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Review

DESIGNS, APPLICATIONS, AND FUTURE PERSPECTIVES OF NANOZYMES IN ORTHOPEDICS AND DENTISTRY

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Abstract

Nanozymes are nanomaterials that mimic enzyme activities by exhibiting notable enzyme-like kinetics and catalyze substrates under physiological conditions. Compared to natural enzymes, nanozymes offer advantages such as lower cost, higher stability, multi-enzymatic activity, and simpler preparation, making them promising alternatives in biomedical applications, particularly in bone and dental-related areas. This review presents a comprehensive overview of recent advances in nanozyme for orthopedic and dental application. The methodologies used to design and construct nanozymes are summarized, including composition regulation, size control, morphology engineering and surface modification. The applications of nanozymes in various stages of bone and dental tissue regeneration are then explored, with detailed discussions on their roles in *in vitro* diagnostics, anti-infection strategies, osteogenic microenvironment regulation, and combating bone tumors. The review highlights how nanozymes address key challenges in the field, such as reactive oxygen species management, immune response modulation, and targeted drug delivery. Furthermore, by addressing current challenges and outlining prospects, this review aims to advance the therapeutic potential of nanozymes in treating bone and dental diseases and bridge the gap towards their clinical translation.

Keywords: Nanozymes, bone, microenvironment, immunomodulation, materials engineering, dentistry.

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Introduction

Bone and dental tissue diseases, such as osteoporosis, osteoarthritis, periodontitis, and osteosarcoma, pose significant public health challenges, affecting millions worldwide [1,2]. According to the World Health Organization, the global burden of these conditions is substantial, with oral diseases affecting nearly 3.5 billion people globally, while bone diseases result in approximately 1.5 million fractures annually [3]. These conditions result in substantial morbidity and diminished quality of life [4]. Traditional treatments, including pharmacotherapy, surgery, and dental restorations, often prove inadequate [4,5]. These treatments carry risks such as incomplete healing, infections, frequent re-treatments, and, in some cases, resistance to therapy. Moreover, conventional methods frequently fail to fully restore lost tissue functionality or halt disease progression, highlighting the urgent need for innovative therapeutic approaches [5].

In inflammatory bone and dental diseases such as periodontitis, osteoporosis, and osteomyelitis, the pathological microenvironment plays a crucial role in disease progression and treatment outcomes [6–8]. This environment is characterized by several detrimental factors. Chronic inflammation, resulting from persistent immune activation from infections, autoimmune diseases, diabetes, aging, and other factors, causes continuous secretion of proinflammatory cytokines [9]. These cytokines promote osteoclast differentiation and activity, leading to excessive bone resorption and disrupted bone remodeling. In addition, elevated levels of reactive oxygen species (ROS), resulting from oxidative stress, can cause significant damage to cellular components, leading to impaired bone regeneration and healing

[10]. Excessive ROS production disrupts the balance between bone resorption and formation, contributing to conditions like osteoporosis and periodontitis [11]. Nevertheless, pathogenic microorganisms, causing osteomyelitis and periodontitis, infiltrate bone and dental tissues, adhere to bone tissue surfaces, and form biofilm communities that resist immune responses and antimicrobial treatments [12]. This biofilm formation allows bacteria to persist, leading to chronic infections, and resulting in delayed bone healing [12]. The combination of these elements creates a hostile environment that hampers the healing process and the effectiveness of traditional treatments.

The body naturally generates enzymes to regulate the stressful microenvironment, such as those that reduce ROS. However, in the stressful microenvironment, cellular functions are compromised, leading to reduced production of these natural enzymes [10]. To make matters worse, these enzymes are quickly deactivated in the harsh microenvironment [13]. The use of exogenous enzymes to supplement this natural process is feasible but faces several limitations. Natural enzymes, primarily globular proteins or bioorganic molecules are sensitive to harsh physiological conditions and can be costly to produce and challenging to store. Their stability under physiological conditions is often poor, and their single-enzyme activity significantly limits their versatility in therapeutic applications [14]. These limitations necessitate the development of more robust alternatives to overcome the intrinsic drawbacks of natural enzymes.

Nanozymes present a revolutionary class of artificial enzymes that combine the catalytic properties of natural enzymes with the unique advantages of nanomaterials [13,14]. These nanomaterials possess intrinsic enzyme-like activities due to their specific atomic arrangements, electronic structures, and surface properties, enabling them to catalyze biochemical reactions under physiological conditions [15]. Compared to their natural counterparts, nanozymes offer several significant advantages in biomedical applications: (a) less expensive to produce and store, exhibiting greater stability under physiological conditions; (b) can mimic multiple enzyme activities; (c) tailorable to meet versatile functionalities such as biosafety, stability, biocompatibility, and catalytic efficiency [16]. For instance, horseradish peroxidase (HRP), a widely used natural enzyme, loses its tertiary structure and catalytic activity at pH values below 6 or above 8, and temperatures exceeding 60 °C cause protein unfolding, leading to complete loss of peroxidase activity [17,18]. In contrast, nanozymes exhibit remarkable stability across a much broader range of conditions. Magnetite (Fe_3O_4) nanozymes, for example, maintain their peroxidase-like activity across a wide pH range (0–12) and can function effectively at temperatures from 4–90 °C [14]. This enhanced stability makes nanozymes particularly valuable for biomedical applications where maintaining catalytic activity under varying physiological conditions is crucial. In the context of bone and dental tissues, nanozymes have demonstrated remarkable potential in addressing multiple pathological conditions simultaneously (Fig. 1). For instance, certain nanozymes can exhibit both superoxide dismutase (SOD) and catalase (CAT)-like activities, effectively neutralizing various ROS that contribute to tissue damage. This dual functionality not only helps reduce oxidative stress but also creates a more favorable environment for bone repair and regeneration [19,20]. Furthermore, nanozymes can be designed to modulate immune response by regulating macrophage polarization, while simultaneously promoting osteogenic differentiation. Their multifunctional nature makes them particularly valuable for treating complex bone and dental conditions where multiple therapeutic actions are required simultaneously.

Overall, nanozymes represent a promising alternative to traditional therapies, offering targeted action, high biocompatibility, and the potential to facilitate tissue regeneration. Their ability to regulate the stressful microenvironment in bone and dental tissues positions them at the forefront of innovative treatments for these challenging conditions. In this review, we aim to elucidate the design principles of nanozymes in biomedical settings. Additionally, we will examine the current and potential applications of nanozymes in diagnosing and managing these conditions, highlighting their improvements over traditional methods. Lastly, we will discuss future perspectives in nanozyme research, technological advancements, and their path to clinical translation, underscoring the significance of nanozymes in regenerative medicine and beyond.

Chemical Design of Nanozymes

Nanozymes are engineered nanomaterials that exhibit enzyme-like catalytic activities through specific atomic and molecular arrangements at their surfaces. Unlike traditional enzymes that rely on protein structures with defined active sites, nanozymes achieve their catalytic functions through various mechanisms including electron transfer, surface adsorption, and coordination chemistry. The synthesis of nanozymes typically involves precise control of nucleation and growth processes, utilizing methods such as chemical reduction, hydrothermal synthesis, sol-gel processing, and template-directed approaches. These fabrication techniques allow for the creation of diverse nanostructures with tailored catalytic properties.

The catalytic activity and biological response of nanozymes are intricately dependent on their nanostructure, which can be systematically engineered at multiple levels. At the atomic level, the arrangement of surface atoms and their electronic states determines the formation of active sites. At the molecular level, the crystal structure and surface defects influence substrate binding and product release. At the nanoscale, the overall architecture affects mass transport and accessibility of active sites. These structural features can be precisely controlled through the regulation of their composition, size, morphology, surface proper-

