydrothermal method using ammonium Fe citrate and dicyandiamide as precursors. In the presence of H<sub>2</sub>O<sub>2</sub>, this material exhibits POD-like activity ( $K_m = 0.423$  mM,  $V_{max} = 12.15 \times 10^{-8}$  M s<sup>-1</sup>), catalyzing the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) into a green product (ox-TMB) (*in vitro*). Based on this property, a highly sensitive colorimetric method was developed for detecting H<sub>2</sub>O<sub>2</sub> and ascorbic acid (AA), with detection limits of 0.40  $\mu$ M and 2.05



$\mu\text{M}$ , respectively. The method has been successfully applied to detect AA in fruit juices, vitamin C tablets, and human serum, showing potential for applications in biotechnology and the food industry. This system demonstrates cytoprotective effects against oxidative damage.

Ce/Se-co doped CDs establish a self-cascading antioxidant system for synergistic diagnosis and immune microenvironment remodeling in RA. Dong *et al.*<sup>30</sup> designed Ce- and Se-co doped red-emitting CDs (CS-CDs) that integrate self-cascading SOD-like and GP<sub>x</sub>-like catalytic activities. By incorporating miRNA-155, CS-CDs enable macrophage sub phenotype identification to accurately monitor RA, regulate pro-inflammatory macrophage polarization (*in vitro*), and synergistically improve RA outcomes (*in vivo*) (Fig. 1c).

### 2.3 Intelligent regulation of RONS

Light-responsive O/N-doped CDs have overcome the challenge of “uncoordinated multi-activity” in nanozymes, enabling spatiotemporally precise and non-invasive dynamic regulation of RONS metabolism. Nanozymes possess multi-enzymatic activities superior to natural enzymes, enabling multi-pathway synergistic effects in various biomedical applications. However, their multi-enzymatic activities often remain co-activated, which significantly reduces synergistic efficiency and becomes a critical bottleneck in intelligent therapy. For example, POD-like nanomaterials such as Fe<sub>3</sub>O<sub>4</sub> effectively increase intracellular ROS levels (*in vitro*), but sustained “open-mode” catalytic activity leads to excessive ROS-induced oxidative damage to cells and tissues, potentially triggering cardiovascular diseases, Alzheimer’s disease, renal diseases, and other conditions. Dynamic regulation of multi-enzymatic activities circumvents these risks, making the development of nanozymes with switchable multi-activities urgent. Such materials must not only enable precise regulation of catalytic activity but also switch between activity modes to avoid oxidative damage and optimize synergistic therapeutic effects.

Fang *et al.*<sup>31</sup> developed O/N-functionalized carbon quantum dots (O/N-CQDs) with dual activities of POD (ROS generation) ( $K_m = 0.13 \text{ mM}$ ,  $V_{\text{max}} = 10.1 \times 10^{-7} \text{ M s}^{-1}$ ) and light-responsive CAT (ROS scavenging). The study demonstrated that the enzymatic activities of O/N-CQDs can be reversibly switched by visible light intensity (*in vitro*). Under illumination, the quinone-N groups on the surface polyaniline precursor of O/N-CQDs undergo polarization, driving ROS consumption to generate O<sub>2</sub> and H<sub>2</sub>O, thereby converting POD activity to CAT activity. As a proof-of-concept, the team successfully regulated intracellular ROS levels non-invasively by toggling light on/off, providing a new strategy for precise drug modulation during disease progression.

Integrating multi-active elements and natural molecular wisdom, the N/S/Cu-doped CDs platform offers a comprehensive “antibacterial-anti-inflammatory-promoting repair” solution for infected wound healing. Aiming at the challenges in inflammation control and treatment of bacterial-infected wounds, and inspired by the antibacterial mechanisms of active elements such as N, S, Cu, and tannic acid (TA), Zhang *et al.*<sup>32</sup> designed an

efficient multifunctional CQDs platform *via* their specific assembly in a solvothermal reaction system. By introducing N, S, and Cu, the platform exhibits antibacterial properties (*in vitro*), while Cu ions enable excellent angiogenesis-promoting performance (Fig. 1d). TA contributes immunomodulatory capacity to the platform, and therapeutic studies in bacterial infection models showed that the multifunctional CQDs accelerate infected wound healing by inhibiting bacterial infection, regulating immune responses, accelerating collagen deposition, and promoting angiogenesis (*in vivo*). This multifunctional CQDs platform demonstrates promising clinical application prospects for treating bacterial-infected wounds.

## 3. Citric acid as green carbon precursors

Citric acid, with its unique molecular modifiability, biocompatibility, and multi-active site characteristics, is driving a leap forward in CDs-based antioxidant research toward low-cost, multifunctional, and precision-oriented applications.<sup>33</sup> Citric acid possesses a unique molecular structure with multiple carboxylic groups and a hydroxyl group, which facilitate dehydration and carbonization under mild conditions, leading to CDs rich in oxygen-containing functional groups. These groups not only enhance water dispersibility and biocompatibility but also serve as active sites for radical scavenging, thereby inherently promoting antioxidant activity. Compared to other carbon sources (*e.g.*, graphite, polymer wastes), citric acid offers superior controllability in size and surface functionality, enabling precise tuning of optical and electronic properties through one-pot synthesis. Furthermore, its low cost, commercial availability, and green nature align with sustainable material design, making it particularly attractive for large-scale biomedical and environmental applications. Citric acid-derived CDs, designed and functionalized from precursors, not only scavenge radicals like traditional antioxidants but also show breakthrough potential in synergistic therapy and intelligent responsiveness across multiple areas including plant stress resistance, wound healing, gut health, and biosensing.

Citric acid-based CDs have demonstrated pioneering application potential in agricultural stress resistance. Prof. Lisak’s team<sup>34</sup> synthesized blue-fluorescent antioxidant CDs (B-CDs) *via* microwave-assisted synthesis using citric acid and ascorbic acid as carbon sources. These B-CDs show promise in enhancing drought resistance in *Pisum sativum* L. (pea plants) (Fig. 2a). Focused on improving biosafety, metal-free citric acid CDs have emerged as highly compatible candidates for colitis treatment. Wang *et al.*<sup>35</sup> developed metal-free CDs (CP-CDs) using citric acid and polyamine precursors for colitis therapy (Fig. 2b). As antioxidants, CP-CDs protect against intestinal inflammation by regulating oxidative stress and gut microbiota in nematode and mouse models (*in vivo*).

