Particuology 76 (2023) 32–45

Contents lists available at ScienceDirect

Particuology

journal homepage: www.elsevier.com/locate/partic

Invited review

Nanozymes for biomedical applications in orthopaedics

Anqi Lin [a, 1], Shujie Liu [a, 1], Hui Wei [a, b, *]

[a] Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, 210023, China
[b] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Chemistry and Biomedicine Innovation Center (ChemBIC), Nanjing University, Nanjing, 210023, China

ARTICLE INFO

Article history:
Received 14 July 2022
Received in revised form
11 August 2022
Accepted 17 August 2022
Available online 29 August 2022

Keywords:
Nanozymes
Artificial enzymes
Orthopaedics
Nanomedicine
Nanomaterials

ABSTRACT

As the next generation of artificial enzymes, nanozymes have attracted increasing attention in biomedical applications due to their multienzyme-like characteristics, multifunctionalities, low cost, and high stability. By taking advantage of their diverse activities, a growing number of nanozyme-mediated therapeutic strategies have been developed for various diseases. Herein, we provide a brief review of the representative studies of nanozymes, especially in orthopaedic diseases over the past decade, which include arthritis, osteoporosis, bone regeneration, bacteria-associated infections, and osteosarcoma. Moreover, the future potential applications and some major challenges are also discussed. This review would not only provide some instructive views of nanozymes but also promote the development of enzyme-mimetic strategies in orthopaedics.

1. Introduction

Since Yan's group demonstrated the peroxidase (POD)-like activity of ferromagnetic nanoparticles (Fe_3O_4 NPs) in 2007 (Gao et al., 2007), numerous nanomaterials such as metals (Y. Lin, Ren, & Qu, 2014; Miao et al., 2020), metal oxides (Soh et al., 2017; Vernekar et al., 2014), metal-organic frameworks (MOFs) (Nath, Chakraborty, & Verpoort, 2016; Wu et al., 2021), carbons (Fan et al., 2018; Sun, Zhou, Ren, & Qu, 2018), and others (Singh, NaveenKumar, Geethika, & Mugesh, 2021; Yin et al., 2016), have been reported to have diverse enzyme-like characteristics. These nanomaterials are defined as "nanozymes", which integrates the merits of nanomaterials and enzymes (H. Wei, Qiao, et al., 2021; Wei & Wang, 2013; Wu et al., 2019; R. Zhang, Yan, & Fan, 2021).

In recent years, nanozymes have attracted increasing attention in various biomedical applications due to their multienzyme-like activities, multifunctionalities, low cost, and high stability (Huang, Ren, & Qu, 2019; Jiang et al., 2019; Wu et al., 2019). They can modulate the levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and some specific biomolecules in cellular metabolism, which is essential to the treatment of different diseases (H. Wang, Wan, & Shi, 2019). Generally, ROS scavenging activities originate from •OH radical eliminating and antioxidant enzyme-mimicking abilities, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase. ROS generating activities originate from POD- and oxidase (OXD)-like abilities, which involve the production of threatening •OH and H_2O_2. Some nanozymes also show RNS (such as NO• radical and $ONOO^-$) scavenging activities (Cao et al., 2020; Mu et al., 2019). In addition, hydrolase-like abilities toward different substrates (such as phosphates, proteins, and saccharides) have been reported as well (S. Li et al., 2022).

Based on the above diverse activities, a growing number of studies have developed nanozyme-mediated therapeutic strategies for different diseases, including local inflammation (Choi, Cha, & Kim, 2020; Kumar, Adjei, Brown, Liseth, & Sharma, 2019; Y. Liu et al., 2020), injury therapies (Han et al., 2018; Y. Liu et al., 2017; Ni et al., 2018), infectious diseases (X. Li, Qi, et al., 2019; Xu et al., 2019), neurodegenerative disorders (Hao et al., 2019; Kwon et al., 2018; Ma et al., 2020), dental diseases (Bao, Zhao, Sun, Hu, & Yang, 2018; X. Chen, Xie, et al., 2021), and tumors (He et al., 2020; Sang et al., 2020; Tang, He, Liu, Yan, & Fan, 2021). In this review, we focused on the biomedical applications of nanozymes in orthopaedics. The symptoms covered in this review include

* Corresponding author. Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, 210023, China.
E-mail address: weihui@nju.edu.cn (H. Wei).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.partic.2022.08.009

inflammatory arthritis, osteoporosis, traumatic bone defects, bacteria-associated infections, and osteosarcoma. Nanozyme-involved systems provide novel insights into strategies of efficient therapeutic methods in bone diseases. In the final section, we discuss the potential applications and some major challenges that the field of nanozymes faces. We hope that this review will not only provide some instructive views of nanozymes but also promote the development of enzyme-mimicking strategies in orthopaedics.

2. Biomedical applications in orthopaedics

2.1. Osteoarthritis (OA)

Osteoarthritis (OA) is a degenerative joint disease which would lead to various levels of loss in joint functions (Neogi, 2013; Rahmati, Nalesso, Mobasheri, & Mozafari, 2017). It is primarily characterized by articular cartilage degradation, subchondral bone thickening, osteophyte formation, and joint degeneration (Varela-Eirin et al., 2018). As the obese and elderly population grows, the prevalence of OA increases, resulting in deleterious effects on more than 250 million people worldwide (Hunter & Bierma-Zeinstra, 2019; Neogi, 2013). Generally, the pathological deterioration is closely related to oxidative stress caused by excessive ROS in joints during the process of OA (Morita et al., 2007; Yudoh et al., 2005). Increased ROS production disturbs the homeostasis of the joint microenvironment induced by lipid peroxidation, protein carbonylation, and DNA damage (Lepetsos & Papavassiliou, 2016; Portal-Nunez, Esbrit, Alcaraz, & Largo, 2016). Furthermore, overproduced ROS also promotes the activation of macrophages and stimulates pro-inflammatory signaling pathways, leading to an aggravated inflammatory response (Y.-C. Liu, Zou, Chai, & Yao, 2014; Shapouri-Moghaddam et al., 2018).

Therefore, recent studies have focused on scavenging excessive ROS to alleviate oxidative stress in OA pathogenesis, such as using nanozymes as natural antioxidant enzyme equivalents (Hou et al., 2021; Kumar et al., 2019). As shown in Fig. 1(a–c), Sharma's group synthesized PEG-MnO_2 nanoparticles (NPs) with a size of less than 20 nm to prolong retention time in the joint cavity for continuous ROS scavenging (Kumar et al., 2019). The PEG-MnO_2 NPs exhibited adequate ROS scavenging ability in an *ex vivo* bovine model of IL-1β-induced chronic OA. They could prevent the development of OA by reducing the loss of glycosaminoglycans, a key component of cartilage extracellular matrix. Moreover, PEG-MnO_2 NPs exhibited chondroprotection effects by reducing the release of stimulated nitric oxide and downregulating oxidative stress-related gene expression in cytokine-challenged chondrocytes. As shown in Fig. 1(d), Cai's group proposed a nanozyme-mediated microenvironment remodeling strategy to protect chondrocytes and delay OA by applying hollow Prussian blue (PB) nanozymes (HPBzymes) *in vitro* and *in vivo* (Hou et al., 2021).

2.2. Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA), a chronic autoimmune disease, causes progressive disability due to severe cartilage and bone damage (Smolen, Aletaha, & McInnes, 2016). As a multifactorial disease, the etiologies of RA involve genetic factors, environmental agents, autoimmunity, and nutritional status (George, Shyni, & Raghu, 2020). The multiplicity mentioned above would induce the infiltration of inflammatory cells in the joint synovium and further promote RA progression (Udalova, Mantovani, & Feldmann, 2016). Anti-inflammatory drugs and immunosuppressants have been exploited for using alone or in combination with clinical RA treatments, which include non-steroidal anti-inflammatory drugs, glucocorticoids, and disease-modifying anti-rheumatic drugs (Burmester & Pope, 2017; Q. Guo et al., 2018). Although they are effective in disease remission and prevention of deformity, nonspecific targeting and prolonged repetitive use of these drugs may lead to serious side effects, such as osteoporosis, muscular atrophy and drug resistance (W. Wang, Zhou, & Liu, 2018).