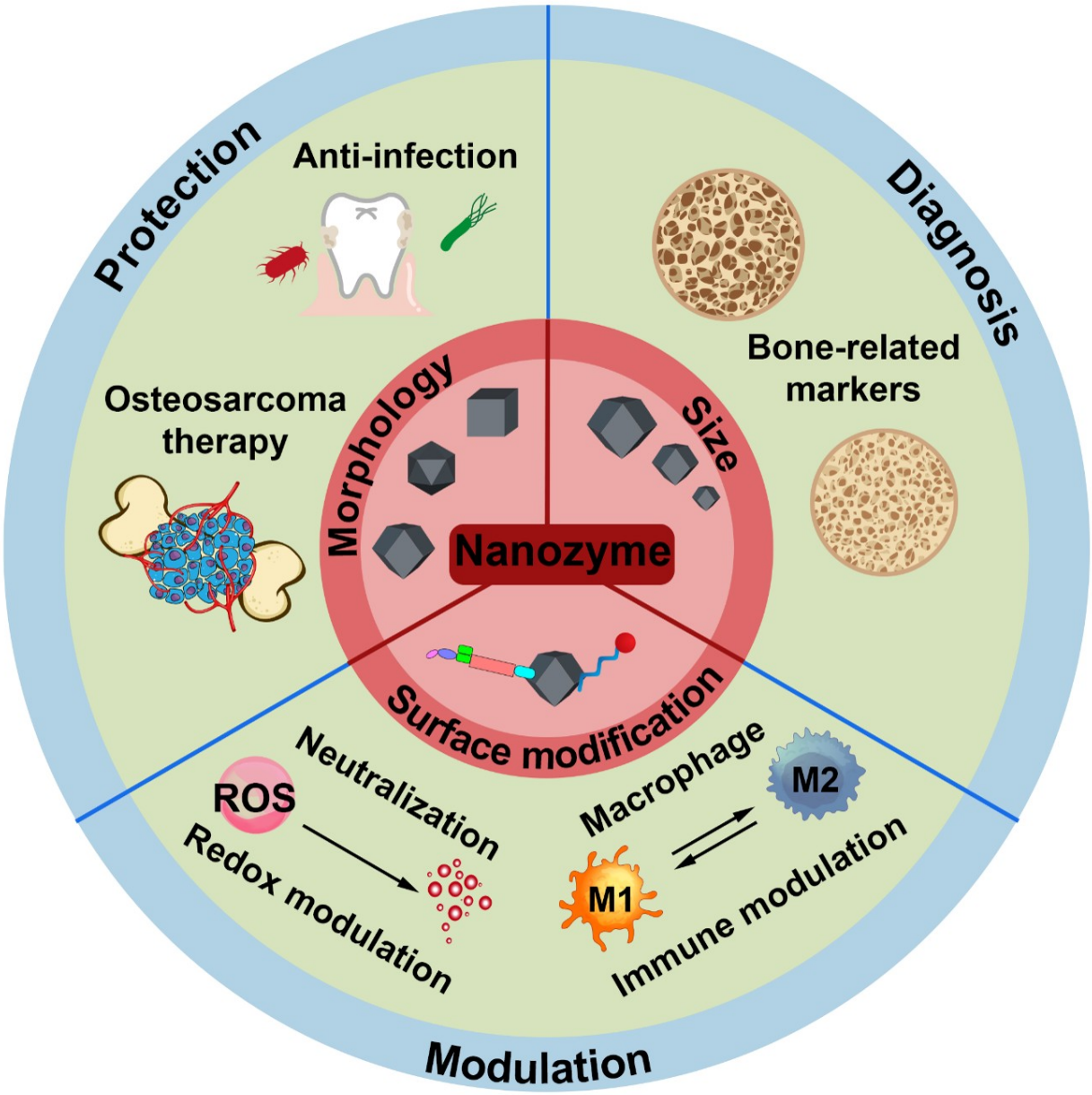

Fig. 1. Schematic summary of chemical designs of nanozymes and their applications for bone and dental tissue diseases. ROS, reactive oxygen species; M1, M1 macrophages; M2, M2 macrophages.

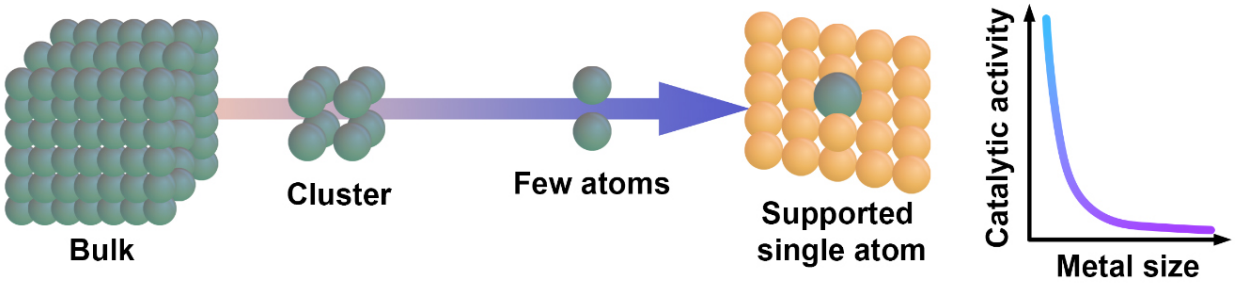

Fig. 2. Schematic illustration of size related catalytic activity of metal nanocatalysts.

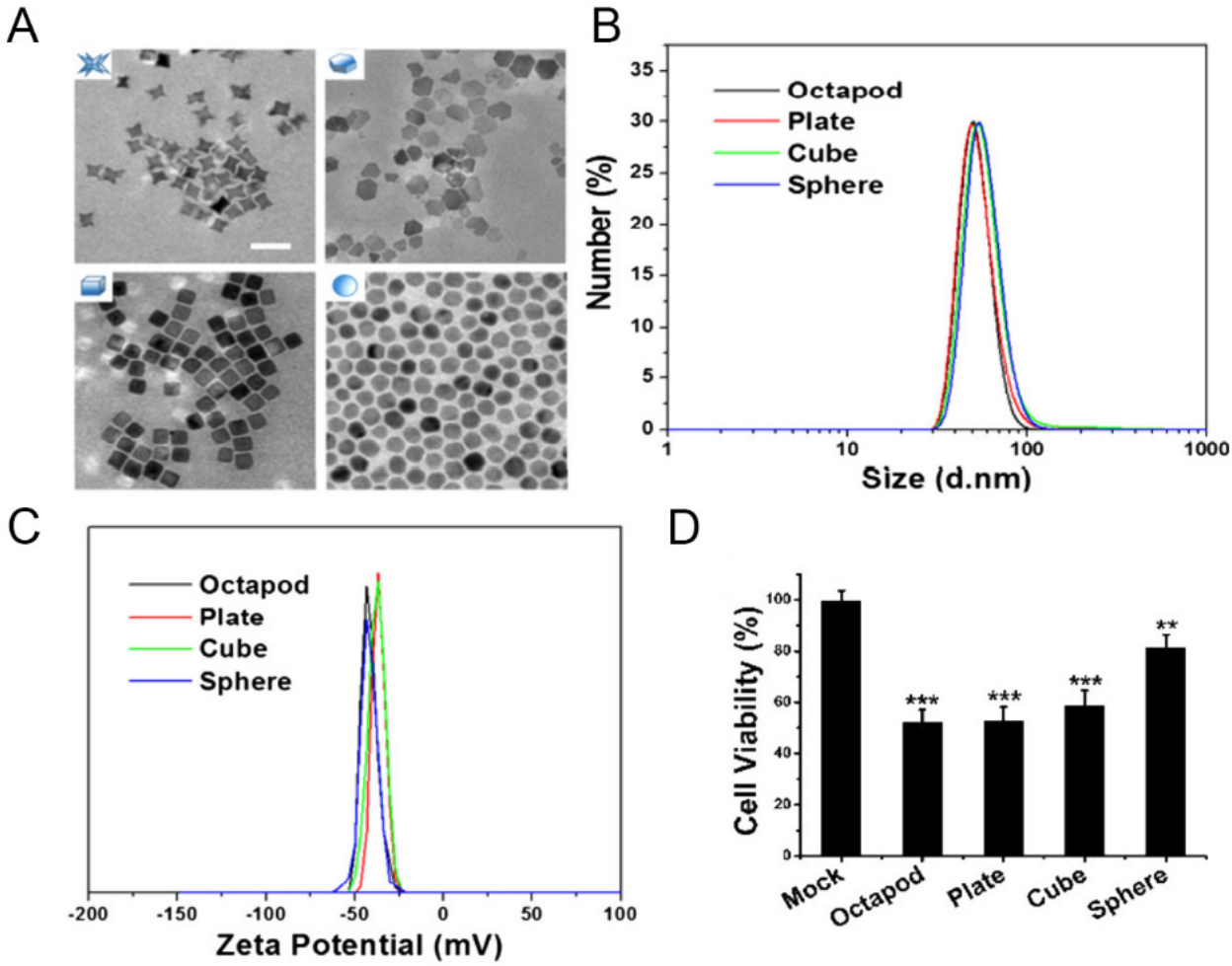

Fig. 3. Characterization of IONPs. (**A**) TEM images of different IONPs with similar (**B**) size and (**C**) zeta potential but shown (**D**) distinguished cytotoxicity. Reprinted (adapted) with permission from [58]. Copyright 2018 American Chemical Society. IONPs, iron oxide nanoparticles; TEM, transmission electron microscopy. Scale bar: 50 nm; $^{**}p < 0.01$; $^{***}p < 0.001$.

ties, and assembly methods, significantly altering their activity, selectivity, and interaction with biological systems.

The design of nanozymes requires careful consideration of several key factors: (1) the selection of appropriate chemical compositions to achieve desired catalytic activities; (2) the optimization of size and morphology to enhance catalytic efficiency and cellular interactions; (3) the modification of surface chemistry to improve biocompatibility and targeting capabilities; and (4) the incorporation of additional functionalities for specific therapeutic applications. In this section, these chemical strategies are comprehensively discussing chemical strategies based on precise structure-activity relationships, examining how each design element influences both the catalytic efficiency and biological interactions of nanozymes. By exploring these aspects, this section provides insights and inspiration for designing highly efficient and biocompatible nanozymes for various biomedical applications.

Composition Regulation of Nanozymes

The compositional design of nanozymes is fundamental to their development and application, significantly influencing their catalytic abilities and suitability for various biomedical interventions. Over 40 elements, including metals such as iron (Fe) [21], cobalt (Co) [22], nickel (Ni) [23], copper (Cu) [20], zinc (Zn) [24], vanadium (V) [25], manganese (Mn) [26], molybdenum (Mo) [27], ruthenium (Ru) [28], rhodium (Rh) [29], palladium (Pd) [30], silver (Ag) [31], cerium (Ce) [32], platinum (Pt) [33], and gold (Au) [34], have been used for the synthesis of nanozymes. The selection and combination of these elements profoundly impact the enzymatic properties of nanozymes, enabling tailored catalytic responses for specific medical needs.