This strategy has been innovatively extended to create multifunctional CDs with photothermal antibacterial and heat-enhanced antioxidant properties for diabetic wound healing. Lin *et al.*<sup>36</sup> developed multifunctional AA-CDs (antibacterial and



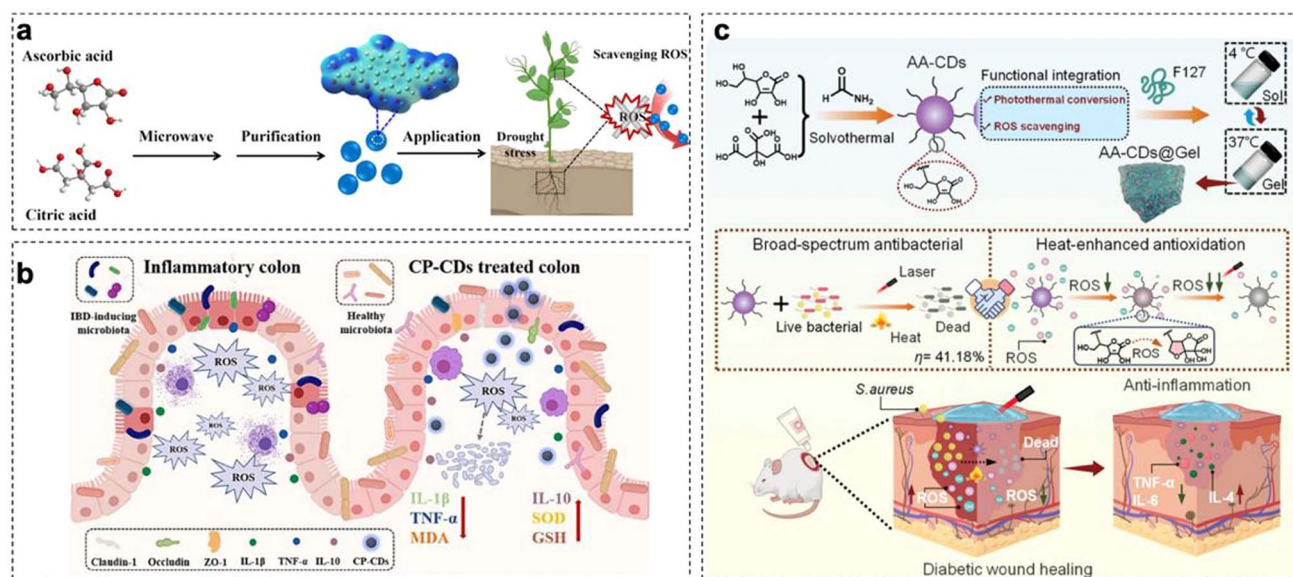


Fig. 2 (a) Formation of B-CDs and application in peas. Reproduced with permission.<sup>34</sup> Copyright 2024, American Chemical Society. (b) Preparation of CP-CDs and their therapeutic mechanism in a DSS-induced colitis model. Reproduced with permission.<sup>35</sup> Copyright 2024, Elsevier. (c) Schematic of AA-CDs synthesis for photothermal antibacterial and heat-enhanced antioxidant therapy in diabetic wound healing. Reproduced with permission.<sup>36</sup> Copyright 2024, Wiley.

antioxidant) using low-cost citric acid and ascorbic acid precursors. These CDs integrate photothermal sterilization and ROS-scavenging capabilities. *In vitro* experiments show that AA-CDs significantly inhibit various bacteria (including multidrug-resistant strains) while protecting cells from oxidative stress *via* ROS scavenging to maintain cellular function. Their heat-enhanced antioxidant activity improves ROS-scavenging efficiency at both solution and cellular levels (*in vitro*), providing additional protection against oxidative damage. *In vivo* studies demonstrate that AA-CD-based hydrogels accelerate diabetic wound healing through triple actions of antibacterial activity, heat-enhanced antioxidant, and anti-inflammation (*in vivo*) (Fig. 2c).

## 4. Amino acids as functional precursors

Amino acids, as ideal precursors combining biocompatibility and tunable functionality, are driving antioxidant CDs research toward precision applications in targeted delivery, environmental protection, and biomimetic catalysis. Their specific moieties (*e.g.*, imidazole rings, thiol groups, chiral centers) endow CDs with intelligent responsiveness beyond traditional antioxidants, offering molecular-level design paradigms to overcome limitations of natural enzymes. Histidine-derived CDs have pioneered cross-system synergy of antioxidation, microbiota regulation, and neuroprotection. He *et al.*<sup>37</sup> synthesized CDs nanozymes using glucose and histidine as precursors, featuring excellent antioxidant capacity and biocompatibility. These CDs effectively reduce oxidative stress (*in vitro*), restore gut microbial balance (*in vivo*), and alleviate depressive symptoms (*in vivo*) (Fig. 3a).

Thiol engineering in cysteine endows CDs with dual functions of full-spectrum UV shielding and anti-photoaging. Li *et al.*<sup>38</sup> introduced thiol groups to synthesize L-cysteine-derived CDs (GLCDs) with UV resistance (Fig. 3b). GLCDs exhibit highly efficient and superior UV absorption capacity (*in vitro*), achieving 200–400 nm UV absorption (99% UVC, 97% UVB, and 86% UVA) at a low concentration of 0.5 mg mL<sup>-1</sup>. Meanwhile, GLCDs reduce UVB-induced oxidative damage and apoptosis in zebrafish cells (*in vitro*), upregulate type I collagen gene expression, and inhibit skin aging in zebrafish (*in vivo*). They also suppress senescence induced by the aging inducer 2,2'-azobis(2-methylpropionamide) dihydrochloride and reduce oxidative damage (*in vitro*).

Through in-depth exploration of structure–function relationships, researchers have revealed that key groups (such as imidazole groups) in specific amino acid-derived CDs endow them with exceptional SOD-like activity and a unique mechanism for regulating gut microbiota. Wang *et al.*<sup>39</sup> synthesized structurally similar CDs using citric acid and 16 amino acids as precursors *via* a one-step hydrothermal method. Among the synthesized CDs, histidine-derived CDs (His-CDs) were selected as representative nanomedicines due to their superior radical-scavenging ability and SOD-like activity (*in vitro*). Studies have found that the surface groups of His-CDs, particularly imidazole groups, play a critical role in their SOD enzymatic activity (*in vitro*). His-CDs restore intestinal homeostasis by reshaping the gut microbiota (*in vivo*), increasing the abundance of *Bacteroidetes* and inhibiting *Proteobacteria*.

Currently, traditional nanomaterials are primarily constructed by mimicking key structural features of natural enzymes, such as amino acid microenvironments, metal-free structures, and coordination structures between active centers