To develop novel therapeutic strategies, various nanozymes have been employed for RA treatment. As shown in Fig. 2(a and b), Hyeon and co-workers developed manganese ferrite and CeO_2-anchored mesoporous SiO_2 NPs (MFC-MSNs) with synergistic ROS scavenging and O_2 generation abilities to efficiently facilitate the polarization of M1-to-M2 macrophages (J. Kim, Kim, Song, et al., 2019). Moreover, MSNs showed better therapeutic effects after encapsulating the antirheumatic drug methotrexate. In another work, Kim's group combined CeO_2 NPs with albumin to target inflamed joints in RA while ameliorating hypoxia, scavenging ROS and polarizing M1 into M2 macrophages (Kalashnikova et al., 2020). Besides metal oxides, MOFs are also excellent candidates for designing high-performance biomedical nanozymes due to their well-defined structures which are similar to proteins (S. Li et al., 2022; Wu et al., 2021). In Fig. 2(c), Shi's group established an *in situ* generated anti-inflammatory nanosystem (Mn-HMSN-PEG-H_2TE-2-PyP^{4+}, denoted as MHPH) with SOD-like and CAT-like abilities for the catalytic treatment of RA (B. Yang, Yao, Yang, Chen, Guo, et al., 2022). Owing to the coordination environment of active Mn sites, manganese porphyrin presented excellent antiarthritic efficacy compared with the typical antioxidant ascorbic acid in an adjuvant-induced arthritis animal model. They also applied the coordination geometry mimicking concept in construction of 2D MOF nanosheets to achieve biomimetic enzyme catalysis (B. Yang, Yao, Yang, Chen, & Shi, 2022). Apart from utilizing the intrinsic enzymatic activity of nanozymes, recent studies have developed a promising strategy of combining nanozymes and other therapeutic approaches, which included using concave-cubic rhodium nanozyme for the enhancement of ultrasound-driven sonodynamic therapy (W. Li, Han, et al., 2021).

2.3. Osteoporosis (OP)

Osteoporosis (OP) is defined as a systemic skeletal disease with clinical manifestations including low bone mass, microarchitectural deterioration of bone tissue, and enhanced skeletal fragility (Compston, McClung, & Leslie, 2019; Vidal, Thibodaux, Neira, & Messina, 2019). Generally, OP is classified into two categories, primary type and secondary type, according to the etiology (Sozen, Ozisik, & Basaran, 2017). The primary OP is mainly attributed to aging and age-related decreases of sex steroids (Ji & Yu, 2015), while the secondary OP is related to undesirable pathological conditions or medications (Mirza & Canalis, 2015). Under normal conditions, there is a dynamic balance between bone resorption and bone formation (Kenkre & Bassett, 2018). However, the unbalanced skeletal homeostasis always results in enhanced bone resorption and decreased bone mass in OP patients (Baron & Hesse, 2012).

The current treatments for OP are largely limited to anti-resorptive drugs and anabolic agents that stimulate bone formation (Kanis, Cooper, Rizzoli, & Reginster, 2019). Due to obvious side effects, these treatments are unable to achieve satisfying therapeutic effects (H. Li, Xiao, et al., 2021; Reginster et al., 2014). Meanwhile, regulating the local immune environment affected by excessive ROS has been considered as a novel strategy to restore the intrinsic balance of bone metabolism (Chen et al., 2015; J. Li, Han, et al., 2021). For example, CeO_2 attracted much attention for OP treatment due to its multienzyme-like activities and outstanding antioxidant effect through switching between the Ce^{3+}/Ce^{4+} oxidation states during redox reactions (Pinna et al., 2021). Ceria

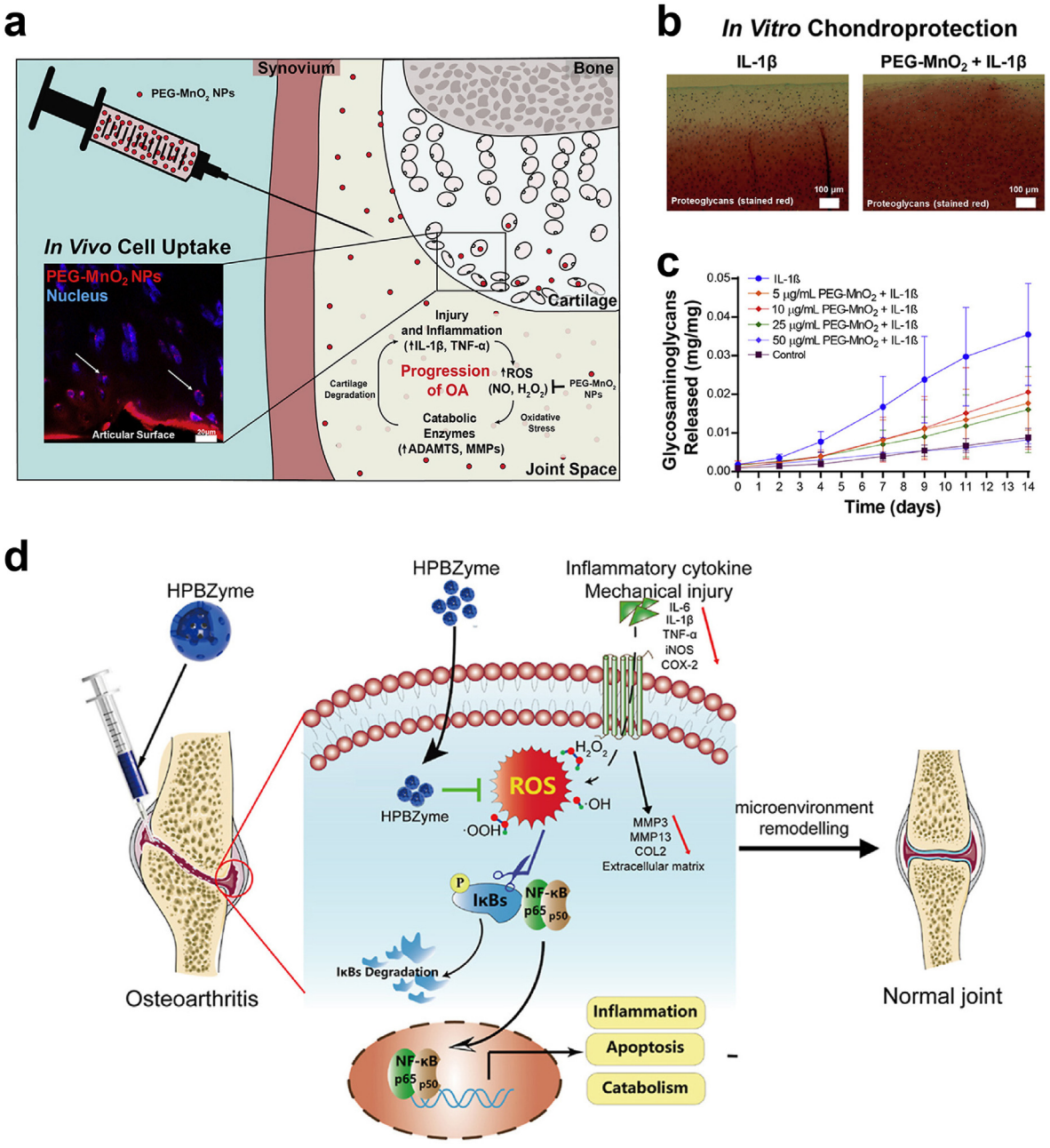

Fig. 1. Nanozymes for OA therapy. (a) Chondroprotective mechanism and intracellular localization of MnO_2 NPs in IL-1β-induced OA model. (b) Safranin-O staining (scale bar = 100 μm). (c) Measurement of released glycosaminoglycans. (d) Therapeutic mechanisms of HPBzymes in OA mouse model. (a–c) Adapted from (Kumar et al., 2019), copyright 2019. (d) Adapted from (Hou et al., 2021), copyright 2021.