Single metallic nanozymes leverage the unique catalytic properties of individual metals, which are particularly effective in oxidation and reduction reactions [16]. The active sites of these catalysts are generally transition metals. These metals have special electron configurations

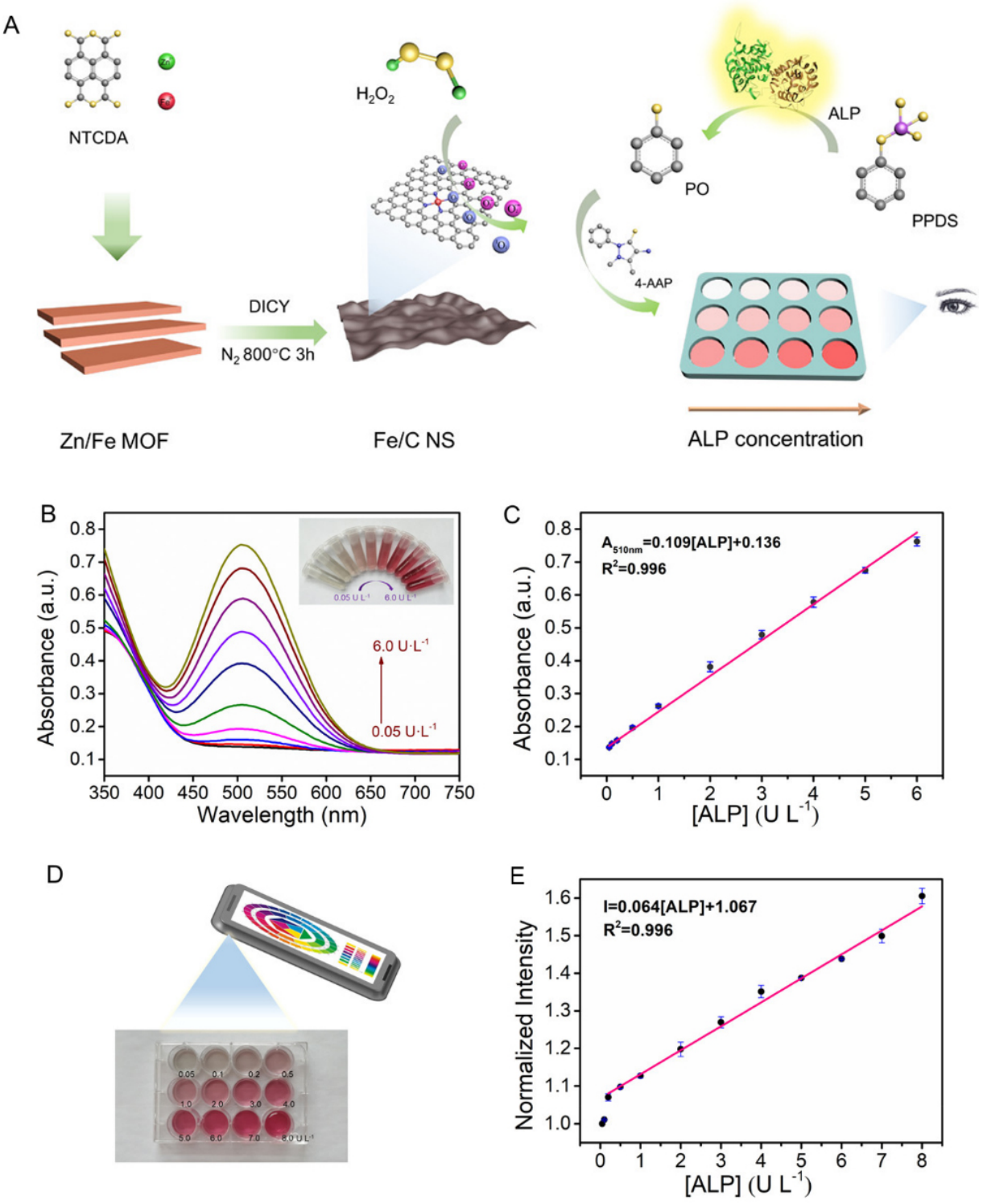

Fig. 4. Colorimetric detection method for alkaline phosphatase (ALP) using an Fe-doped carbon-based nanozyme. (**A**) Schematic illustration of the preparation of the Fe/C NS, and the colorimetric assay for ALP activity based on the Fe/C NS. (**B**) UV-Vis spectra and color variances of the system with different ALP concentrations. (**C**) Changes of A652 vs. the concentrations of ALP. (**D**) Color variances of the system with various amounts of ALP and the schematic of the smartphone taking photos. (**E**) Change of RGB values vs. the concentrations of ALP. Reprinted (adapted) with permission from [79]. Copyright 2021 American Chemical Society. ALP, alkaline phosphatase; MOF, metal-organic framework; H_2O_2, hydrogen peroxide; Fe, iron; Zn, zinc; Fe/C NS, Fe-doped carbon nanosheet; UV-Vis, ultraviolet-visible; RGB, red, green, and blue; NTCDA, 1,4,5,8-naphthalenetetracarboxylic dianhydride; DICY, dicyandiamide; PPDS, disodium phenyl phosphate; PO, phenol; 4-AAP, 4-aminoantipyrine.

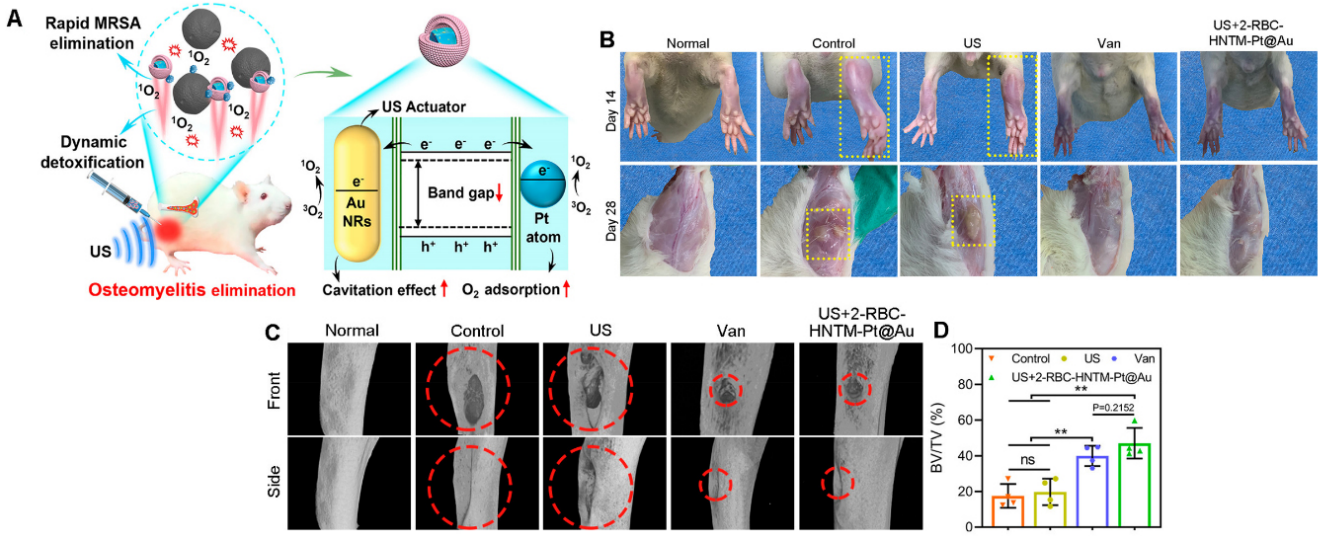

Fig. 5. Single-atom catalysis (SAC) for sonodynamic therapy (SDT) to treat methicillin-resistant *Staphylococcus aureus* (MRSA)-infected osteomyelitis. (**A**) Schematic illustration of RBC-HNTM-Pt@Au in treating osteomyelitis. (**B**) Surgical site images of rat osteomyelitis models after 2 and 4 weeks of treatment. (**C**) Micro-CT images of tibial defects after 4 weeks of treatment. (**D**) Bone volume (BV) vs. tissue volume (TV). Reprinted (adapted) with permission from [85]. Copyright 2021 American Chemical Society. MRSA, methicillin-resistant *Staphylococcus aureus*; AuNRs, gold nanorods; Pt, platinum; O_2, oxygen; RBC, red blood cell; RBC-HNTM-Pt@Au, red blood cell coated Au nanorod (NRs)-actuated single-atom-doped porphyrin metal-organic framework; CT, computed tomography; US, ultrasound. $^{**}p < 0.01$.

with partially filled outer electron shells (known as *d* or *f* orbitals), which can form coordination bonds with substrate molecules, creating transition states with lower energy barriers [21,35]. This reduces the activation energy of the reaction and accelerates chemical processes. Since the pioneering development of Fe_3O_4 nanozymes in 2007, magnetic metals and their oxides have been extensively studied for their potential in nanozyme applications [36]. One notable advancement in this field is the development of a single-atom Fe-N_4 catalytic site anchored on N-doped porous carbon materials (Fe-SAs/NC), as illustrated by Ma *et al*. [37]. This innovative design mimics the bifunctional activities of natural antioxidative enzymes CAT and SOD, offering a robust solution for scavenging ROS [37]. The Fe-SAs/NC nanozyme exhibits superior catalytic properties by decomposing ROS, thereby protecting cells from oxidative stress with minimized cytotoxicity. This work highlights the significant potential of metal-organic framework (MOF)-based Fe nanozymes in achieving precise ROS regulation, advancing the application of nanotechnology in biomedical interventions [37].