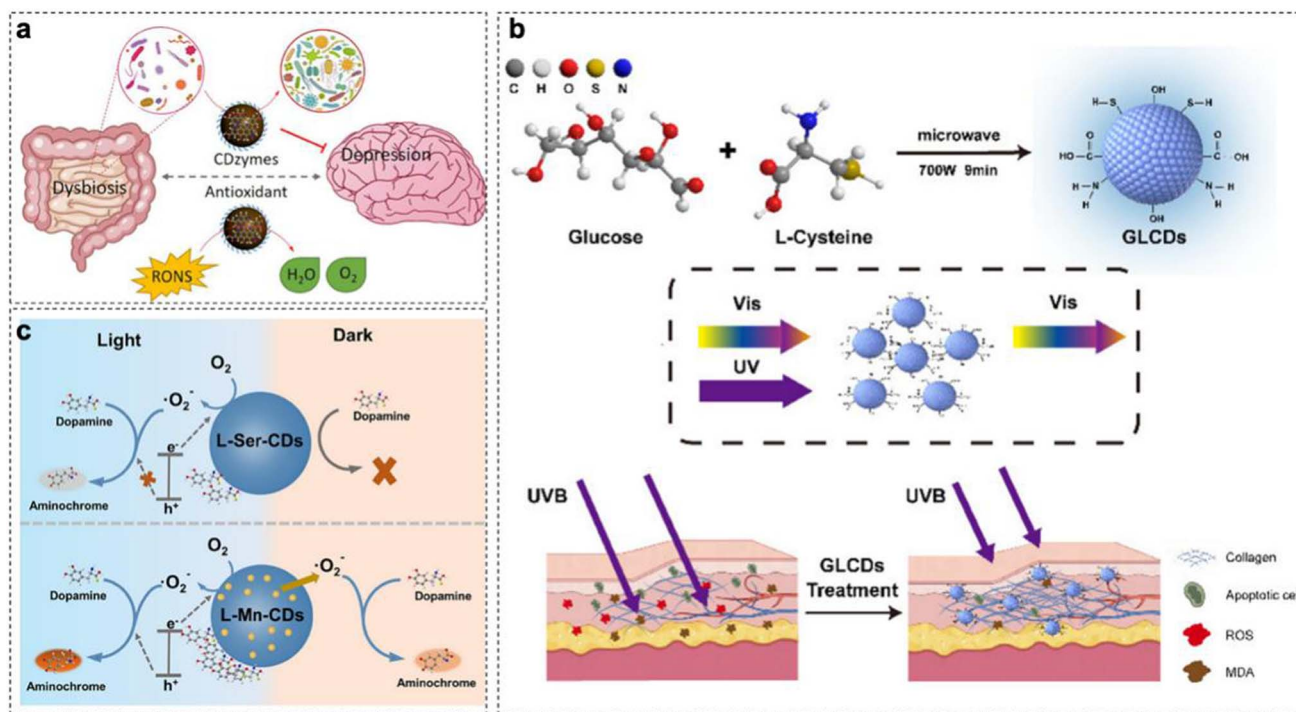


Fig. 3 (a) Schematic of glucose- and histidine-derived CDs nanozymes for relieving depression *via* antioxidant restoration of gut microbial balance. Reproduced with permission.<sup>37</sup> Copyright 2024, ACS publications. (b) Mechanism of L-cysteine-derived CDs (GLCDs) in inhibiting zebrafish skin aging and cellular senescence through efficient 200–400 nm UV absorption, oxidative damage reduction, and collagen synthesis promotion. Reproduced with permission.<sup>38</sup> Copyright 2024, American Chemical Society. (c) Catalytic mechanism comparison between L-Ser-CDs and L-Mn-CDs. Reproduced with permission.<sup>40</sup> Copyright 2024, Elsevier.

and attached cofactors. However, for these artificial enzymes with ill-defined nanostructures, there is a lack of relevant catalyst design strategies, which severely hinders their further development in the biomedical field. While traditional nanozymes struggle to mimic the active centers of natural enzymes due to structural ambiguity, chiral amino acids open new pathways for the precise construction of biomimetic catalysts. Chiral CDs, which possess both inherent enzyme-like activity and specific recognition capabilities, have emerged as potential enzyme-like catalysts, although their catalytic activity still lags that of natural enzymes and requires further improvement. Kang *et al.*<sup>40</sup> regulated the chiral structure and oxidase (OXD)-like catalytic performance of chiral CDs *via* manganese doping (Fig. 3c). Results showed that under light irradiation, chiral CDs derived from chiral serine (Ser) (L-Ser-CDs and D-Ser-CDs) exhibited weak catalytic activity and low selectivity toward the oxidation of L-dopamine (L-DOPA) (*in vitro*). In contrast, Mn-doped chiral CDs (L-Mn-CDs or D-Mn-CDs) displayed catalytic activity ( $K_m = 3.04$  mM for D-Mn-CDs) and selectivity ratio (SR) 6.9-fold and 2.9-fold higher than those of Ser-CDs, respectively. The catalytic process of Mn-CDs follows a dual-mode mechanism: photogenerated electrons reduce  $O_2$  to  $\cdot O_2^-$  as reactive species, while holes directly oxidize DOPA. Additionally, Mn doping enhances the generation of  $\cdot O_2^-$  in CDs. Notably, L-Mn-CDs showed higher catalytic activity than D-Mn-CDs, and chiral Mn-CDs exhibited stronger enantioselective adsorption toward chiral DOPA compared to Ser-CDs. However, chiral CDs face

three core challenges in *in vivo* biological applications: first, their potential immunogenicity and unpredictable pharmacokinetics may trigger immune responses or lead to rapid clearance; second, the chiral centers exhibit insufficient stability in physiological environments, and are prone to structural degradation under the influence of proteases or pH variations; third, although metal doping enhances their catalytic performance, their enantioselectivity and efficiency are still far lower than those of natural enzymes. Relying on non-specific reaction mechanisms results in insufficient precision, making it difficult to meet the requirements of biological applications with high selectivity demands.

Apart from histidine, cysteine, and serine, amino acid-derived CDs offer untapped potential for the design of functional nanomaterials with customized properties. Tryptophan-derived CDs, with their indole group, can enhance electron-donating ability and UV absorption, making them promising for photodynamic therapy or as fluorescent probes for biomarkers in neurodegenerative diseases. Lysine-derived CDs, with their amine-rich surface, promote pH-responsive drug release and enhance cellular uptake, making them especially suitable for acidic tumor microenvironments or inflammation sites. Tyrosine-derived CDs, with their phenolic hydroxyl groups, can exhibit excellent free radical scavenging effects through hydrogen donation, mimicking the functions of natural antioxidants like tyrosine-based melanin. Systematic comparative studies are still needed to evaluate the structure-



activity relationships of different amino acid precursors, focusing on how aromatic rings, charged residues, and other side chain groups affect the catalytic activity, stability, and biocompatibility of CDs.

## 5. Drug molecules as functional precursors

Drug molecules, as precursors for functionalized CDs, are revolutionizing disease treatment through their inherent bioactivity and nanocarrier properties. These “drug-CDs” hybrid systems not only retain the targeting mechanisms of

parent drugs but also integrate CDs-mediated ROS regulation and imaging capabilities, achieving breakthroughs in therapeutic integration across inflammatory, metabolic, and neurodegenerative diseases. Bi *et al.*<sup>41</sup> reported a novel biofunctional CDs synthesized by integrating metformin and tea polyphenols. The resultant metformin-TP CDs (MTCDs) exhibit excellent biocompatibility and accumulate in lysosomes *via* endocytosis (*in vitro*) (Fig. 4a). Biological assays demonstrated that MTCDs significantly reduce oxidative stress and lipid deposition in hepatocytes (*in vitro*). Mechanistically MTCDs preserve lysosomal integrity activate the AMPK signaling pathway and initiate lysosome-associated adipophagy accelerating lipolysis and fatty acid oxidation. This biofunctional CDs design