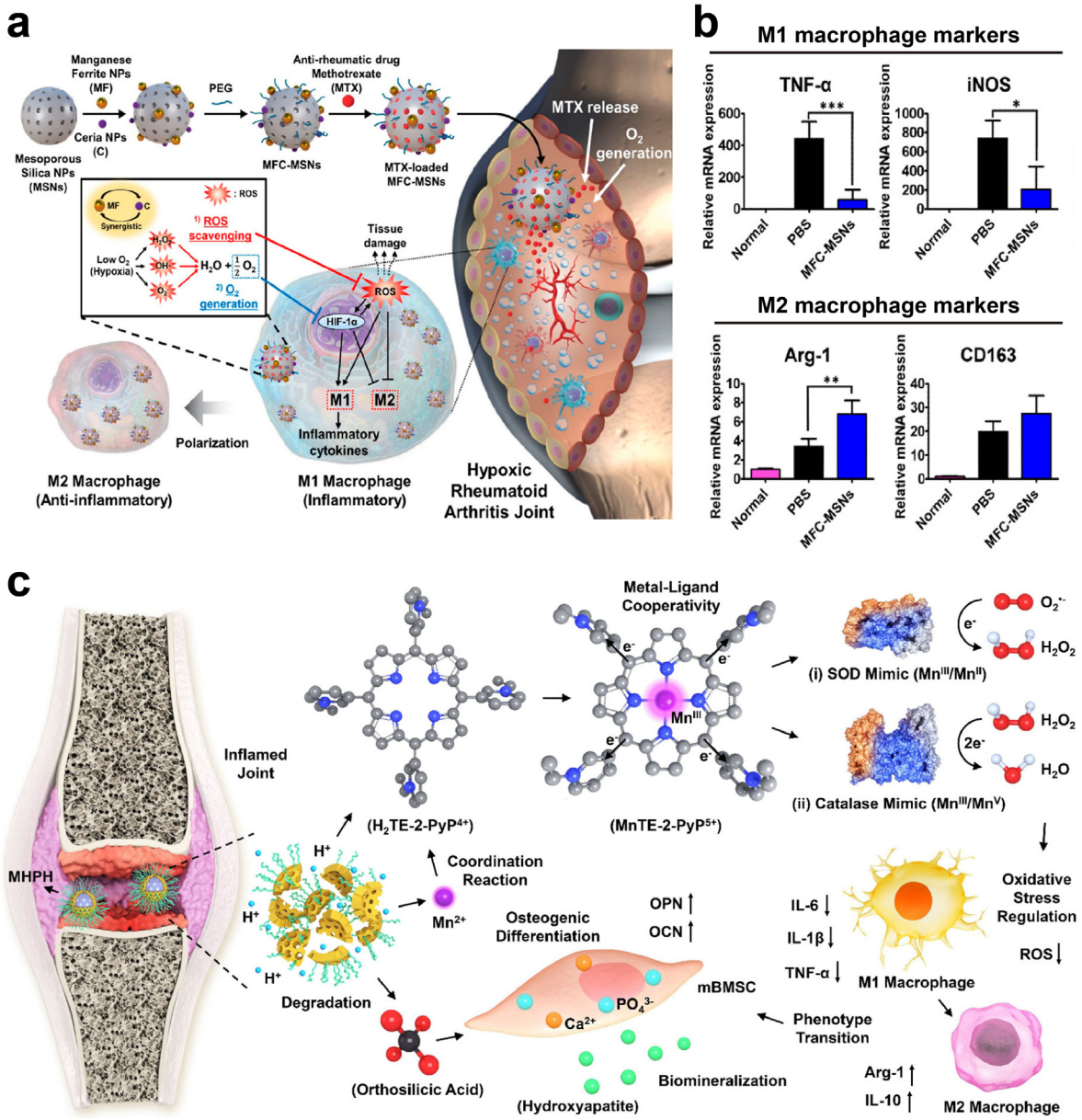

Fig. 2. Nanozymes for RA therapy. (a) Therapeutic mechanisms of MFC-MSNs in RA treatment. (b) qRT-PCR analysis of M1 and M2 macrophage markers in synovial tissue 30 days after injection. (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001. (c) Schematic illustration of the therapeutic concept of MHPH nanomedicine for catalytic anti-inflammatory RA treatments. (a, b) Adapted from (J. Kim, Kim, Song, et al., 2019), copyright 2019. (c) Adapted from (B. Yang, Yao, Yang, Chen, Guo, et al., 2022), copyright 2022.

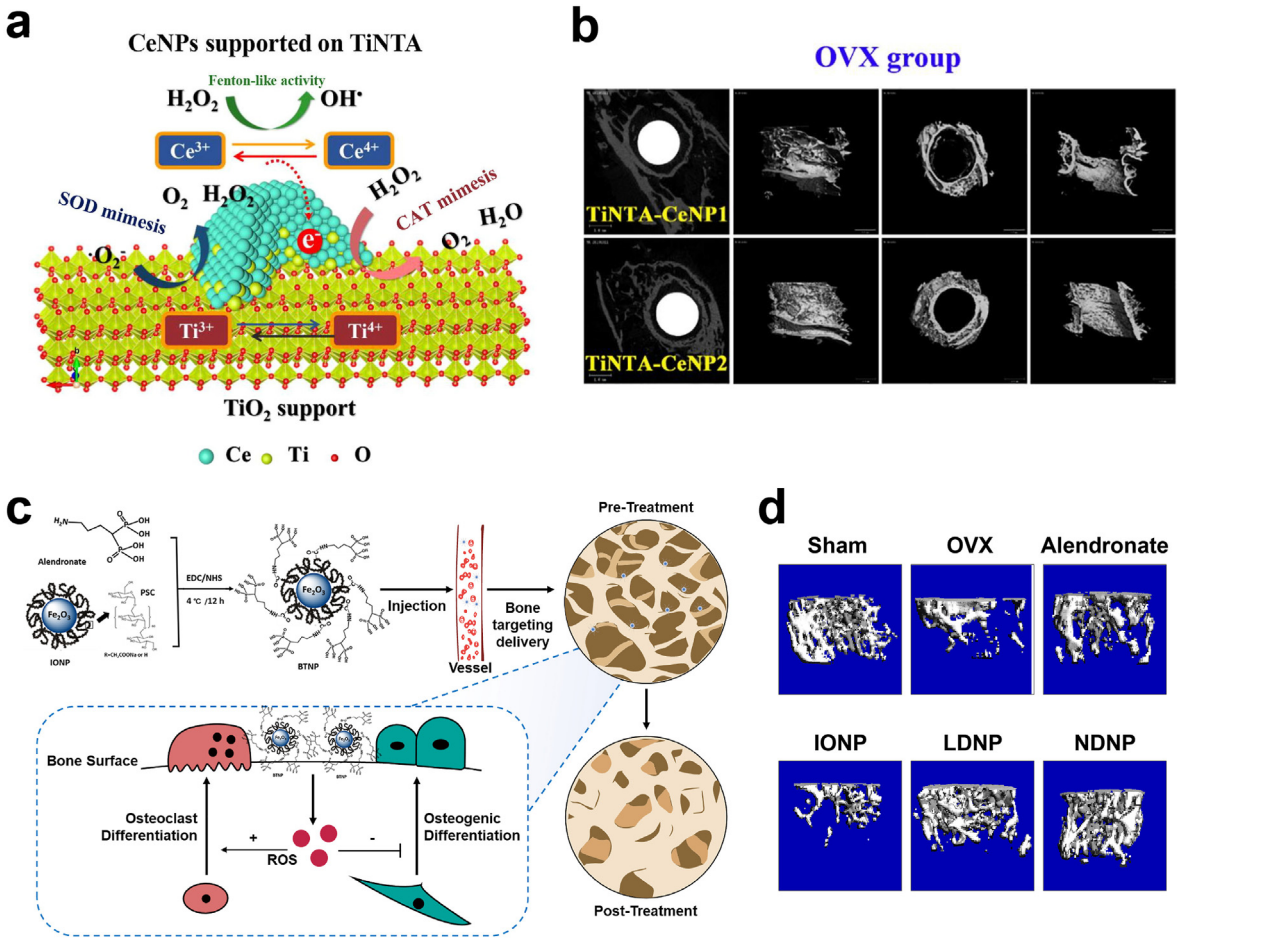

Fig. 3. Nanozymes for OA therapy. (a) Schematic illustration of CeO_2 NPs loaded on a vertically aligned TiO_2 nanotube array. (b) Cross-sectional 2D images and 3D reconstruction images of the peri-implant trabecular bone structure. Scale bar = 1.0 mm *p < 0.05, **p < 0.01. (c) Synthesis of bone targeting iron oxide NPs loaded with alendronate. (d) Micro-CT reconstruction images of the distal femurs in postmenopausal OP mouse model. (a, b) Adapted from (Shao et al., 2021), copyright 2021. (c, d) Adapted from (Zheng et al., 2022), copyright 2022.

nanozymes were also employed as an additive in orthopedic implants for improving osseointegration at the bio-interface. In Fig. 3(a and b), it showed that the interaction between vertically aligned TiO_2 nanotube array and CeO_2 NPs (TiNTA-CeNPs) contributed to ROS elimination (Shao et al., 2021). TiNTA-CeNPs maintained cycling stability between Ce^{3+} and Ce^{4+} in PBS with H_2O_2, which was possibly due to the preferable phosphate adsorption on surface Ti^{3+} in TiNTA. Moreover, TiNTA-CeNPs accelerated the regeneration of new bone in the oxidative stress-related rat OP model. In addition, as shown in Fig. 3(c and d), Jiang's group further extended the treatment of iron oxide NPs in postmenopausal OP by loading the iron oxide NPs with bone targeting bisphosphonates (Zheng et al., 2022).