Cobalt (Co) and cerium (Ce)-based nanozymes have also garnered significant attention. For Co-based nanozymes, Mandakhbayar *et al*. [38] developed a bioactive nanozyme based on cobalt-doped nanoglass (CoNZ) to treat acute and diabetic wounds. The CoNZ nanozymes were synthesized using a modified sol-gel method and demonstrated superior ability to scavenge ROS and promote angiogenesis by enhancing endothelial migration and tubule formation through the activation of hypoxia-inducible factor 1-alpha (HIF-1α) [38]. Fore cerium-based nanozyme, cerium oxide (CeO_2) has been developed to prevent and treat ionizing radiation (IR)-induced bone loss due to their unique catalytic properties and ability to mimic antioxidant enzymes [39]. These nanozymes exhibit a higher fraction of trivalent cerium (Ce^{3+}) surface sites, which enhances their capacity to neutralize ROS generated by IR exposure, protecting bone tissue from oxidative damage, reducing inflammation, and inhibiting osteoclastogenesis, thereby maintaining bone architecture and strength [39].

Noble metals, such as Ru, Pd, Rh, and Au, have also drawn significant attention. These noble metal-based nanozymes possess unique characteristics such as excellent electron conductivity, numerous reactive corners, and high specific surface area. For instance, Au-based nanomaterials have been particularly well-studied due to their unique plasmonic features at the nanoscale and excellent biocompatibility [40]. For example, gold nanoclusters have been incorporated into zirconium-based porphyrin metal-organic framework (TP-Au@PCN) for the treatment of osteoarthritis (OA) [41]. This nanozyme significantly alleviates oxidative stress by scavenging different types of ROS, repairs mitochondrial function and improves impaired autophagic flux in chondrocytes, which are crucial for maintaining cartilage integrity [41].

By strategically selecting and combining these elements, researchers can design nanozymes with specific catalytic properties tailored to various biomedical applica-

tions, enhancing their effectiveness in treating and diagnosing medical conditions.

Size Regulation of Nanozymes

The size of nanocatalysts is crucial in determining their catalytic performance. As the size of metal particles decreases, the catalytic activity per metal atom improves due to the higher proportion of low-coordinated metal atoms serving as active sites (Fig. 2) [15,42]. Recent research highlights the critical role of particle size in the catalytic efficiency of nanozymes [43]. For instance, the study by Herzing *et al*. [44] revealed that subnanometer gold (Au) clusters on iron oxide supports exhibit significantly higher catalytic activity for carbon monoxide (CO) oxidation compared to larger Au nanoparticles. Specifically, bilayer clusters approximately 0.5 nm in diameter, containing around 10 Au atoms, were found to be the most active, whereas clusters in the 2–5 nm range exhibited much lower activity. This increased activity is attributed to the higher fraction of low-coordinated Au atoms, which have electronic structures that favor reactant adsorption and activation [44]. Additionally, single-atom catalysts (SACs), which contain only isolated single metal atoms on various substrates, represent the pinnacle of metal atom utilization, achieving nearly 100 % efficiency [45,46]. SACs were first reported by Qiao *et al*. [21] for catalyzing CO oxidation using single platinum (Pt) atoms anchored on an iron oxide (FeO_x). The unique features of SACs, such as quantum confinement of electrons, low-coordination environments of metal centers, and active valence electrons, contribute to their superior catalytic activity and selectivity [21].

Additionally, the size of nanoparticles significantly influences their interaction with cells [1]. Immune cells uptake, degrade, and process nanoparticles in a size-dependent manner [47]. Nanoparticles smaller than 10 nm can passively diffuse across the cell membrane [48]. Nanoparticles approximately 20–200 nm are primarily internalized via clathrin-mediated endocytosis, where clathrin-coated vesicles fuse with early endosomes for further processing [49]. Nanoparticles ranging from 200 nm to several micrometers can be internalized through macropinocytosis, a process involving actin-dependent membrane ruffling and the formation of large, irregular vesicles [50]. Nanoparticles larger than 500 nm are typically taken up through phagocytosis, where macrophage membranes engulf the particles, forming phagosomes through actin polymerization and pseudopod formation [51].

Studies indicate that gold nanoparticles (AuNPs) smaller than 15 nm are cleared from the body within 24 hours, while those larger than 15 nm can persist for up to two weeks. For AuNPs that are around 40 nm, they remain detectable in Kupffer cells for at least six months [52]. Besides the circulation time, nanoparticle size also exhibits distinguished biocompatibility. The 1.5 nm AuNP showed significantly higher cytotoxicity on stem cells compared to 4 nm and 14 nm AuNPs, causing a significant decrease in global deoxyribonucleic acid (DNA) methylation and disrupting the formation of embryoid bodies and neural rosettes, leading to adverse effects on neuronal differentiation [52]. Conversely, iron oxide nanoparticles (IONPs) around 50–100 nm offer better reactivity, optimal uptake efficiency, and reduced clearance issues, making them more suitable for biomedical applications compared to their smaller counterparts [53]. These findings underscore the importance of considering size-related cellular responses when preparing nanozymes, despite the higher catalytic efficiency of smaller nanozymes. Balancing catalytic performance with biocompatibility is essential for effectively applying nanozymes in biomedical fields.

Morphology Regulation of Nanozymes

The morphology of nanoparticles significantly influences their nanostructures, including crystal planes, surface facets, and surface energy, which in turn affects their enzyme-like activities. As a result, nanozymes with various shapes, such as nanowires, nanorods, nanospheres, nanofibers, and spiny nanomaterials, have gained considerable attention in recent years. For instance, Tian *et al*. [33] demonstrated that the catalytic efficiency of platinum (Pt) nanocrystals can be significantly enhanced by engineering high-index facets on their surfaces. They synthesized tetrahexahedral (THH) Pt nanocrystals with high-index facets like {730}, {210}, and {520}, which have a high density of atomic steps and dangling bonds. These high-index facets exhibit much higher catalytic activity compared to low-index facets such as {111}, {100}, and {110}, due to the increased number of active sites for chemical reactions [33]. Similarly, Puvvada *et al*. [54] investigated the influence of nanoparticle shape on the peroxidase mimetic activity of magnetite (Fe_3O_4) nanoparticles. They found that truncated octahedron (TO)-shaped magnetite nanoparticles exhibited significantly higher peroxidase-like activity towards the oxidation of pyrogallol by hydrogen peroxide than spherical magnetite nanoparticles. This enhanced activity is attributed to the different surface energy facets present on the two types of nanoparticles [54].

Beyond catalytic efficiency, morphology also significantly affects the immune response. It is generally accepted that rod-like nanoparticles are more likely to boost an inflammatory response compared to cubic or spherical nanoparticles [55]. For example, rod-shaped AuNPs stimulate dendritic cells to produce more interleukin (IL)-1β and IL-18, while cube- and spherical-shaped AuNPs result in higher production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-17, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [56]. Additionally, studies have revealed that the geometry of a target, rather than its size, significantly influences whether macrophages will proceed with