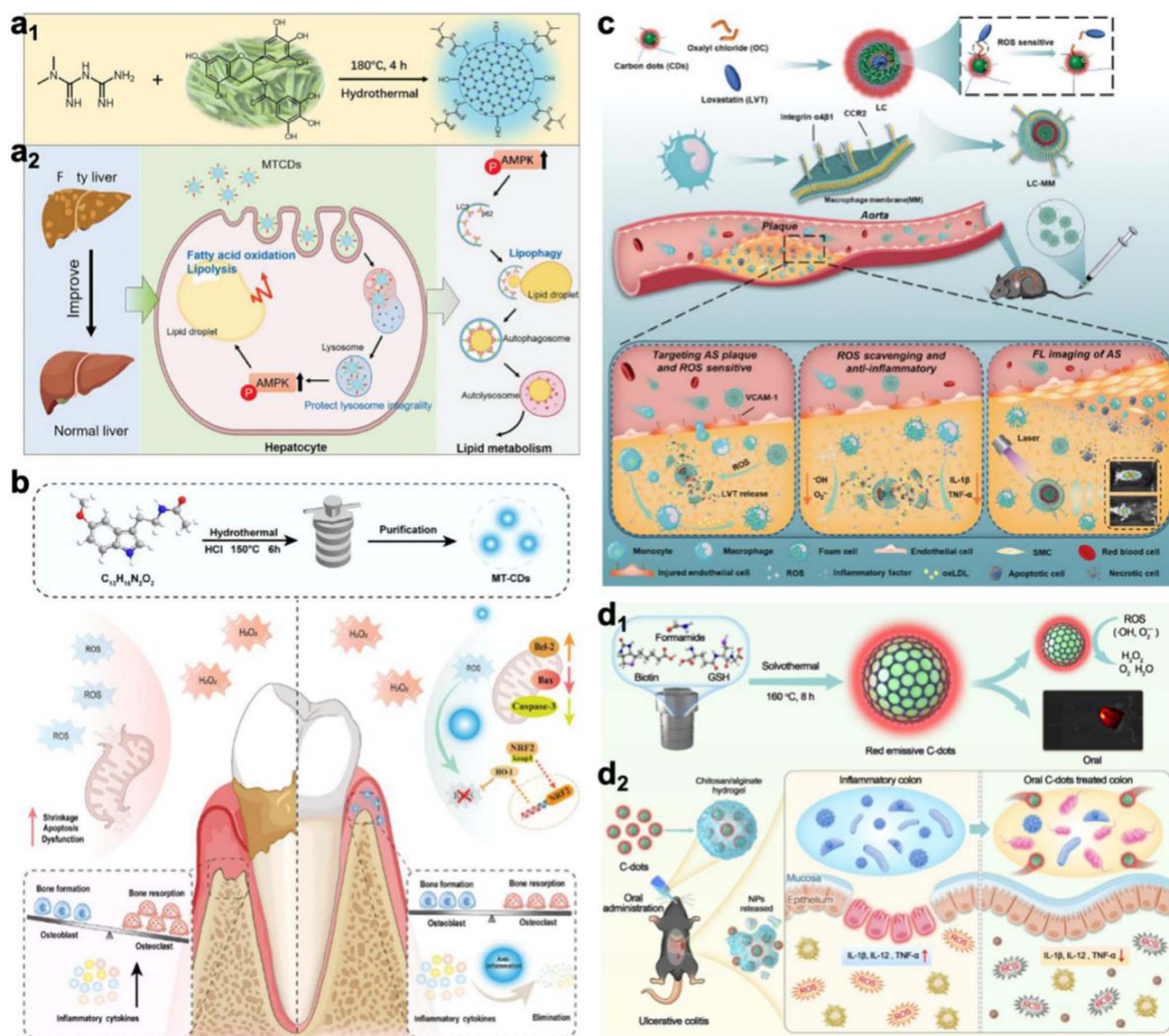


Fig. 4 (a<sub>1</sub>) MTCDs synthesis schematic; (a<sub>2</sub>) mechanism of MTCDs in promoting lipid metabolism and improving MAFLD. Reproduced with permission.<sup>41</sup> Copyright 2024, Wiley-VCH Verlag. (b) Design of MT-CDs and their anti-inflammatory/antioxidant applications in periodontal diseases. Reproduced with permission.<sup>42</sup> Copyright 2024, American Chemical Society. (c) LC-MM preparation and its application in AS management. Reproduced with permission.<sup>43</sup> Copyright 2024, Wiley-VCH Verlag. (d) Schematic of C-dots nanozyme synthesis and UC therapy: (d<sub>1</sub>) ROS-scavenging and bioimaging properties; (d<sub>2</sub>) oral delivery *via* chitosan/alginate hydrogel for UC treatment by regulating ROS and intestinal microbiota. Reproduced with permission.<sup>44</sup> Copyright 2024, Elsevier.



addresses both lipid deposition and oxidative stress in metabolic-associated fatty liver disease (MAFLD) through a dual-drug synergistic strategy (*in vivo*).

Melatonin-derived CDs innovatively reverse the balance of bone destruction in periodontitis with mitochondrial homeostasis as the core hub. Yu *et al.*<sup>42</sup> synthesized melatonin-derived CDs (MT-CDs) *via* a hydrothermal method. MT-CDs exhibit antioxidant activity by scavenging ROS protecting cells and maintaining mitochondrial homeostasis (*in vitro*). Concurrently MT-CDs restore the balance between osteoblasts and osteoclasts by suppressing periodontal tissue inflammation and ROS accumulation effectively alleviating periodontitis (*in vivo*) (Fig. 4b).

Biomimetic macrophage membrane coating technology enables statin-CDs hybrid systems to overcome dual challenges of precise lesion accumulation and ROS-responsive release. Wu *et al.*<sup>43</sup> constructed a ROS-responsive biomimetic nanomedicine (LC-MM) for targeted theranostics of atherosclerosis (AS) (Fig. 4c). This system uses reduced glutathione-derived CDs as carriers, with their deep red emission and strong antioxidant capacity serving as a foundation for nanomedicine development. The classic cholesterol-lowering drug lovastatin (LVT) is loaded to inhibit pro-inflammatory cytokines (*in vitro*). LVT is conjugated to CDs *via* an oxalyl chloride (OC) chemical bonding strategy to ensure ROS-triggered rapid drug release. Further coating with macrophage membranes (MM) enhances the enrichment efficiency of nanomedicines in AS plaques through specific binding between membrane protein integrin  $\alpha 4\beta 1$  and overexpressed vascular cell adhesion molecule-1 (VCAM-1) on inflamed endothelial cell surfaces (*in vitro*). This multifunctional integrated design establishes LC-MM as a safe and efficient AS theranostic platform (*in vivo*), providing new strategies for precise management of atherosclerosis.

Glutathione-biotin bifunctional CDs have pioneered synergistic intervention of ROS scavenging, microbiota remodeling, and inflammation imaging. Zhang *et al.*<sup>44</sup> prepared glutathione- and biotin-derived CDs (C-dots) nanozymes *via* a solvothermal method. The synthesized C-dots nanozymes were found to possess both SOD-like activity and the ability to regulate gut microbiota (Fig. 4d). *In vitro* experiments showed that C-dots nanozymes can scavenge excessive ROS, protect cells from oxidative stress damage, and reduce the expression of pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin-6 (IL-6), and IL-1 $\beta$ . In a UC mouse model, oral administration of C-dots nanozymes encapsulated in chitosan/alginate hydrogels significantly alleviated colonic inflammation, as evidenced by improved endoscopic and histopathological findings, with both preventive and therapeutic effects. Additionally, C-dots significantly regulated gut microbiota composition by increasing potential probiotics and reducing pathogenic microorganisms. Meanwhile, C-dots exhibited excellent red fluorescence properties for bioimaging of intestinal inflammation.