2.4. Bone regeneration

Bone regeneration is the essential management approach for massive bone defects following traumatic fracture, tumor resection, infection and skeletal abnormalities (Verrier et al., 2016). Autografts, allografts, and xenografts are the most commonly used bone grafts to accelerate bone regeneration, but their clinical applications are limited by the disadvantages such as donor-site pain, antigenic response, and transmission of zoonotic diseases (Oryan, Alidadi, Moshiri, & Maffulli, 2014). Therefore, bone tissue engineering has been introduced to develop bone substitutes via the synergistic combination of biomaterials, cells, and growth factors (Koons, Diba, & Mikos, 2020).

Nanozymes, as a kind of novel catalytic biomaterial, have been recently used in bone tissue engineering (Golchin et al., 2017). The most representative ceria nanozymes play an essential role due to their remarkable antioxidant ability, signaling pathway regulation, and osteogenic differentiation enhancement (K. Li, Xie, You, Huang, & Zheng, 2016; F. Wei, Qiao, et al., 2021; S. Yang, Ji, Luo, Li, & Gao, 2021; Yu et al., 2022). As shown in Fig. 4(a–e), Coathup's group demonstrated the immunomodulatory and pro-osteogenic capacities of CeO_2 NPs under both acute and chronic inflammatory conditions induced by LPS (F. Wei, Qiao, et al., 2021). CeO_2 NPs not only increased the expression of anti-inflammatory cytokines in macrophages but also enhanced mesenchymal stem cell proliferation, osteogenic differentiation, and mineralization at the same concentration. Furthermore, ceria nanozymes have been incorporated in conventional scaffolds as functional additives to optimize the mechanical, topographical, and biological properties of scaffolds (Karakoti et al., 2010; Mandoli et al., 2010). In addition, other types of nanozymes also show their potential in bone tissue engineering. Taking carbon-based nanozyme as an example, as shown in Fig. 4(f and g), Xu and co-workers constructed fullerol-embedded hydrogel microfluidic spheres (FMSs) with robust ROS eliminating capability at the extracellular and intracellular levels, aiming to protect bone marrow mesenchymal stem cells (BMSCs) from oxidative stress (J. Yang, Liang, et al., 2021). Moreover, FMSs

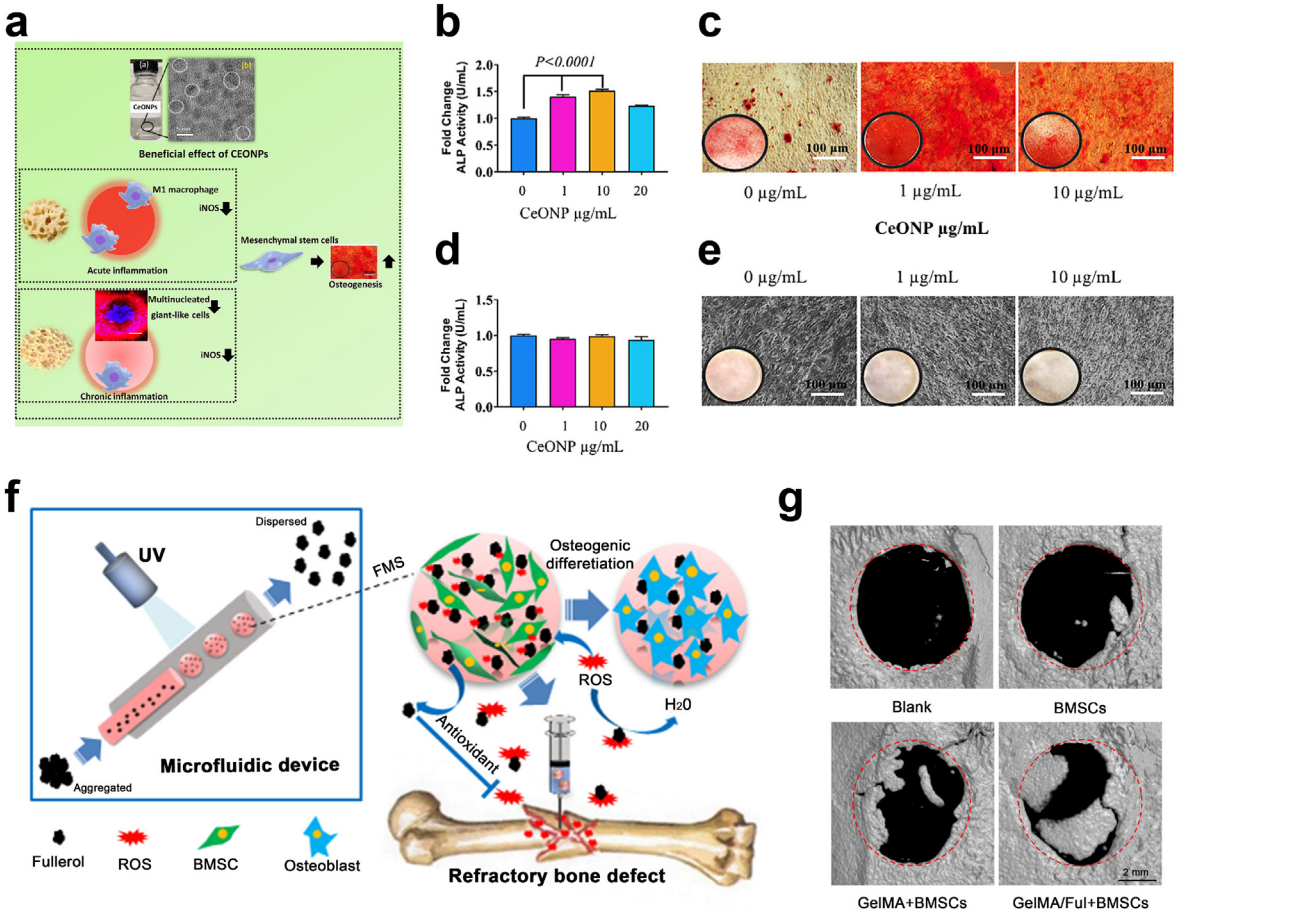

Fig. 4. Applications of nanozymes in bone regeneration. (a) Schematic illustration of the immunomodulatory and pro-osteogenic capacities of CeO_2 NPs, (b) ALP activity in osteogenic media. (c) Alizarin Red-stained mineral deposits red in the osteogenic media groups. (d) ALP activity in non-osteogenic media. (e) Alizarin Red-stained mineral deposits red in the non-osteogenic media groups. (f) Microfluidic synthesis of antioxidative hydrogel microspheres (FMSs) containing highly dispersed fullerol nanocrystals. (g) Micro-CT 3D reconstruction images of new bone in the defect areas (circled by red dot line). (a–e) Adapted from (F. Wei, Qiao, et al., 2021), copyright 2021. (f, g) Adapted from (J. Yang, Liang, et al., 2021), copyright 2021. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exhibited the osteogenic properties and significantly increased the amount of new bone in calvarial-defect rats.

2.5. Gout

Gout is the most prevalent form of inflammatory arthritis, which results from the deposition of excessive monosodium urate (MSU) crystals in joints or periarticular tissues induced by uric acid (UA) disturbance (Dalbeth, Gosling, Gaffo, & Abhishek, 2021). Clinical manifestations of gout include the onset of persistent inflammation and subsequent intense pain (Taylor et al., 2015). The level of UA depends on the balance between dietary intake, endogenous metabolism synthesis, and kidneys/intestine excretion (Mandal & Mount, 2015). Many factors could contribute to hyperuricaemia, such as metabolic syndrome, high purine meals, alcohol intake, and medications (Dehlin, Jacobsson, & Roddy, 2020).