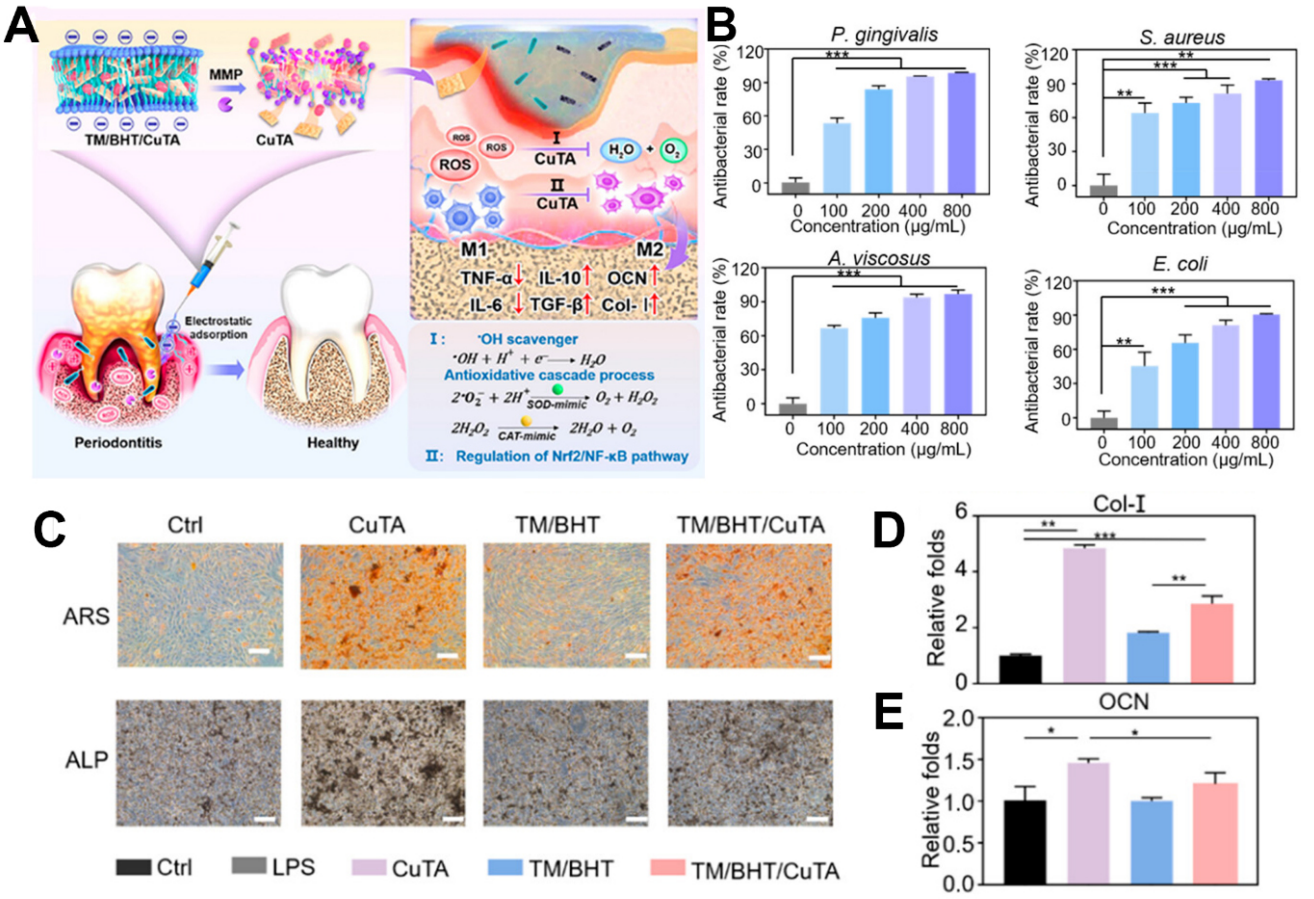

Fig. 6. Microenvironment-responsive metal-phenolic nanozyme release platform for the treatment of periodontitis. (**A**) Schematic illustration of the synthesis of TM/BHT/CuTA hydrogel and its application in periodontitis. (**B**) Antibacterial and antibiofilm activities of CuTA NSs against common bacteria. (**C**) Alizarin Red S (ARS) and ALP staining of MC3T3-E1 cells with different treatments (scale bar = 200 μm). Relative mRNA expression of (**D**) collagen I (Col-I) and (**E**) OCN. Reprinted (adapted) with permission from [92]. Copyright 2021 American Chemical Society. TM, triglycerol monostearate; BHT, 2,6-di-tert-butyl-4-methylphenol; CuTA NSs, copper tannic acid coordination nanosheets; TNF-α, tumor necrosis factor-alpha; *S. aureus*, *Staphylococcus aureus*; IL-10, interleukin-10; IL-6, interleukin-6; LPS, lipopolysaccharide; SOD, superoxide dismutase; CAT, catalase; Nrf2, nuclear factor erythroid 2-related factor 2; NFκB, nuclear factor kappa B; MC3T3-E1, embryonic mouse calvarial cell line; OCN, osteocalcin; MMP, matrix metalloproteinases; TGF-β, transforming growth factor beta; *E.coli*, *Escherichia coli*; *P. gingivalis*, *Porphyromonas gingivalis*; *A. viscosus*, *Actinomyces viscosus*; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

phagocytosis or simply spread on the particle [57]. Particles with specific orientations, such as elliptical disks approached along their major axis, are rapidly internalized, while those approached from other orientations result in spreading but not internalization [57]. Moreover, particle morphology is a critical determinant for iron oxide nanoparticle (IONP)-induced inflammasome activation (Fig. 3, Ref. [58]). Among four IONPs of similar size and zeta potential but different shapes (Fig. 3**A–C**), the octopod and plate-shaped IONPs showed higher inflammasome-activating capacities than cubic and spherical IONPs due to their ability to induce higher levels of ROS generation, lysosomal damage, and potassium efflux (Fig. 3**D**) [58]. These findings underscore the importance of considering particle shape in addition to size and surface properties when designing safer and more effective nanozymes for biomedical applications.

Surface Modification of Nanozymes

Surface modification is essential for enhancing biocompatibility, water dispersibility, and biological targeting of nanozymes, as well as their catalytic activities. This section explores various surface modification techniques, such as polyethylene glycol (PEG)ylation, biomimetic coatings, hydrophilicity adjustments, chemical engineering, and defect engineering.

The surface charge of nanoparticles (NPs) significantly impacts their cellular uptake and cytotoxicity, with

different responses observed in phagocytic and nonphagocytic cells. In general, cationic NPs are more readily internalized by nonphagocytic cells compared to their anionic counterparts [59]. Cationic (positively charged) NPs are more readily internalized by nonphagocytic cells compared to their anionic (negatively charged) counterparts. This higher uptake rate of positively charged NPs is associated with increased cytotoxicity due to membrane disruption, mitochondrial damage, and lysosomal degradation [59]. Additionally, cationic NPs tend to absorb more serum proteins *in vivo*, facilitating opsonization and subsequent phagocytosis by macrophages [60]. Since most nanozymes are positively charged, it is crucial to balance surface charge to achieve desired therapeutic outcomes while minimizing adverse effects. Combining surface modification techniques, such as PEGylation or ligand exchange, can fine-tune NP interactions with biological systems.

PEGylation is a common method used to extend the circulation time and improve the biocompatibility of nanoparticles. For example, Yang *et al*. [20] modified copper single-atom nanozymes (CuSAzymes) with PEGylation to create a SOD-like antioxidant for treating sepsis. PEGylation reduced the zeta potential of CuSAzyme from +41.52 mV to –3.51 mV, shifting the surface charge from positive to nearly neutral. This reduction in surface charge minimizes nonspecific interactions with cells and proteins, enhancing biocompatibility [20]. Biomimetic coatings, such as cell membrane coatings [61], provide a way to enhance targeting, evade the immune system, and increase biocompatibility by endowing negatively charged cell membranes on the surface of NPs [62]. Feng *et al*. [63] developed a neutrophil-like cell membrane-coated mesoporous Prussian blue nanozyme (MPBzyme@NCM) for treating ischemic brain damage. This coating facilitated targeted delivery to inflamed brain microvascular endothelial cells, enhanced blood-brain barrier penetration, and promoted therapeutic effects such as reduced neutrophil recruitment, microglia polarization from M1 macrophages (M1) to M2 macrophages (M2), decreased neuronal apoptosis, and increased neurogenesis [63]. A cancer cell membrane biomimetic mesoporous nanozyme system (Fe SAZs/DDP) was designed to enhance ROS generation and overcome tumor chemoresistance [64]. This cancer cell membrane coating provided homology targeting, biosafety, and improved circulation time by evading interception by the liver and kidneys [64].

The surface hydrophilicity of nanozymes is another crucial factor that affects their functionalities in biomedical applications. Hydrophilic or neutral copolymer surfaces inhibit macrophage adhesion and fusion into foreign body giant cells (FBGCs), while hydrophobic materials tend to improve monocyte adhesion, resulting in local immune reactions [55]. Surface wettability also influences the catalytic activities of nanozymes in biological systems. For example, Liu *et al*. [65] converted hydrophobic nanoparticles into hydrophilic ionic nanoparticles through ligand exchange and ionization. These ionic nanoparticles exhibited good dispersion in water due to their surface charges, with either positive or negative zeta potentials preventing aggregation. Ionic nanoparticles demonstrated peroxidase-like activity, with amino-functionalized nanoparticles (positive charge) showing lower Michaelis constant (Km) values and higher substrate affinity than carboxyl-functionalized nanoparticles (negative charge) [65].

Chemical engineering techniques can also be used to conjugate functional groups on the surface of nanozymes, modify their properties, and introduce additional functions. Various chemical reactions, such as glutaraldehyde crosslinking, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) conjugation [66], and biotin-streptavidin reactions [67], are employed. For instance, Zhu *et al*. [68] developed a universal nanozyme-integrated sensing platform leveraging the biotin-streptavidin conjugation system. Streptavidin-coated gold nanoparticles (AuNPs) were used to immobilize biotinylated antibodies, enabling a robust and efficient binding mechanism. This design facilitated the quantitative detection of analytes, significantly improving sensitivity and specificity [68].

Defect engineering is an approach to creating vacancies and substitutional defects in nanoscale catalysts and has been utilized to enhance the catalytic efficiency of nanozymes. These defects simulate the spatial and compositional cooperation found in natural enzymes, enriching, organizing, and activating substrates [45,69]. Fan *et al*. [45] modulated the defect environment of neighboring metal sites via edge-site engineering to boost the CAT-like activity of Fe-N_4 nanozymes. This method involved a three-step pyrolysis-leaching process, creating defects around edge-hosted Fe-N_4 sites that transferred charges from Fe atoms to the carbon matrix, enhancing the hydrogen peroxide (H_2O_2) interaction and weakening the chemical bond between two oxygen atoms (O-O) bond [70]. Similarly, Yu *et al*. [71] constructed iron-doped molybdenum oxide with oxygen vacancies (FeMoOV) nanozymes using a defect strategy. Iron doping in molybdenum oxide (MoO_x) introduced abundant iron substitution and oxygen vacancy defects, improving the multienzyme-mimic catalytic activities and inducing a significant surface plasmon resonance effect in the near-infrared (NIR) region [71].