The integration of drug molecules as precursors fundamentally reshapes the physicochemical and biological properties of CDs through molecular-level functional inheritance. Unlike conventional carbon sources, drug molecules introduce

specific bioactive moieties that are partially preserved during carbonization, directly conferring targeting capabilities, signaling pathway modulation, and metabolic activities. For instance, the AMPK-activating property of metformin-derived CDs originates from retained biguanide-like fragments, while melatonin-derived CDs inherit radical-scavenging indole motifs. This “structure–activity retention” principle enables precise tuning of CDs' electronic structure, surface reactivity, and biological interactions. However, the extent of functional group preservation depends heavily on synthesis parameters, and excessive carbonization may degrade critical pharmacophores. A systematic comparative analysis of how different drug classes influence CDs properties is urgently needed to establish rational design rules.

## 6. Chinese herbal medicines as precursors

Chinese herbal medicines are revolutionizing traditional nanozyme design paradigms through their multi-component synergistic pharmacology. By reconstructing active molecules into stable nanostructures *via* carbonization, CDs inherit native antioxidant/anti-inflammatory moieties while integrating catalytic properties and fluorescence functions, achieving a therapeutic upgrade from “chemical component delivery” to “bioactive nanonization”.

Nanozymes based on Se- or metal-doped CDs hold promise as antioxidants, but their high cost and potential biotoxicity of metal elements hinder widespread application. Leveraging the natural bioactivity of Chinese herbal medicines, such as polyphenols/flavonoids, to circumvent metal toxicity and reduce costs, Jiang *et al.*<sup>45</sup> prepared SB-HHD-CDs *via* hydrothermal synthesis using *Scutellaria barbata* (SB) and *Hedyotis diffusa* (HHD) as precursors. These SB-HHD-CDs exhibit excellent thermal and pH stability, rapidly scavenging multiple RONS, including superoxide anions, hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl radicals, and nitric oxide radicals at low concentrations (*in vitro*). As a proof-of-concept, SB-HHD-CDs integrated into cigarette filter systems effectively remove over 80% of RONS generated by cigarette combustion. This study provides a new strategy for developing low-cost, highly biocompatible antioxidant nanozymes (Fig. 5a).

Hydrothermal treatment converts natural flavonoid quercetin into 5 nm-diameter CDs, preserving its antimicrobial/antioxidant active groups (catechol structures) and forming a five-layer graphene-like structure to enhance stability (Fig. 5b).<sup>46</sup> Li *et al.*<sup>47</sup> prepared ROS nano modulators by extracting CDs from *Lonicera japonica* (HOCD), *Taxus chinensis* leaves (TACD), and *Taraxacum officinale* (DACD) for dynamic ROS regulation to achieve precise treatment of chronic inflammation and infections (Fig. 5c). Zhang *et al.*<sup>48</sup> used *Lonicera japonica* as a common carbon precursor to synthesize CDs with both catalytic properties and pharmacological activities *via* hydrothermal (Hy-CDs) and carbonization (Ca-CDs) methods. *In vitro* experiments showed that Hy-CDs effectively alleviate cellular oxidative stress and inhibit pro-inflammatory



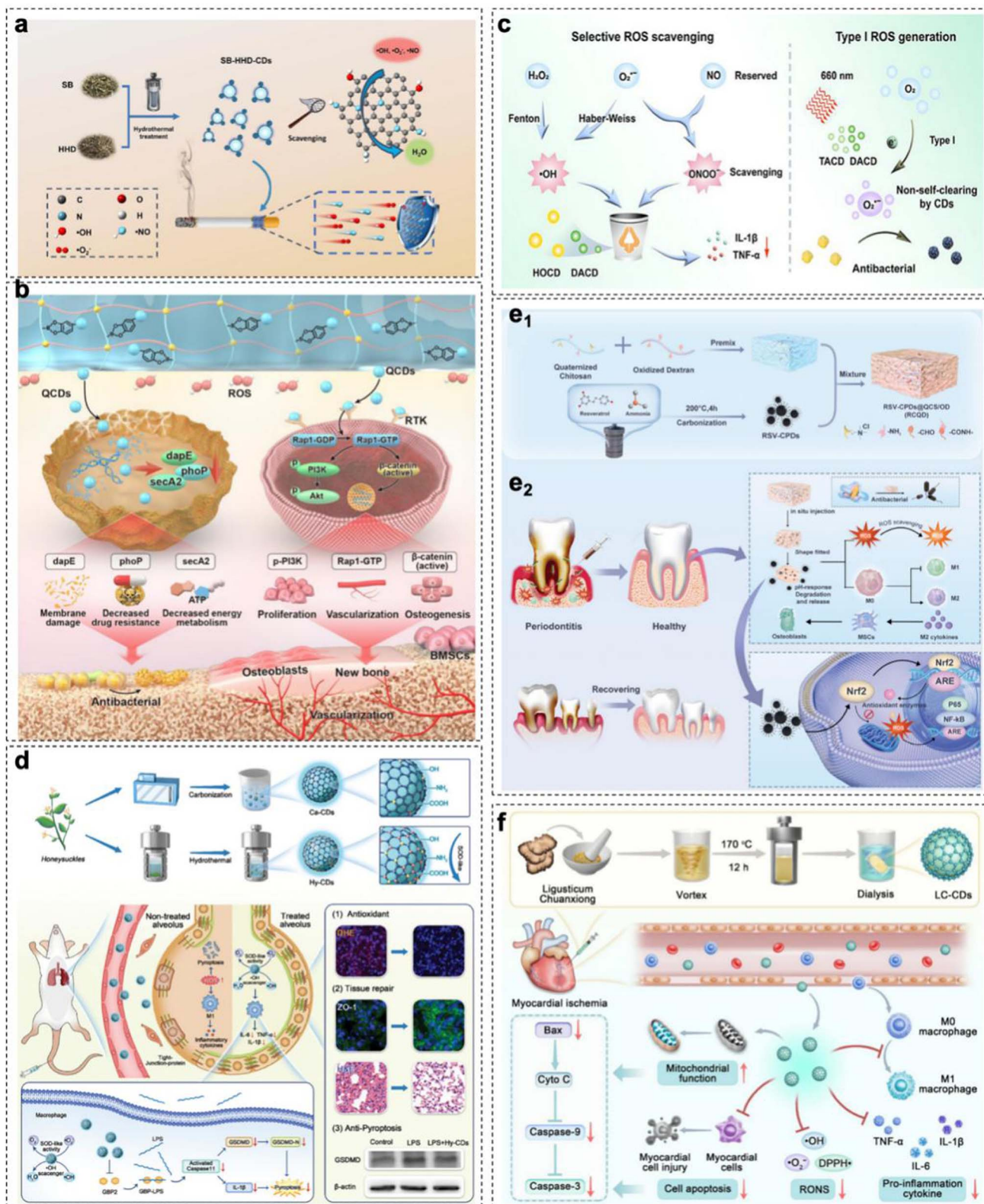


Fig. 5 (a) Schematic of SB-HHD-CDs preparation and their application in cigarette filter development. Reproduced with permission.<sup>45</sup> Copyright 2023, Elsevier. (b) Composite hydrogels containing QCDs for infected bone injury repair: five-layer graphene-like QCDs disrupt bacterial walls, scavenge ROS via borate bond degradation, and activate Rap1 signaling for cell proliferation and osteogenesis. Reproduced with permission.<sup>46</sup> Copyright 2025, Wiley-VCH Verlag. (c) Preparation of three herbal-derived CDs as dynamic ROS nano modulators for anti-inflammatory and antibacterial applications. Reproduced with permission.<sup>47</sup> Copyright 2024, American Chemical Society. (d) Synthesis of honeysuckle-derived Hy-CDs (hydrothermal) and Ca-CDs (carbonization) for lung inflammation alleviation: Hy-CDs retain more surface functional groups, exhibit enhanced catalytic activity, scavenge ROS, and inhibit GBP2-mediated non-classical pyroptosis. Reproduced with permission.<sup>48</sup> Copyright 2025,