In the acute course of gout, major focus of managements on rapidly suppressing inflammation and joint pain. It is recommended to used one or more potent anti-inflammatory agents (*e.g.*, nonsteroidal anti-inflammatory drugs, colchicine, corticosteroids, and anti-IL-1β biologics). Long-term gout management targets lowering UA continuously by medications (*e.g.*, allopurinol, febuxostat, uricosuric medications, and uricase) (Pillinger & Mandell, 2020). In particular, uricase-based therapies have been regarded as effective methods to treat gout due to the lack of uricase in humans. However, the accumulation of the toxic by-product H_2O_2 in joints during the uricase-mediated UA degradation would cause serious side effects. Therefore, earlier studies combined uricase with CAT or CAT-like nanozymes through complex multi-step designs, to achieve H_2O_2 elimination in time (S. Kim, Kim, Song, et al., 2019; X. Liu et al., 2016; Z. Zhang et al., 2018). Recently, Wei et al. developed a self-cascade Pt/CeO_2 nanozyme with synergistic uricase/CAT-like activities as an efficient therapeutic agent for alleviating acute gout (Fig. 5) (A. Lin et al., 2022). They demonstrated the best UA-degradation capability of Pt in comparison with other platinum-group metals (*i.e.*, Ir, Rh, and Pd). Moreover, the synthesized Pt/CeO_2 structure with small Pt NPs anchored on CeO_2 nanorods facilitated the catalytic stability *in vivo*. In the MSU-induced acute gout rats, Pt/CeO_2 nanozyme markedly alleviated joint pain and improved gait claudication, showing great potential for gout treatment.

2.6. Osteomyelitis (OM)

Osteomyelitis (OM) is an inflammatory state of bone caused by infectious microorganisms, leading to continuous local bone destruction and necrosis (Lew & Waldvogel, 2004). Based on the source of infection, OM can be classified into three categories: (1) hematogenous OM, (2) secondary OM (associated with vascular or neurologic insufficiency), and (3) contiguously infected OM (caused

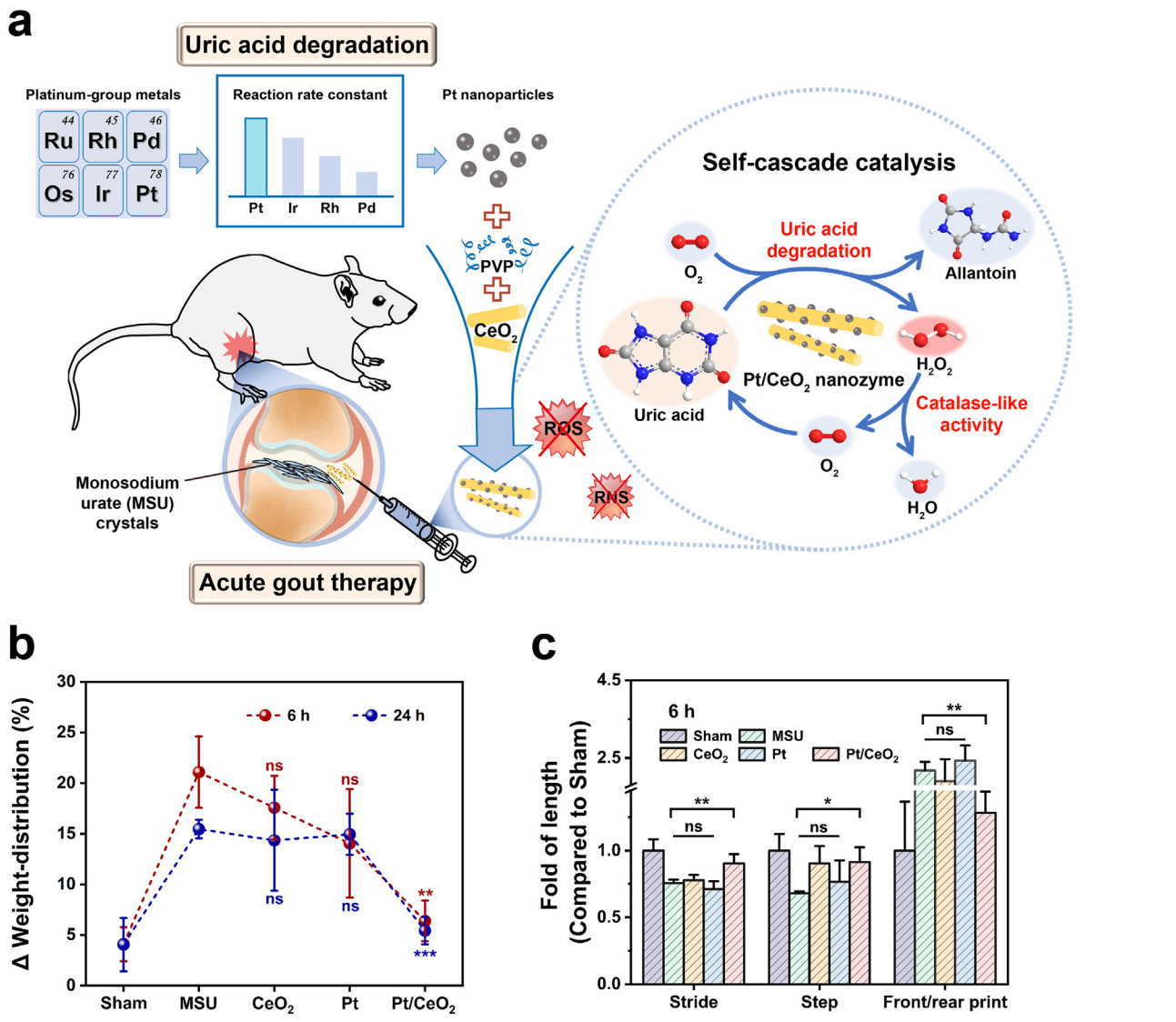

Fig. 5. Nanozymes for gout therapy. (a) Schematic illustration of the design and synthesis of the Pt/CeO_2 nanozyme and self-cascade UA degradation for MSU-induced acute gout therapy. (b) Differences in weight distribution between bilateral hind limbs of rats at 6 and 24 h (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and ns means not significant). (c) Quantification of stride length, step length, and the length of front/rear paw prints at 6 h (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and ns means not significant). (a–c) Adapted from (A. Lin et al., 2022), copyright 2022.

by open fractures and orthopaedic joint replacement) (Birt, Anderson, Toby, & Wang, 2017).

To develop efficient treatments of bacteria-infected OM, some nanozyme-based antibacterial platforms have been constructed with considerable efforts. As shown in Fig. 6(a–c), Wu and co-workers proposed a rapid therapeutic strategy by utilizing microwave and Na^+ insertion to accelerate the release of Fe(II)/Fe(III) ions from PB, which further activated the Fenton reaction to produce toxic $^{\bullet}OH$ for killing bacteria (S. Wei, Qiao, et al., 2021). Nie's group developed an integrated accurate diagnosis platform (denoted as BMUIG) of bacteria-involved OM by encapsulating indocyanine green into MnO_2 conjugated with cationic human antimicrobial peptide (Lu et al., 2019). The synergistic antibacterial capabilities of BMUIG combined with photodynamic therapy and gentamicin could also be proved in the acute OM mouse model. With an increasing number of implant surgeries, OM is often related to the infected prostheses after total hip arthroplasties and total knee arthroplasties (Song et al., 2013). The crucial managements of implant-associated OM often focus on the undermining of biofilm adhered to the surfaces of prostheses and bone (Zimmerli & Sendi, 2017). As shown in Fig. 6(d–f), Zhang et al. designed $CuFe_5O_8$ nanocubes (NCs) with controlled Fenton-like activity regulated by pH and H_2O_2 concentration in the biofilm microenvironment (G. Guo et al., 2020). It demonstrated that $CuFe_5O_8$ NCs showed superior effects on the elimination of biofilms in an implant-related infection model by cleaving extracellular DNA and reversing immunosuppressive microenvironment.

2.7. Osteosarcoma (OS)

Osteosarcoma (OS) is a kind of bone neoplasm derived from bone-forming mesenchymal cells (Heymann, Lezot, & Heymann, 2019). As one of the malignant bone tumors, OS causes horrendous local invasion and systemic metastasis (preferentially to the lung parenchyma), leading to pain, fracture, and even death (C. Chen, Xie, et al., 2021). Currently, chemotherapy, wide surgical resection, and radiotherapy are the most commonly used in OS therapies (Thanindratarn, Dean, Nelson, Hornicek, & Duan, 2019).