Application of Nanozymes in Bone and Dental Diseases

In Vitro Diagnostics

In vitro diagnostics (IVD) tests are essential tools for detecting diseases using biological samples such as blood and saliva. Nanozymes, particularly those with peroxidase-like activity, have emerged as powerful alternatives to natural enzymes in biosensing applications due to their high

specificity, stability, and efficiency. These nanozymes have been utilized in various detection methods, including fluorescence, electrochemical, and colorimetric assays, to identify and quantify critical biomarkers associated with bone remodeling, bone turnover, and dental health [72–74]. Their ability to catalyze colored reactions with excellent sensitivity makes them ideal for biosensing.

Osteoprotegerin (OPG) is crucial in bone remodeling, primarily by inhibiting the formation and activity of osteoclasts through the receptor activator of nuclear factor κB (RANK)/receptor activator of nuclear factor kappa-B ligand (RANKL)/OPG pathway [75]. Its role in regulating bone resorption makes it vital for maintaining bone density and strength and a potential therapeutic target for bone-related diseases. Arshad *et al*. [76] developed a highly sensitive dual-mode immunosensor for OPG detection using a gold-silver-platinum trimetallic nanozyme. This novel nanozyme exhibits excellent peroxidase-like activity due to the synergistic effects of the three metals. Unlike traditional monometallic nanoparticles that tend to aggregate and form nanoclusters with reduced enzyme-mimicking properties, the trimetallic system maintains superior stability and catalytic performance through enhanced electronic charge transfer between adjacent metal nanoparticles. The unique three-metal structure exposes specific side chains to the substrate, facilitating improved electron transfer and catalytic function within the heterostructure. The dual-mode immunosensor leverages both electrochemical and colorimetric assays, enhancing detection sensitivity and specificity [76]. Utilizing the trimetallic nanozyme's electrocatalytic activity towards reducing hydrogen peroxide (H_2O_2), the electrochemical detection achieved a low detection limit of 1.81 pg/mL with a linear range from 0.1 fg/mL to 10 ng/mL. The colorimetric detection, based on the same nanozyme, showed a detection limit of 1.87 pg/mL with a linear range from 1 fg/mL to 100 ng/mL. This dual-mode sensor demonstrated excellent performance in human serum samples, showcasing its potential for reliable and sensitive detection of OPG in clinical settings [76].

Similarly, alkaline phosphatase (ALP) is a marker for bone turnover, particularly in conditions like osteoporosis [77]. Elevated serum ALP levels in postmenopausal women are linked to high bone turnover [78]. Zhou *et al*. [79] developed a colorimetric detection method for ALP using an Fe-doped carbon-based nanozyme (Fig. 4**A**, Ref. [79]). This nanozyme catalyzes the decomposition of H_2O_2 to ROS, which oxidize phenol produced by ALP, resulting in a detectable color change (Fig. 4**B**). The method shows high sensitivity with a detection limit of 0.03 U/L (Fig. 4**C**) and can quantify ALP activity using a smartphone, making it practical and efficient for biological analysis (Fig. 4**D,E**) [79].

In the context of dental health, rapid and direct detection of oral pathogens is crucial for preventing and diagnosing dental caries [80]. Zhang *et al*. [81] developed DNA-engineered nanozyme interfaces for the rapid and sensitive detection of *Streptococcus mutans*, a key bacterium in dental caries. They constructed three biointerfaces using iron oxide nanozymes functionalized with DNA aptamers to enhance recognition capabilities. Among them, the Fe_3O_4/samarium (III) ions (Sm3) system, incorporating a split DNAzyme, showed the highest sensitivity with a detection limit of 12 CFU/mL and excellent specificity. This colorimetric biosensor allowed for rapid detection within 15 minutes, highlighting its potential for clinical applications in dental disease diagnosis and prevention [81].

Anti-Infection

The intricate relationship between bacterial infections and the bone and dental microenvironment significantly impacts clinical and biomedical approaches. Pathogenic microorganisms, such as those responsible for osteomyelitis and periodontitis, infiltrate bone and dental tissues, adhere to surfaces, and form resilient biofilm communities [82]. These biofilms enable bacteria to evade immune responses and antimicrobial treatments, leading to persistent and chronic infections [83]. The presence of these infections often triggers the release of pro-inflammatory agents, which exacerbate tissue damage, promote bone resorption, and contribute to the destruction of periodontal tissues, ultimately resulting in tooth loss [84]. Developing effective anti-infection strategies is crucial to interrupt this cycle, safeguard bone and dental health, and enhance treatment outcomes.

Yu *et al*. [85] developed a novel SAC for sonodynamic therapy (SDT) to treat methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA)-infected osteomyelitis. The SAC comprised gold nanorods actuating a single-atom-doped porphyrin metal-organic framework (HNTM-Pt@Au) coated with a red blood cell (RBC) membrane (RBC-HNTM-Pt@Au) to enhance biocompatibility and toxin neutralization (Fig. 5**A**, Ref. [85]). The platinum single atoms within the hollow Zr-porphyrin metal-organic framework (HNTM) significantly improved sonocatalytic activity by enhancing electron transfer and oxygen adsorption, facilitating the generation of ROS under ultrasound irradiation. This innovative system achieved an antibacterial efficiency of 99.9 % against MRSA within 15 minutes of ultrasound exposure. *In vivo* experiments using a rat model of osteomyelitis showed successful infection eradication, minimal bone loss, and reduced inflammation, highlighting the nanozyme's potential for rapid and effective treatment of deep-seated bone infections (Fig. 5**B–D**). This multifunctional SAC system offers a promising antibiotic-free strategy for combating persistent infections in bone and dental applications by leveraging enhanced catalytic activity and targeted delivery [85].

In another study, Yu *et al*. [86] used MOFs to develop single-atom catalysts targeting biofilm-induced periodontitis. These SACs, featuring single cobalt atoms dis-

persed on MOF supports, exhibited remarkable catalytic efficiency in producing ROS, which disrupted the biofilm matrix and killed embedded bacteria. The study demonstrated that zirconium-based porphyrinic metal-organic framework decorated with platinum atoms (PCN-222-Pt) MOF-based SACs achieved exceptional anti-biofilm performance, with 98.69 % elimination of *S. aureus* biofilm and 99.91 % elimination of *Escherichia coli* (*E. coli*) biofilm within just 1 hour of treatment. Crystal violet staining assays further confirmed the biofilm disruption efficiency, showing 86.66 % removal of *S. aureus* biofilm matrix. The catalytic performance of the SACs was validated through both computational modeling, which showed reduced oxygen (O_2) activation energy (0.922 eV), and experimental assays demonstrating strong oxidase-like and peroxidase-like activities. *In vivo* studies using a rat periodontitis model showed that the PCN-222-Pt ointment significantly reduced inflammation and alveolar bone loss compared to clinical antibiotic treatments. This approach offers a novel therapeutic strategy for periodontitis, with rapid action and higher efficacy than traditional antibiotic treatments such as Periocline, which showed only 52.40 % anti-biofilm efficiency under the same conditions [86].

Sun *et al*. [87] focused on developing single-atom catalysts based on iridium and ruthenium atoms anchored on sp^2-carbon linked covalent organic frameworks (COFs) to induce bacterial ferroptosis-like cell death, characterized by lipid peroxidation and oxidative damage. Under light irradiation or in the presence of hydrogen peroxide, the SACs generated high levels of ROS, leading to bacterial cell death. The study demonstrated that these COF-based SACs were effective against various bacteria, including MRSA and biofilms, with minimal toxicity to mammalian cells. These findings suggest that SACs can be used to develop potent antibacterial therapies that circumvent traditional antibiotic resistance mechanisms [87].

Osteogenic Microenvironment Regulation

The osteogenic microenvironment is crucial for bone regeneration and healing [1]. During acute inflammation, immune cells such as macrophages, neutrophils, and lymphocytes are recruited to the injury site [88]. These cells generate inflammatory factors and ROS to clean up debris and remove pathogens. Proper acute inflammation creates an environment conducive to bone repair by enhancing osteoblast differentiation and activity, leading to effective bone formation and healing. However, chronic inflammation, resulting from persistent immune activation due to infections, autoimmune diseases, diabetes, aging, and other factors, can lead to continuous secretion of pro-inflammatory cytokines and ROS [89]. Elevated levels of these cytokines promote osteoclast differentiation and activity, leading to excessive bone resorption and disrupting the balance of bone remodeling [90]. Additionally, excessive ROS production causes oxidative stress and damage to osteoblasts and other bone cells, leading to reduced new bone formation. Conditions such as osteoporosis, osteoarthritis, and periodontitis are associated with chronic inflammation, resulting in weakened bones and compromised bone regeneration [91]. Effective regulation of osteogenic environment can significantly enhance osteogenesis and improve outcomes in bone-related diseases. Key aspects of regulating the osteogenic microenvironment include managing ROS levels, modulating immune responses, and regulating balanced bone remodeling. Nanozymes, with their unique catalytic properties, offer innovative approaches to achieve these goals by mimicking natural enzyme activities to reduce the ROS and influencing cellular processes.