cytokine secretion, while *in vivo* studies demonstrated that Hy-CDs' significant biological activity and catalytic properties aid in treating acute lung injury (ALI) and lung ischemia/reperfusion injury (LRI). Mechanistically, Hy-CDs suppress caspase11/GSDMD-dependent non-canonical pyroptosis by downregulating GBP2 protein expression, thereby inhibiting pulmonary inflammation (Fig. 5d).

Miao *et al.*<sup>49</sup> developed a multifunctional hydrogel platform RCQD for periodontitis treatment (Fig. 5e). This system consists of quaternized chitosan/oxidized dextran (QCS/OD) hydrogel and resveratrol-derived carbonized polymer dots (RSV-CPDs): the former provides high-efficiency bactericidal capability (*in vitro*), while the latter activates the Nrf2/NF- $\kappa$ B signaling pathway *via* immunomodulatory functions. RCQD creates a favorable microenvironment for periodontal hard and soft tissue regeneration by inhibiting bacterial proliferation, scavenging oxidative stress (*in vitro*), and alleviating inflammatory responses (*in vivo*). Zhang *et al.*<sup>50</sup> synthesized *Ligusticum chuanxiong*-derived CDs (LC-CDs) *via* hydrothermal method, which exhibit strong radical-scavenging capacity, effectively reduce oxidative stress, and prevent cell apoptosis, contributing to combating myocardial ischemia-reperfusion injury (MIRI) (Fig. 5f). CDs derived from *Lophatherum gracile* use near-infrared fluorescent probes as carriers to simultaneously achieve Fe ion monitoring, mitochondrial autophagy inhibition (*in vitro*), and ferroptosis blockade, inaugurating a synergistic nano-traditional Chinese medicine therapy for acute kidney injury.<sup>51</sup>

While the aforementioned case studies highlight the potential of herbal-derived CDs, a systematic comparison with other precursor classes (*e.g.*, citric acid, amino acids, drug molecules) is essential to contextualize their unique advantages and limitations. Herbal precursors stand out due to their inherent multi-component complexity, which often translates into CDs with synergistic bioactivities that are difficult to achieve with single-molecule precursors. For instance, compared to citric acid-derived CDs, herbal CDs like those from *Scutellaria barbata* and *Hedyotis diffusa* inherit diversified pharmacophores that enable broad-spectrum RONS scavenging and enhanced biocompatibility. Conversely, herbal CDs may suffer from batch-to-batch variability due to natural heterogeneity in plant composition, whereas synthetic precursors (*e.g.*, amino acids, drugs) ensure higher reproducibility. Drug-derived CDs excel in target specificity but often require complex synthesis to retain bioactivity, while herbal CDs inherently encapsulate multi-target functionalities through carbonization-preserved native motifs. A critical evaluation of trade-offs in activity, reproducibility, cost, and design controllability across precursor categories is needed to guide selection for specific applications.

## 7. Biological waste as precursors

Biological waste is transforming from an environmental burden into a strategic resource for functional CDs. Through molecular reconstruction, fruit-vegetable residues, hair, and even bacterial cell walls are converted into intelligent nanomaterials, achieving zero-waste recycling while endowing CDs with precise biological functions beyond traditional materials, such as temperature sensing, dynamic antibacterial activity, and immune regulation.

CDs synthesized from carbonaceous residues of Tahitian lemon (*Citrus latifolia*) *via* a simple and low-cost method were used as nanothermometers and antioxidants (Fig. 6a).<sup>52</sup> Following the concept of waste valorization, Sun *et al.*<sup>53</sup> developed an economical, green, rapid, and sensitive rare earth element detection method using banana peel-derived N-doped CDs (BP-N-CDs) as fluorescent sensors. Using *o*-phenylenediamine (OPD) as a N source further enhanced the photoluminescence properties of BP-N-CDs, yielding bright fluorescence and excellent stability. Fluorescence of BP-N-CDs was rapidly quenched by Res within 10 seconds *via* an internal filtering effect. This method exhibited good selectivity and sensitivity toward Res, with a limit of detection (LOD) of 2.21 ng mL<sup>-1</sup>, and was applicable to food sample analysis (Fig. 6b).

Breaking through the limitations of static antibacterial strategies, celery-derived CDs<sup>54</sup> exhibiting OXD-like activity ( $K_m = 0.15$  mM,  $V_{max} = 1.68 \times 10^{-7}$  M s<sup>-1</sup>) pioneered a dual-switch mechanism of "light-controlled ROS generation/pH-triggered scavenging" to achieve adaptive precise regulation of infected microenvironments (Fig. 6c). Integrating traditional Chinese medicine wisdom, human hair-derived CDs from *Xueyutan* (charred human hair) form self-assembled oleogel dressings that synergistically integrate four functions: hemostasis, antibiosis, anti-inflammation, and angiogenesis. Mao *et al.*<sup>55</sup> successfully prepared *Xueyutan*-derived CDs (CrCi-CDs) using hair as a precursor. The team further developed a multifunctional CrCi-CDs-loaded oleogel (CrCi-CDs/OG) wound dressing *via* a simple self-assembly method. This dressing not only achieves rapid hemostasis but also effectively inhibits bacterial growth (*in vitro*), regulates the oxidative stress and inflammatory microenvironment around wounds, promotes neovascularization, and accelerates wound healing (*in vivo*). With a simple preparation process and low cost, it holds broad application prospects in biomedical materials (Fig. 6d). Exploring the frontiers of immune regulation, *Escherichia coli* cell wall-derived CDs breakthrough sepsis treatment bottlenecks *via* dual pathways of TLR4 degradation/STING inhibition, inaugurating pathogen-derived nano-immunotherapy.<sup>56</sup>

Wiley-VCH Verlag. (e) Construction of RCQD hydrogel for periodontitis treatment: (e<sub>1</sub>) encapsulation of RSV-CPDs in QCS/OD hydrogel; (e<sub>2</sub>) pH-responsive antibacterial, ROS-scavenging, and M2 macrophage polarization *via* Nrf2/NF- $\kappa$ B pathway to promote alveolar bone regeneration. Reproduced with permission.<sup>49</sup> Copyright 2025, Wiley-VCH Verlag. (f) One-step hydrothermal synthesis of *Ligusticum Chuanxiong*-derived LC-CDs for mitigating MIRI *via* multi-radical scavenging, anti-inflammation, and mitochondrial function restoration. Reproduced with permission.<sup>50</sup> Copyright 2025, BioMed Central.