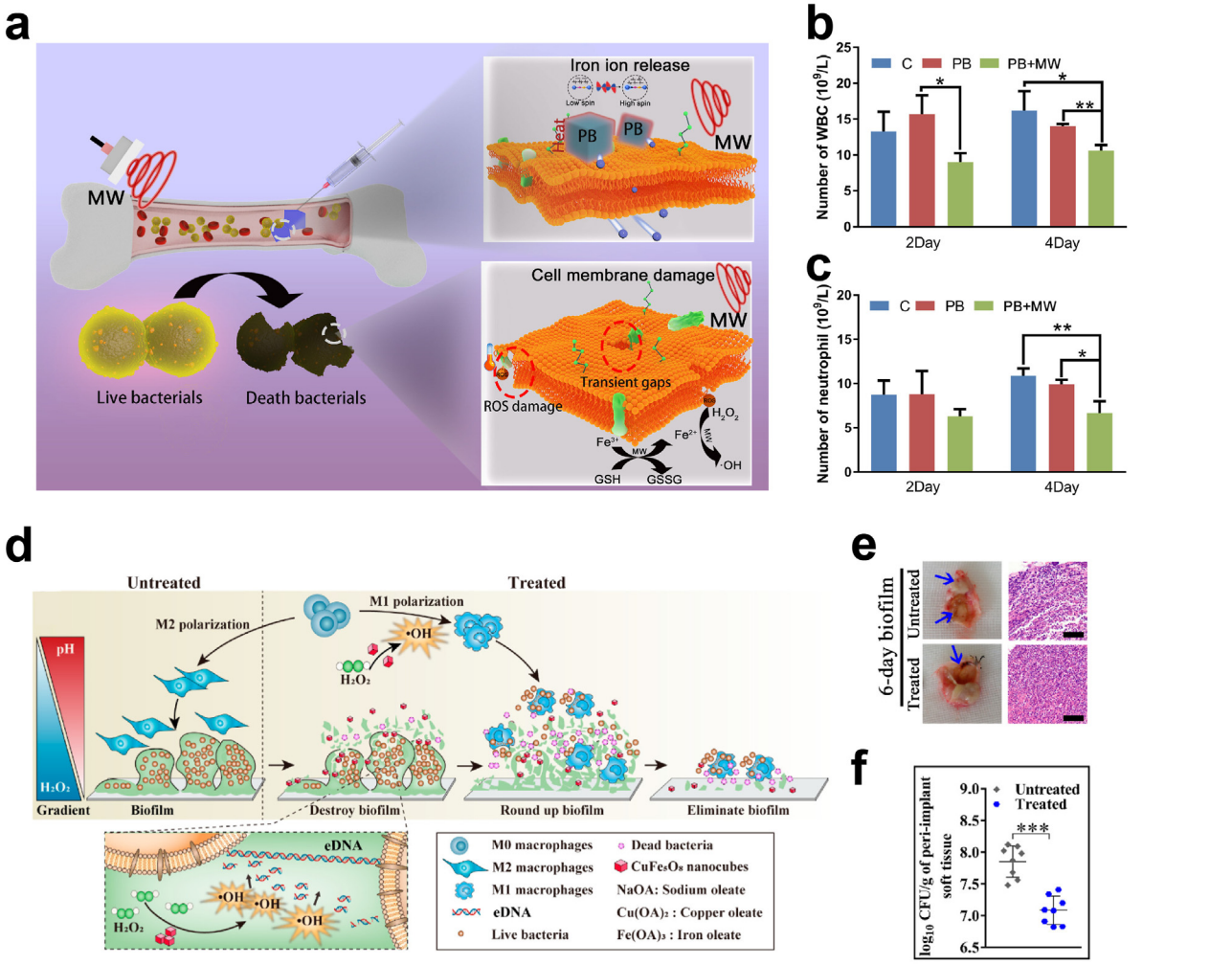

Fig. 6. Nanozymes for OM therapy. (a) Schematic diagram of PB with microwave response for osteomyelitis treatment. (b, c) Blood routine examination of the white blood cells (b) and neutrophils (c) (n = 3, *p < 0.05, **p < 0.01, ***p < 0.001). (d) Schematic illustration of proposed antibiofilm and immunomodulatory mechanisms of $CuFe_5O_8$ NCs. (e, f) Pathologic results (general observation and histologic section) (e) and bacterial burden of peri-implant soft tissues (f) at 7 days after surgery. Blue arrows indicated the white suppuration and necrotic tissues in the gross specimens of peri-implant soft tissues (**p < 0.01, ***p < 0.001). (a–c) Adapted from (S. Wei, Qiao, et al., 2021), copyright 2021. (d–f) Adapted from (G. Guo et al., 2020), copyright 2020. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

However, chemotherapy and radiotherapy may increase the risk of systemic side effects as well as the drug resistance (Eaton et al., 2021). Moreover, invasion and residual tumor tissues are inevitable as well. The massive bone defects after surgical resection also give rise to the poor prognosis.

Considering the current clinical difficulties, it is essential for new management strategies of OS to simultaneously achieve tumor killing and osteogenesis promoting (Liao, Han, Wu, & Qian, 2021). Previous studies have verified the anti-OS ability of nanozymes in physiological environments (Alpaslan, Yazici, Golshan, Ziemer, & Webster, 2015; Mehmood, Wang, Koshy, Yang, & Sorrell, 2018). Recently, more studies have focused on combining nanozymes with other therapeutic agents. As shown in Fig. 7(a–c), Wang and co-workers constructed a multifunctional scaffold AKT-Fe_3O_4–CaO_2 by loading CaO_2 and Fe_3O_4 nanozymes into a 3D printing akermanite scaffold (S. Dong, Chen, Yu, Lin, & Wang, 2019). The synergistic anti-tumor effect of AKT-Fe_3O_4–CaO_2 was attributed to the cooperation between hyperthermia and the H_2O_2-self-supplying Fenton reaction. In addition, the integrated CaO_2 released Ca^{2+} to enhance the repair of bone defects. In another work, as shown in Fig. 7(d–f), Xu's group reported the single-atom iron nanozyme FeSAC-integrated 3D-printed bioactive glass scaffold (FeSAC-BG), which attained a therapeutic effect against OS and bactericidal properties simultaneously due to hyperthermia-enhanced toxic •OH generation (L. Wang, Wu, et al., 2021). Chen and co-workers developed a metal-drug nanozyme HA@FeZOL by combining ferric ions with the anti-osteoclastogenesis drug zoledronate (ZOL) (Geng et al., 2021). HA@FeZOL could increase the ZOL level in tumor tissues and simultaneously generated •OH to enhance OS radiotherapy.

3. Challenges and outlooks

We have highlighted representative studies of nanozymes in orthopaedics over the past decade. All orthopaedic applications mentioned above are summarized in Table S1 (see the Supplementary Material), with their corresponding nanozymes used for the therapy. Meanwhile, there is great potential to extend their biomedical applications into other bone diseases. Also, the field of nanozymes is confronted with exciting challenges in the future.