Periodontitis is a chronic inflammatory disease caused by dental plaque that leads to excessive ROS accumulation and tissue destruction. Xu *et al*. [92] developed a multifunctional hydrogel system (TM/BHT/CuTA) that combines copper tannic acid coordination nanosheets (CuTA NSs) with a triglycerol monostearate/2,6-di-tert-butyl-4-methylphenol (TM/BHT) hydrogel (Fig. 6**A**, Ref. [92]). The CuTA NSs exhibited broad-spectrum antibacterial properties (Fig. 6**B**) and simulated the activities of SOD and CAT to scavenge multiple ROS. This dual action reduces oxidative stress and modulates the immune response by shifting macrophages from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype through the nuclear factor erythroid 2-related factor 2 (Nrf2)/nuclear factor kappa B (NFκB) pathway, promoting tissue regeneration (Fig. 6**C**–**E**) [92]. Similarly, cerium oxide nanoparticles (CeO_2 NPs) showed similar properties in treating periodontitis. CeO_2 NPs significantly reduced intracellular ROS levels in lipopolysaccharide (LPS)-stimulated macrophages, leading to decreased inflammation by inhibiting the mitogen-activated protein kinase (MAPK)-NFκB signaling pathway, which is responsible for producing inflammatory cytokines like TNF-α and IL-1β. Additionally, CeO_2 NPs activate the Nrf2-antioxidant response element (ARE) pathway, an important antioxidant regulatory pathway that balances oxidative and antioxidative activities in healthy cells [93]. In addition, glutathione peroxidase (GPx) has also been mimicked by nanozyme to treat periodontitis. Zhu *et al*. [94] developed a MIL-47(V)-F (MVF) nanozyme mimicking the function of GPx to alleviate ROS and promote bone regeneration in periodontitis. The MVF nanozyme, synthesized by assembling vanadium ions with a fluorinated ligand, specifically targets and eliminates H_2O_2, the predominant ROS in periodontitis. By selectively scavenging H_2O_2, MVF mitigates oxidative stress without disrupting the physiological functions of ROS crucial for cell signaling and metabolism. The MVF nanozyme demonstrates excellent biocompatibility and cytoprotective effects *in vitro*, protecting cells from H_2O_2-induced oxidative damage by activating the Nrf2/heme oxygenase-1 (HO-1) signaling pathway. This activation enhances the antioxidant defense mechanism of cells, reducing lipid peroxi-

dation and DNA damage. Furthermore, MVF modulates the immune response by inducing macrophage polarization from M1 phenotype to M2 phenotype, creating a favorable microenvironment for bone regeneration. *In vivo* studies using a rat model of periodontitis showed that MVF treatment significantly reduced alveolar bone loss, decreased osteoclast activity, and improved periodontal tissue integrity [94].

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes progressive cartilage destruction and severely affects physical activity. Jia *et al*. [95] designed a nanozyme system to treat RA based on nanoscale manganese dioxide (MnO_2) encapsulated with dexamethasone sodium phosphate (DSP) and cloaked with microvesicles derived from macrophages (MMV). This system, referred to as MMV-MnO_2@DSP, aims to treat RA by targeting and reprogramming the inflammatory microenvironment. The MnO_2 nanozyme possesses SOD and CAT-like activities, which enable it to modulate the imbalances in superoxide anion and hydrogen peroxide metabolism commonly seen in RA. The macrophage-derived microvesicle coating provides the inflammation-homing ability and helps the nanozyme evade immune detection, facilitating targeted delivery to inflamed joints [95]. MMV-MnO_2@DSP nanozymes accumulate in activated macrophages and reprogram the cellular environment by reducing oxidative stress and inhibiting pro-inflammatory cytokine feedback loops, crucial for reducing inflammation and promoting tissue repair [95].

Similar to RA, osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation, presents distressing symptoms such as pain, stiffness, and impaired joint function. Guo *et al*. [96] developed a novel Zn-doped hollow mesoporous cerium oxide nanozyme (HMZC) encapsulating kartogenin (KGN) and coated with hyaluronic acid (HA) to create KGN@HMZC@HA nanozymes for OA treatment. This design leverages the SOD-like and CAT-like catalytic activities of Zn-doped HMZC to effectively scavenge ROS and generate oxygen (O_2), to facilitate the remodeling of OA microenvironment. The nanozyme initiated the conversion of pro-inflammatory M1 macrophages to anti-inflammatory M2 phenotypes, thus suppressing inflammation and promoting cartilage differentiation. The pH-responsive release of KGN achieved by the HMZC@HA system ensures targeted drug delivery, enhancing therapeutic precision and minimizing side effects. *In vitro* studies demonstrated significant ROS scavenging, reduced inflammation, and improved chondrocyte viability. *In vivo* experiments in an OA rat model showed effective cartilage protection and repair, confirming the potential of KGN@HMZC@HA as a promising treatment for OA through microenvironment remodeling, oxidative stress mitigation, and targeted anti-inflammatory effects [96].

Osteoporosis is characterized by weakened bones and compromised bone regeneration, predominantly affecting postmenopausal women and the elderly. Li *et al*. [97] designed a composite nanoparticle to target ROS-induced osteoblast senescence and excessive receptor activator of nuclear factor kappa-B ligand (RANKL) production, key factors in osteoporosis. They synthesized Prussian blue nanozymes (PBzymes) grown on sub-50 nm hollow mesoporous silica nanoparticles (HPB), creating a composite called HPB@RANKL-clustered regularly interspaced short palindromic repeats/associated protein 9 (CRISPR/Cas9)-alendrenate (RC-ALN). This platform combines the ROS-scavenging abilities of PBzymes, exhibiting SOD and CAT-like activities, with a CRISPR/Cas9 system for RANKL gene editing and alendronate for bone targeting. HPB@RC-ALN nanozyme showed strong ROS scavenging capabilities, reducing oxidative stress and reversing osteoblast senescence. *In vitro* assays demonstrated that the nanozyme preserved osteogenic activity in senescent osteoblasts and reduced RANKL production, thereby inhibiting osteoclast formation. *In vivo* studies using ovariectomized (OVX) mice, a model for postmenopausal osteoporosis, indicated that HPB@RC-ALN significantly improved bone volume and density and reduced osteoclast numbers [97].

Similarly, Wang *et al*. [98] developed polyacrylic acid-modified cerium oxide nanoparticles (PCNPs) to address bone regeneration challenges in type 2 diabetes mellitus (T2DM). Elevated blood sugar levels in T2DM lead to excessive ROS production, causing mitochondrial dysfunction and inducing senescence in bone marrow mesenchymal stem cells (BMSCs), impairing their osteogenic potential. PCNPs exhibit SOD and CAT-like activities, effectively scavenging ROS and restoring mitochondrial function. *In vitro* experiments showed that PCNPs reduced intracellular and mitochondrial ROS levels, maintained mitochondrial membrane potential, and preserved normal mitochondrial morphology. PCNPs also upregulated the adenosine monophosphate-activated protein kinase (AMPK)-sirtuin 1 (SIRT1)-peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) signaling pathway, a key cellular energy-sensing and mitochondrial regulatory axis. AMPK, an energy sensor activated under low-energy conditions, stimulates SIRT1, a stress-responsive deacetylase, which in turn promotes mitochondrial biogenesis via PGC1α. This cascade enhances mitochondrial function and biogenesis, ultimately alleviating senescence in bone marrow-derived mesenchymal stem cells (BMSCs). *In vivo*, a sustained-release hydrogel model loaded with PCNPs significantly improved bone regeneration in T2DM rats with cranial bone defects, highlighting the potential of PCNPs as a multifunctional therapeutic tool for bone-related diseases in diabetic conditions [98].

Combating Bone Tumors

Bone tumors present significant challenges due to their aggressive nature and the complexities of treatment. Osteosarcoma is the most common primary malignant bone tumour, which involves several possible genetic drivers of disease linked to bone formation, causing malignant progression and metastasis. The rapid multiplication of residual tumour cells and poor reconstruction quality of new bone are considered the major challenges in the postoperative treatment of osteosarcoma. The combination of nanozyme-based therapy and bone grafting materials exhibited great potential for the postoperative treatment of bone tumours. For example, Yan *et al*. [99] developed an innovative approach for osteosarcoma treatment using single-atom copper (Cu) nanozyme-loaded bone scaffolds. These nanozymes, embedded in a mesoporous silica framework, exhibit peroxidase-like and glutathione oxidase-like activities, enabling the generation of ROS under mild photothermal therapy (PTT). The ROS induce ferroptosis in osteosarcoma cells, enhancing antitumor efficacy. The nanozymes were incorporated into a composite scaffold made of poly(L-lactic acid) (PLLA) and bioactive glass (BG), which facilitated tumor inhibition and promoted bone regeneration through the continuous release of bioactive ions [99].

Similarly, Liang *et al*. [100] developed a rhodium-ruthenium (RhRu) alloy-anchored family of two-dimensional nanomaterials composed of transition metal carbides, nitrides, or carbonitrides (MXene) nanozymes for osteosarcoma therapy. These nanozymes combine chemodynamic therapy (CDT), photodynamic therapy (PDT), and PTT by synthesizing RhRu nanoparticles anchored on titanium carbide ($Ti_3C_2T_x$) MXene nanosheets. The nanozymes exhibit CAT and peroxidase (POD)-like activities, enabling efficient ROS generation and heat conversion under NIR irradiation. *In vitro* and *in vivo* studies showed significant tumor growth inhibition, superior to single-mode treatments, with excellent biocompatibility and minimal toxicity to healthy tissues, highlighting their potential as a safe and powerful platform for osteosarcoma therapy [100].