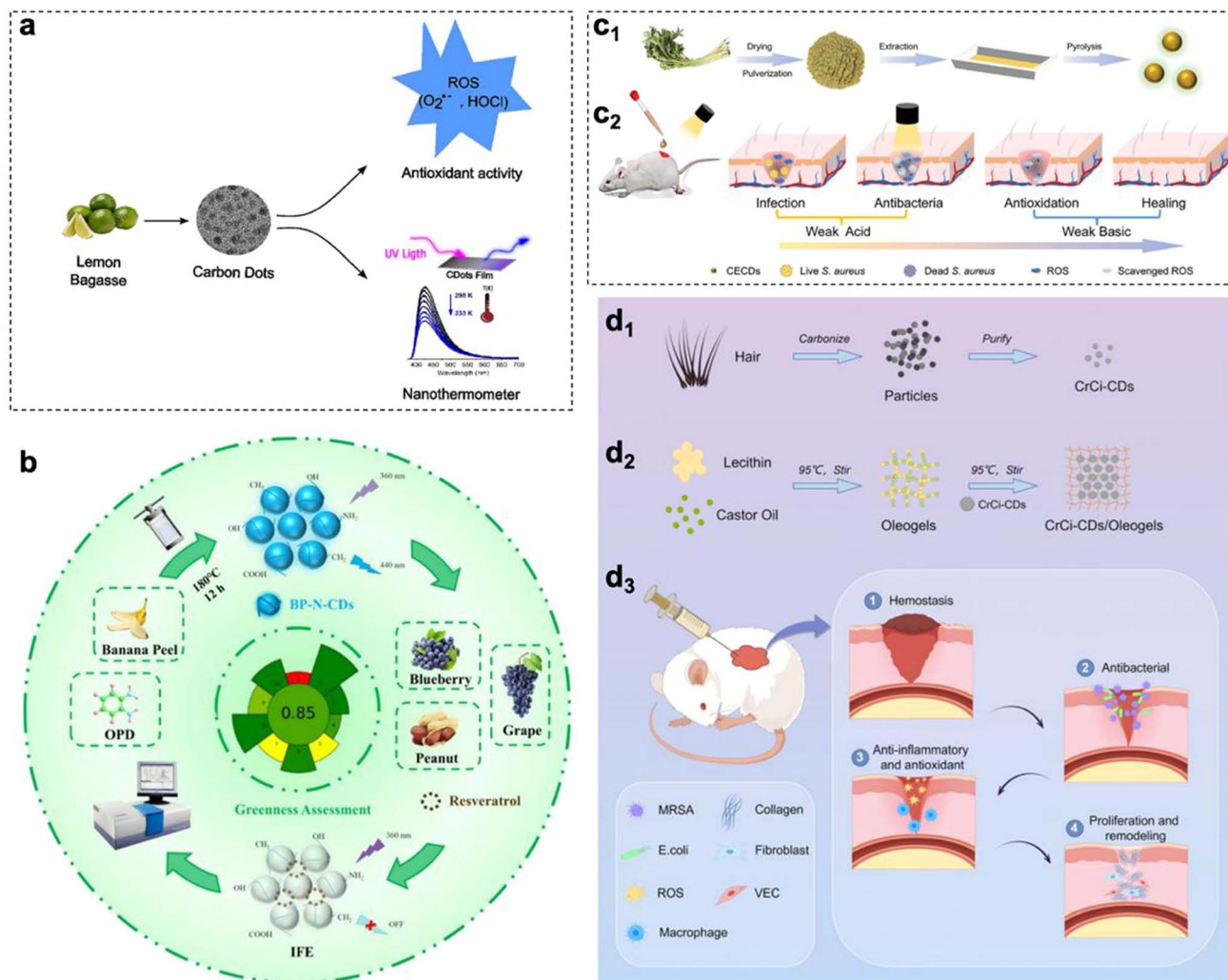


Fig. 6 (a) Schematic of CDs synthesized from Tahitian lemon (*Citrus latifolia*) carbonaceous residues for nano thermometry and antioxidant applications. Reproduced with permission.<sup>52</sup> Copyright 2023, Elsevier. (b) BP-N-CDs as green, fluorescent sensors for economical, rapid, and sensitive detection of Res in foods. Reproduced with permission.<sup>55</sup> Copyright 2025, Elsevier Ltd. (c<sub>1</sub>) Preparation of CECDs; (c<sub>2</sub>) schematic of CECDs for antibacterial and wound-healing applications. Reproduced with permission.<sup>54</sup> Copyright 2024, Wiley-VCH Verlag. (d<sub>1</sub>) Synthesis of CrCi-CDs from human hair; (d<sub>2</sub>) preparation of CrCi-CDs/OG by mixing lecithin, castor oil, and CrCi-CDs; (d<sub>3</sub>) *in vivo* wound-healing application of CrCi-CDs/OG, demonstrating superior performance over controls across all treatment phases. Reproduced with permission.<sup>55</sup> Copyright 2025, Wiley-VCH.

Table 1 summarizes the enzyme-like activities,  $K_m$ , and  $V_{max}$  values of different nanozymes, including POD-like and OXD-like activities, with varying catalytic efficiencies based on their types and compositions. The data highlights the diverse performance characteristics of different carbon-based nanozymes. The utilization of bacterial waste as precursors for CDs,

while innovative, raises significant biosafety concerns that must be critically evaluated before biomedical application. Pathogen-derived nanomaterials may retain residual pathogen-associated molecular patterns, such as lipopolysaccharides or peptidoglycans, which could trigger unintended immune activation or systemic inflammatory responses *in vivo*. Even after

Table 1 Comparison of enzymatic kinetics parameters of different CDs-based nanozymes

Nanozyme type	Enzyme activity	$K_m$ (mM)	$V_{max}$ ( $M s^{-1}$ )	Reference
N <sub>1</sub> Fe-CDs	POD-like	0.423	$12.15 \times 10^{-8}$	29
O/N-CQDs	POD-like	0.13	$10.1 \times 10^{-7}$	31
D-Mn-CDs	OXD-like	3.04	—	40
Celery-based CDs	OXD-like	0.15	$1.68 \times 10^{-7}$	54
Mg-CDs	OXD-like	0.18	$1 \times 10^{-7}$	57



carbonization, the complete degradation of these immunogenic components cannot be assumed, necessitating rigorous purification and characterization to confirm the absence of bioactive contaminants. Furthermore, the potential for horizontal gene transfer or residual antibiotic resistance genes in bacteria-derived CDs requires thorough investigation.

The molecular-level interactions between CDs and biological systems are fundamentally driven by the dynamic adaptation of their surface chemistry to biological molecules, biological membranes, and cellular signaling pathways. Specifically, this occurs through surface functional groups mediating specific molecular recognition (*e.g.*, carboxyl groups electrostatically binding to protein amines, positively charged CDs forming complexes with the nucleic acid phosphate backbone, surface charge affecting initial cell membrane binding efficiency, and modified targeting ligands enabling precise docking), transmembrane transport regulation (receptor-mediated endocytosis is a key pathway for targeted delivery; non-targeted, positively charged CDs can enter cells *via* non-specific endocytosis, while very small CDs modified with membrane-penetrating ligands may directly permeate the membrane), and intracellular distribution and subsequent interactions with biomolecules (*e.g.*, amino-modified CDs achieving lysosomal escape through the “proton sponge effect”, modified with specific ligands for organelle targeting, and surface functional groups or drug-loaded CDs interacting with intracellular enzymes to exert function). Based on these mechanisms, the molecular design of CDs in targeted delivery should focus on “specific recognition – efficient transmembrane transport – safe release”, enhancing targeting precision *via* dual-targeting ligand modification and environment-responsive ligand activation, improving intracellular delivery efficiency with pH/GSH dual-responsive designs and synergistic membrane penetrants, and reducing biotoxicity through surface passivation modifications and precise impurity removal.