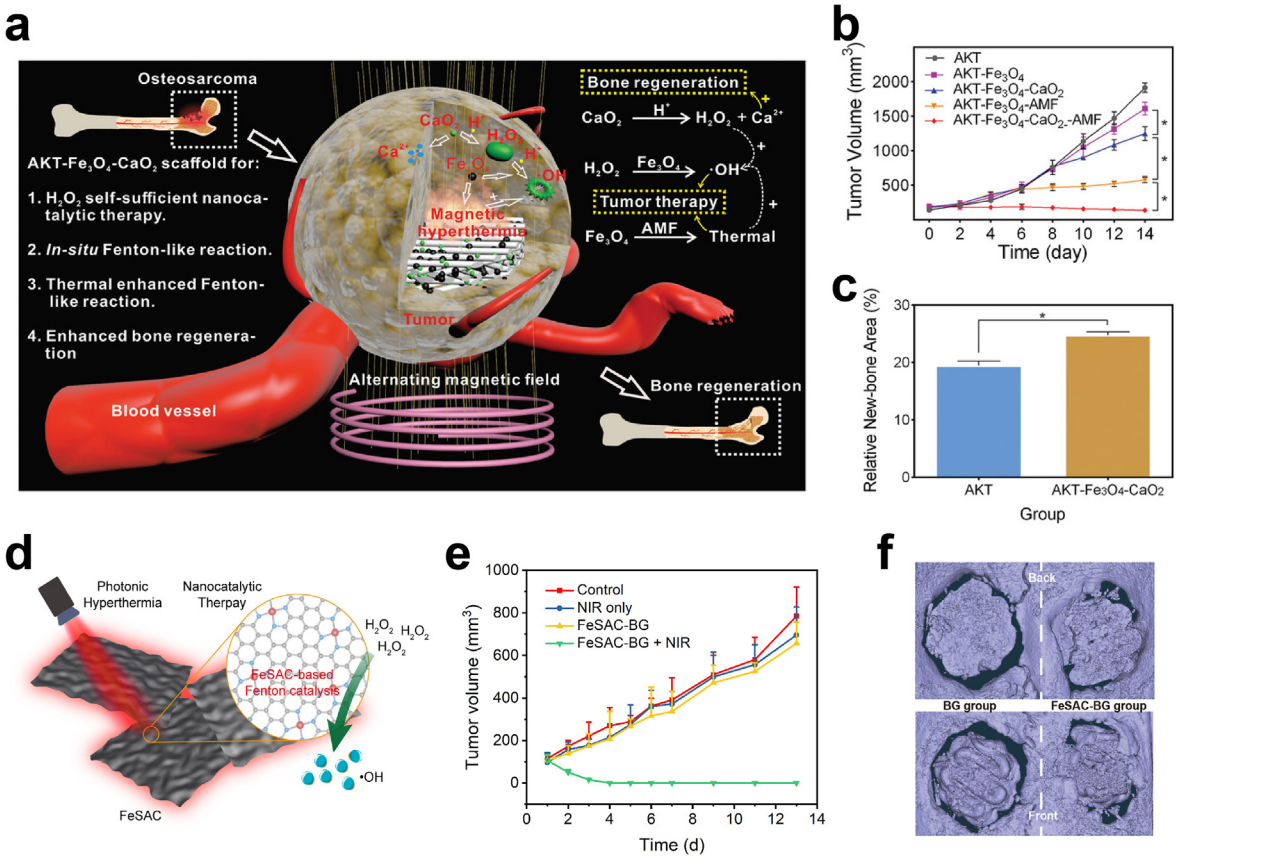

Fig. 7. Nanozymes for OS therapy. (a) Schematic illustration of the cancer-therapeutic performance and bone-regeneration bioactivity of 3D-printed scaffolds coloaded with Fe_3O_4 and CaO_2 NPs (AKT-Fe_3O_4–CaO_2). (b) Time-dependent tumor-volume curves OS-bearing mice treated as described in different groups (n = 5, *p < 0.05). (c) Quantitative analysis of the VG-stained area of newborn bone tissues with ImageJ software (n = 3, *p < 0.05). (d) Schematic illustration of FeSAC for hyperthermia-enhanced Fenton reaction. (e) Tumor volume of mice in each group. (f) Corresponding enlarged images of cranial defect area with BG and FeSAC-BG scaffold implantation. (a–c) Adapted from (S. Dong et al., 2019), copyright 2019. (d–f) Adapted from (L. Wang, Wu, et al., 2021), copyright 2021.

3.1. Other potential applications

Generally, phosphate anion (PO_4^{3-}) is crucial to osteogenesis in the process of bone remodeling. It could be supplied with alkaline phosphatase (ALP)-mediated hydrolysis toward phosphate molecules (Chande & Bergwitz, 2018). Thus, ALP enzymatic activity is directly related to initial mineralization (N. Li, Qi, et al., 2019). In 2010, Kuchma et al. found the hydrolytic activity of CeO_2 NPs with cleaving phosphate ester bonds of *p*-nitrophenylphosphate (*p*NPP) and other biologically relevant molecules (Kuchma et al., 2010). As shown in Fig. 8(a and b), Wang's group established the structure–property relationships of CeO_2 nanocrystals with different shapes, indicating that oxygen vacancies on the CeO_2 surfaces are active sites for the dephosphorylation of *p*NPP (Manto, Xie, & Wang, 2017). Moreover, Wei and co-workers optimized the ALP-like activity of CeO_2 via a simple temperature-regulated synthesis (Fig. 8(c)) (X. Liu et al., 2021). In addition, as shown in Fig. 8(d and e), Wei's group developed a highly active Ce-FMA nanozyme using a data-informed strategy, achieving pH-dependent phosphatase-like activity toward different substrates. These results indicate that phosphatase-like nanozymes have essential implications for their use in potential osteogenic therapies.

As mentioned in last section, MSU crystals deposited in joints and periarticular tissues lead to the occurrence of gout with intense pain and an inflammatory response. Thus, efficient degradation of UA could be an effective strategy for the treatment of gout. In 2011, Chi et al. first demonstrated that Pt NPs, as uricase mimics, had catalytic degradation activity toward UA with high efficiency (Fig. 9(a)) (Y. Dong et al., 2011). Afterwards, as shown in Fig. 9(b), Wei's group found similar catalytic abilities of other platinum-group metals (such as Ir, Rh, and Pd NPs), with a trend of Pt > Ir > Rh > Pd (A. Lin et al., 2022). Moreover, Gao and co-workers developed an artificial *pero*-nanozysome with atomic Fe clusters as reversible cofactors and Fe–N_4 coordination as a prosthetic group (Fig. 9(c)). As shown in Fig. 9(d), this Fe–N-doped carbon nanozyme also exhibited good uricase-like activity (Xi et al., 2020). These nanozymes show great potential in protecting joint tissues from gout.

In addition, nanozymes exhibit their large potential in the applications of aseptic or septic loosening involved in the joint arthroplasty. Wear particle-induced aseptic loosening is attributed to chronic inflammation caused by the interaction between resident immune cells and implant wear debris (Luo et al., 2016). However, septic loosening generally involves biofilm-associated chronic infection at the implant sites (Hodges, Sussman, & Stegemann, 2021). For both aseptic and septic loosening, ROS plays a crucial role in the therapeutic process. Nanozymes with highly active ROS scavenging activity could be applied as antioxidative catalysts in the treatment of aseptic loosening. On the other hand, some nanozymes would generate highly toxic ROS to kill bacteria and disrupt biofilms at the implant sites, exhibiting excellent POD- or OXD-like activities. These nanozyme-based strategies provide novel insights into the treatment of orthopaedic diseases.