Apart from nanozyme-based PDT and PTT applications, researchers also developed nanozymes to regulate the tumour microenvironment. For instance, Xia *et al*. [101] developed copper-loaded nanoheterojunctions (Cu-TiO_2) for effective orthotopic osteosarcoma therapy. These nanoheterojunctions, formed by loading copper nanoparticles onto titanium dioxide (TiO_2) nanoplates, enhanced ROS production and depleted intracellular glutathione (GSH), leading to increased oxidative stress and apoptosis in osteosarcoma cells. *In vitro* studies showed significant cancer cell death due to ROS amplification and GSH depletion, while *in vivo* experiments using orthotopic osteosarcoma models demonstrated substantial tumor growth inhibition and improved survival rates. The nanoheterojunctions exhibited good biocompatibility with minimal adverse effects on healthy tissues, highlighting their potential as a powerful and safe therapeutic tool for combating bone tumors [101].

Challenges and Future Perspectives

Nanozymes have demonstrated significant potential in addressing challenges associated with bone and dental tissue diseases. The advancement of nanotechnology has enabled the fine-tuning of catalytic activity and biological responses through meticulous chemical design and engineering strategies to regulate the surface properties of nanozymes. These properties can be adjusted by controlling size, morphology, and surface engineering. In this review, we systematically summarized various factors that influence the catalytic activity and biological response of nanozymes, aiming to maximize their effectiveness in biomedical applications. With innovative designs, the flexibility of nanozymes has expanded their application in various bone and dental-related fields, including diagnostics, anti-infection treatments, osteogenic microenvironment regulation, and bone cancer therapy.

Although nanozymes have overcome many challenges in treating bone and dental diseases, there remains substantial room for progress. Firstly, ensuring the biosafety and biocompatibility of nanozymes is paramount for their successful integration into clinical applications. Metal-based nanozymes, while effective, can exhibit cytotoxicity due to the release of metal ions or degradation products. Therefore, selecting appropriate metal atoms is critical to minimize toxicity. Using metals that are less likely to release harmful ions or that have been proven safe in biomedical applications can mitigate potential risks. For successful clinical translation, systematic investigations of nanozyme behaviour in biological systems are essential, including detailed analysis of their circulation time, tissue distribution, metabolic pathways, and clearance mechanisms. Recent biodistribution research has shown that most nanozymes tend to accumulate predominantly in reticuloendothelial system organs such as the liver, spleen, and lungs when administered without targeting ligands. This non-specific distribution pattern emphasizes the importance of incorporating targeting strategies through surface modification to improve therapeutic outcomes and reduce potential systemic effects. Additionally, the support materials for nanozymes, such as graphene oxide (GO) or metal-organic frameworks (MOFs), should be designed to minimize cytotoxic effects. Surface modifications and biocompatible coatings can not only enhance the biocompatibility of these materials but also provide targeting capabilities to reduce systemic accumulation. Developing biodegradable nanozymes that can be safely digested and eliminated from the body will also enhance their clinical applicability. Additionally, enhancing the catalytic efficiency of nanozymes can also minimize their usage, thereby reducing the risk of cytotoxicity. En-

suring that both the metal components and the support materials are non-toxic and biocompatible, while maintaining precise control over their biodistribution, is crucial for the long-term success of nanozyme-based therapies.

Currently, the primary application of nanozymes is the reduction of ROS, while fewer studies explore their potential in other enzymatic activities. To unlock the full therapeutic potential of nanozymes, it is essential to develop multiple types of nanozymes capable of meeting the needs of a broader range of biochemical reactions. For example, creating cascade reaction systems that integrate different types of nanozymes can enhance their functional diversity and efficacy. These systems can mimic complex biological pathways, enabling more sophisticated therapeutic interventions. For instance, developing nanozymes that can simultaneously modulate multiple metabolic or signaling pathways could provide comprehensive solutions for treating multifaceted diseases such as bone cancer or age-related bone disorders.

Conclusions

Nanozyme technology has emerged as a powerful tool in orthopedics and dentistry, offering unique solutions to long-standing challenges in disease treatment. The systematic design and engineering of nanozymes, from composition to surface properties, has enabled precise control over their catalytic activities and biological interactions. Through various applications demonstrated in this review—from diagnostics and infection control to microenvironment regulation and tumor treatment—nanozymes have shown remarkable versatility and effectiveness. Their ability to perform multiple enzyme-like functions while maintaining stability under physiological conditions presents distinct advantages over traditional therapeutic approaches. The growing body of research in this field provides compelling evidence for the potential of nanozymes to fundamentally transform treatment strategies in bone and dental medicine. As this technology continues to mature, it promises to deliver more effective, personalized therapeutic solutions that could significantly improve patient care in orthopedic and dental applications.

List of Abbreviations

ALP, alkaline phosphatase; AMPK, adenosine monophosphate-activated protein kinase; ARE, antioxidant response element; AuNPs, gold nanoparticles; BG, bioactive glass; BMSCs, bone marrow mesenchymal stem cells; CAT, catalase; CDT, chemodynamic therapy; Ce, cerium; Co, cobalt; COFs, covalent organic frameworks; Cu, copper; DNA, deoxyribonucleic acid; DSP, dexamethasone sodium phosphate; FBGCs, foreign body giant cells; Fe, iron; GM-CSF, granulocyte-macrophage colony-stimulating factor; GO, graphene oxide; GPx, glutathione peroxidase; GSH, glutathione; H_2O_2, hydrogen peroxide; HIF-1α, hypoxia-inducible factor 1-alpha; HMZC, hollow mesoporous cerium oxide nanozyme; HO-1, heme oxygenase-1; HRP, horseradish peroxidase; IL, interleukin; IONPs, iron oxide nanoparticles; IR, ionizing radiation; IVD, *in vitro* diagnostics; KGN, kartogenin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Mn, manganese; MOFs, metal-organic frameworks; MRSA, methicillin-resistant *Staphylococcus aureus*; NFκB, nuclear factor kappa B; NIR, near-infrared; Nrf2, nuclear factor erythroid 2-related factor 2; O_2, oxygen; OA, osteoarthritis; OPG, osteoprotegerin; OVX, ovariectomized; PDT, photodynamic therapy; PEG, polyethylene glycol; PLLA, poly(L-lactic acid); POD, peroxidase; PTT, photothermal therapy; RA, rheumatoid arthritis; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor kappa-B ligand; RBC, red blood cell; Rh, rhodium; ROS, reactive oxygen species; Ru, ruthenium; SACs, single-atom catalysts; SDT, sonodynamic therapy; SIRT1, sirtuin 1; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; TiO_2, titanium dioxide; TNF-α, tumor necrosis factor-alpha; THH, tetrahexahedral; V, vanadium; Zn, zinc; Fe_3O_4, magnetite; Fe-SAs/NC, single-atom Fe-N_4 catalytic site anchored on N-doped porous carbon materials; CoNZ, cobalt-doped nanoglass; CeO_2, cerium oxide; Ce^{3+}, trivalent cerium; Pd, palladium; Au, gold; CO, carbon monoxide; Pt, platinum; FeO_x, iron oxide; NPs, nanoparticles; *S. aureus*, *Staphylococcus aureus*; CuTA NSs, copper tannic acid coordination nanosheets; MVF, MIL-47(V)-F; MnO_2, manganese dioxide; HA, hyaluronic acid; PBzymes, Prussian blue nanozymes; PCNPs, polyacrylic acid-modified cerium oxide nanoparticles; RhRu, rhodium-ruthenium; MMV, microvesicles derived from macrophages; HPB, hollow mesoporous silica nanoparticles; AuNRs, gold nanorods; TM, triglycerol monostearate; M1, M1 macrophages; M2, M2 macrophages; TEM, transmission electron microscopy; Fe/C NS, Fe-doped carbon nanosheet; UV-Vis, ultraviolet-visible; RGB, red, green, and blue; NTCDA, 1,4,5,8-naphthalenetetracarboxylic dianhydride; DICY, dicyandiamide; PPDS, disodium phenyl phosphate; PO, phenol; 4-AAP, 4-aminoantipyrine; RBC-HNTM-Pt@Au, red blood cell coated Au nanorod (NRs)-actuated single-atom-doped porphyrin metal-organic framework; CT, computed tomography; US, ultrasound; MC3T3-E1, embryonic mouse calvarial cell line; OCN, osteocalcin; MMP, matrix metalloproteinases; TGF-β, transforming growth factor beta; *E.coli*, *Escherichia coli*; *P. gingivalis*, *Porphyromonas gingivalis*; *A. viscosus*, *Actinomyces viscosus*; EDC/NHS, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide; HNTM, a hollow Zr-porphyrin metal-organic framework; PCN-222-Pt, zirconium-based porphyrinic metal-organic framework decorated with platinum atoms; Sm3, samarium (III) ions; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; RC-ALN, RANKL-CRISPR/Cas9-alendrenate;

CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/associated protein 9; MXene, a family of two-dimensional nanomaterials composed of transition metal carbides, nitrides, or carbonitrides.

Availability of Data and Materials

Not applicable.

Author Contributions

WDG, YQM, LX, and YX contributed to the design and data analysis of this work. WDG and YQM drafted the work. LX and YX revised the manuscript and critically contributed to the intellectual content. All authors read and approved the final manuscript for this submission. All authors agreed to be accountable for all aspects of the work and to ensure that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare that they have no competing interests.

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