These innovations in molecular design are also reflected in the patent landscape of this field. Current patents mainly focus on two directions: the selection of different precursors (such as the quercetin CDs in Chinese patent CN120097325A, the traditional Chinese medicine biomass CDs in Chinese patent CN116986583A, and the sulfur-doped CDs in Chinese patent CN114656959A) and targeted modification (such as the cancer cell ligand molecules in Chinese patent CN118458749A, and folic acid modification in Chinese patent CN114209850A). However, patent translation still faces challenges such as “insufficient molecular mechanism – clinical effect validation” and “poor compatibility with large-scale production”. In the future, development will shift toward “multi-mechanism synergy”, “personalized medicine”, and “green synthesis”. Overall, the molecular-level interaction of CDs with biological systems is the “core code” for their targeted delivery. The synergy between molecular design and patent layout can address the bottlenecks in targeted delivery. Future efforts should focus on using single-molecule imaging technologies to track dynamic processes, providing support for precise design and patent translation, and driving CDs toward clinical targeted therapeutic applications.

## 8. Conclusion and prospect

CDs have evolved from simple fluorescent probes to sophisticated multifunctional nanoplateforms capable of dynamically regulating oxidative stress and inflammation. The ability of CDs to mimic enzymatic activity lies in their unique structure and surface properties. On one hand, the surface of CDs typically contains functional groups such as  $-OH$ ,  $-COOH$ , and  $-NH_2$  groups. These abundant surface-active sites serve as active centers for catalytic reactions, similar to the active sites of natural enzymes, and can interact specifically with substrate molecules through hydrogen bonds, electrostatic interactions, and other forces, thus lowering the activation energy of reactions. On the other hand, the carbon core of CDs possesses a conjugated structure, providing excellent electron transfer capabilities. Many enzyme-catalyzed reactions, such as redox reactions, fundamentally rely on electron transfer. Therefore, CDs can replace metal cofactors like  $Fe^{3+}$  and  $Cu^{2+}$ , or coenzymes such as NADH, in enzymes, facilitating electron transfer between substrates and oxidants/reductants. Moreover, by adjusting synthesis temperature, precursor ratios, and other parameters, CDs of varying sizes and morphologies can be fabricated. This controlled size and shape enable precise matching with substrate molecules, optimizing the spatial hindrance effect of catalytic reactions and enhancing the specificity of enzyme mimics. Therefore, optimizing surface modification, doping, size, and morphology can further improve catalytic performance. This review summarizes the design and application advancements of CDs, focusing on four key aspects: First, heteroatom doping regulates the redox properties and enzyme-mimicking activities of CDs, enhancing their therapeutic specificity. Second, precursor-directed bioactivity, such as citric acid-derived CDs used for drought resistance and wound healing, as well as amino acid and drug-derived CDs with anti-inflammatory and antioxidant functions. Third, stimulus-responsive smart CDs, such as photo-switchable and pH-responsive CDs, can autonomously adapt their activities based on environmental changes. Finally, biomass-derived sustainable CDs exhibit good biocompatibility and multifunctionality, showing promise as versatile theranostic agents.

Despite the broad application prospects of CDs due to their excellent physicochemical properties and biocompatibility, their development and clinical translation still face multiple key challenges, primarily in the following aspects. Firstly, at the synthesis and material level, achieving standardization and reproducibility of the synthesis process remains the core bottleneck. Even with the same process, differences in precursor batches or low-purity precursors can introduce unintended catalysis or competitive reactions, leading to significant variations in CDs properties such as particle size, fluorescence performance, surface charge, and dispersion. Therefore, controlling the doping element ratio, surface functional group density, and bioactive molecules derived from precursors are key to achieving precise structural control and improving batch-to-batch consistency. Secondly, in terms of understanding the mechanisms, there is still a need for systematic clarification of how the atomic-level fine structure of CDs regulates their ROS



modulation mechanisms and enzyme-mimicking functions. In addition, intelligent design based on stimuli-response to improve therapeutic specificity is another key direction that requires breakthroughs. Thirdly, in terms of biosafety and clinical translation, the long-term toxicity, *in vivo* distribution and clearance pathways, degradation products, and their biological effects of CDs remain unclear. Heavy metal-doped systems may pose potential biosafety risks, and their *in vivo* behavior is strongly dependent on size, charge, and surface chemistry.

Future research should focus on *in vivo* fate studies, structure–activity relationships at the atomic level *via* advanced characterization, and integration with emerging modalities. In terms of *in vivo* fate studies, to address clinical translation bottlenecks, CDs need to be conjugated with stable isotopes or long-lived fluorescence probes, combined with multimodal imaging techniques to monitor their absorption, distribution, metabolism, and excretion in real time, as well as their penetration in target tissues and intracellular localization. Additionally, single-cell sequencing and proteomics can be employed to assess the long-term effects of CDs on cellular signaling pathways, gut microbiota, and immune function, providing insights for biosafety evaluation and metabolic pathway optimization.

In atomic-level structure–activity relationship (SAR) studies, it is essential to break through the current limitations of macroscopic correlations. The inability to establish an atom-level SAR in current CDs research stems mainly from the high heterogeneity of their structure, limitations of characterization techniques, and insufficient research paradigms. Techniques such as aberration-corrected transmission electron microscopy and X-ray photoelectron spectroscopy should be used to analyze the hybridization ratio, defect configuration, and chemical states of doped elements in the carbon core of CDs. Combined with density functional theory calculations, these methods can quantify the binding energy and electronic transfer barriers between functional groups and substrates, establishing a quantitative “atomic structure-kinetic parameter” model to support the design of high-activity, high-specificity CDs-based nanozymes. For integration with emerging technologies, the multifunctionality of CDs should be leveraged to advance their combination with immunotherapy, gene editing, and biomimetic materials, expanding applications in “integrated diagnosis and therapy” and tissue regeneration.

Furthermore, future research should address critical industrialization challenges by establishing a standardized framework along the entire production chain. This includes developing high-purity biomass extraction and purification technologies, designing continuous-flow systems to precisely control reaction parameters for uniform production, and implementing multidimensional standards to rigorously control metal impurities and biological toxicity, supporting GMP-scale production. Additionally, clinical demand-driven interdisciplinary collaboration should be strengthened, such as developing pH/GSH dual-responsive CDs delivery systems for RA based on the CIA model, light-responsive antibacterial CDs for antibiotic resistance, and mucus-penetrating CDs carriers for cystic fibrosis, accelerating “laboratory-to-clinic”

translation. By synergizing materials science, nanotechnology, and molecular medicine, engineered CDs hold immense promise as next-generation precision nanomedicines for oxidative stress-related pathologies.

## Conflicts of interest

The authors declare no conflict of interest.

## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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