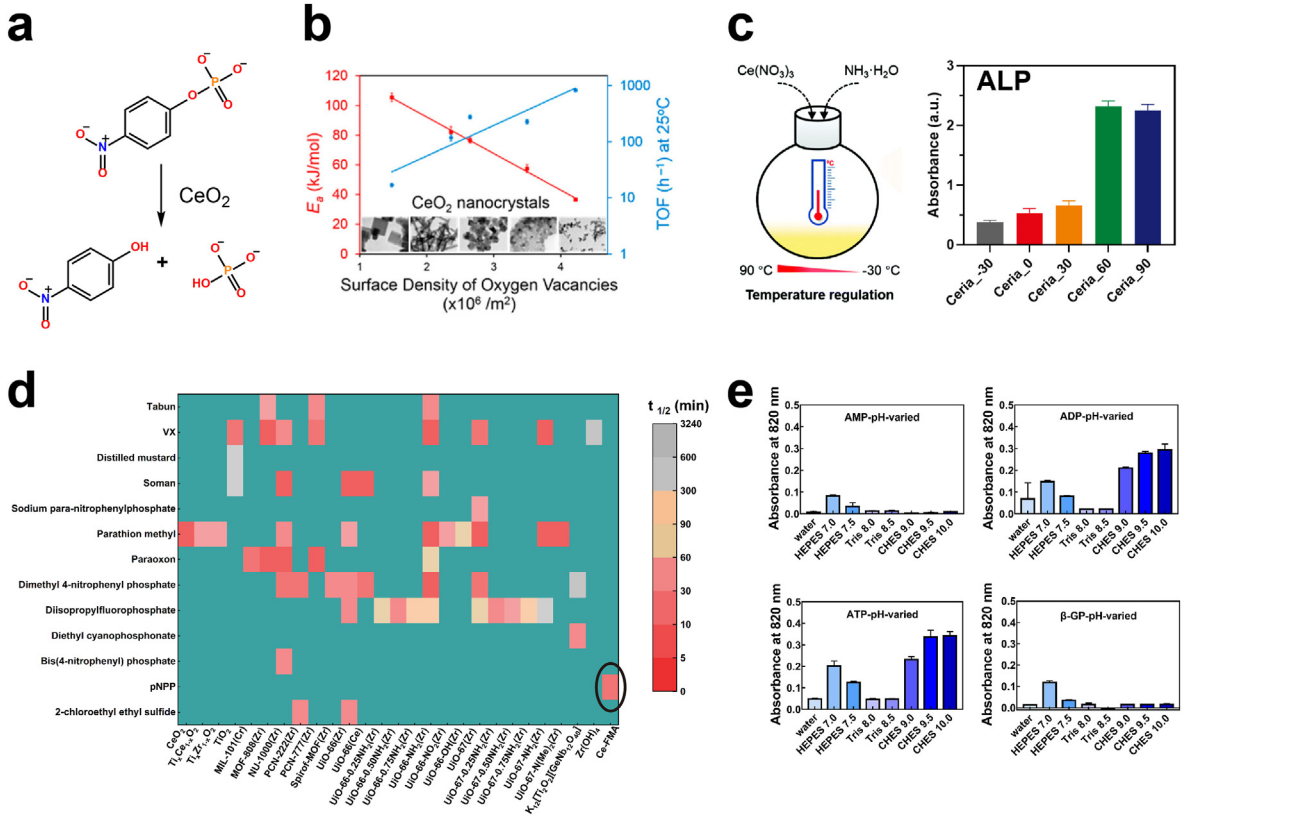

Fig. 8. Phosphatase-like activity of nanozymes. (a) Illustration of the dephosphorylation of *p*NPP catalyzed by CeO_2. (b) Correlations of activation energy and turnover frequency (TOF) to the surface density of oxygen vacancies estimated based on the amount of absorbed O_2 derived from temperature-programmed desorption of oxygen analysis. (c) Synthesis of CeO_2 nanozymes via temperature regulation to optimize ALP-like activity. (d) Heat map of the half-lives of various phosphatase-like nanozymes and their corresponding substrates. Each half-life is presented as the mean unless there is only one sample. The circled one represents the data-informed Ce-FMA nanozyme. (e) pH-dependent phosphatase-like activity of Ce-FMA toward different substrates. (a, b) Adapted from (Manto et al., 2017), copyright 2017. (c) Adapted from (X. Liu et al., 2021), copyright 2021. (d, e) Adapted from (S. Li et al., 2022), copyright 2022.

3.2. Optimization of structure–function and catalytic mechanisms

Though the activities of nanozymes could be well-regulated by different factors (such as size- or shape-control), a precise relationship between the structure and catalytic function is still required to achieve the rational design of nanozymes (X. Liu et al., 2021; Naganuma, 2016; X. Wang, Wan, & Shi, 2019). For this purpose, multidisciplinary collaboration between experimental science and computational research is eagerly desirable to explore the detailed catalytic mechanisms of nanozymes. Gao's group has been devoted to explaining the underlying mechanisms of the diverse enzymatic activities of nanozymes via density functional theory investigation (J. Li, Liu, Wu, & Gao, 2015; Shen et al., 2015). And the theoretical principles established in their work could reliably predict the SOD-like activities of MOFs by high-throughput computational screening (Z. Wang, Wu, et al., 2021). This encourages us to transform the nanozyme area from an empirical science to a theory-experiment combined one.

3.3. Multiple modulatory approaches

In bone physiology, bone resorption and formation involve interconnected and complicated processes mediated by diverse cell types and signaling pathways (Redlich & Smolen, 2012). Although various nanozymes can modulate ROS (or RNS) levels in response to different pathological conditions, the change in ROS (or RNS) levels only plays as a small part in the whole inflammatory regulation. What matters is that diverse cytokines affected by redox homeostasis activate different cellular signaling pathways and augment substantial effects in bone tissues step by step. Taking CeO_2 and Au NPs as examples, Dong and co-workers found that CeO_2 NPs enhanced endochondral ossification-based bone regeneration by promoting the hypertrophic differentiation of BMSCs through DHX15 activation in the p38 MAPK pathway (J. Li, Qi, et al., 2019). Jiang et al. demonstrated that PEGylated Au NPs could increase bone mineralization by the Wnt/β-catenin signaling pathway (Y. Zhang, Yan, & Fan, 2021). Thus, more biological mechanism studies will also be needed for the future translation of nanozymes. The effects of nanozymes on various modulatory approaches are not clear enough to illustrate the detailed therapeutic mechanism of nanozymes, which may bring underlying concerns of applications.

3.4. Biocompatibility and biosafety

An increasing number of studies on nanozymes have reported their significant findings in biomedical applications, yet there are several essential neglected parts regarding clinical biomedicine. It is crucial to maintain the balance between therapeutic efficacy and potential biosafety. Due to the different physicochemical properties of nanozymes (such as size, surface charge, and hydrophilicity), the highest priority is to systematically evaluate toxicity, hemolysis, and stability in blood (or body fluid) circulation. When nanozymes are employed as nanomedicine *in vivo*, it is required to ascertain several momentous factors in pharmacodynamics (PD) studies, including the therapeutic window, minimal effective dose (MED),

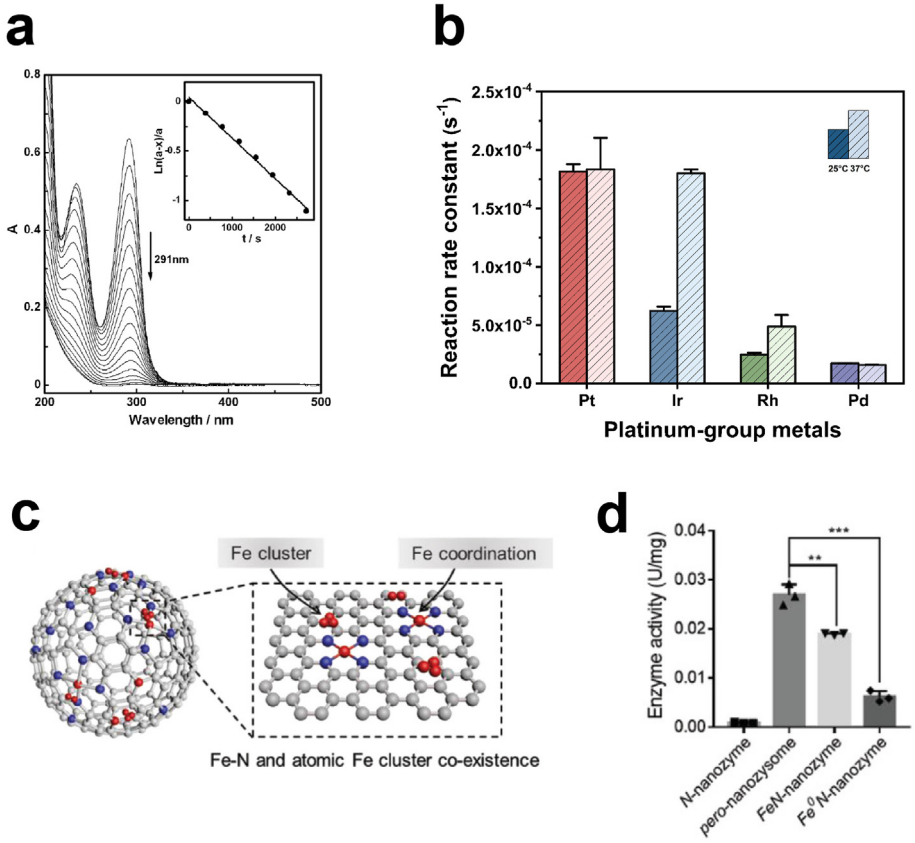

Fig. 9. Uricase-like activity of nanozymes. (a) UV–vis spectrum recorded every 6.5 min for the PtNPs catalytic degradation of UA. Inset: Relationship between degradation percentage and time for the PtNPs catalytic degradation of UA. (b) Comparison of UA degradation activities for four platinum-group metals at 25 and 37 °C. (c) Model for the surface of *pero*-nanozysome, Fe (red), N (blue), and C (gray). (d) Comparison of uricase-like activities between *pero*-nanozyme, FeN-nanozyme, N-nanozyme and Fe^{0}N-nanozyme. (a) Adapted from (Y. Dong et al., 2011), copyright 2011. (b) Adapted from (A. Lin et al., 2022), copyright 2022. (c, d) Adapted from (Xi et al., 2020), copyright 2020. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and maximal tolerated dose (MTD) (Ghorbani et al., 2021). Moreover, post-treatment evaluations should be strictly studied as well, which involve detailed pharmacokinetics (PK), absorption, metabolism, biodistribution, and reversible (or irreversible even delayed) toxicity of nanozymes (H. Wang, Wan, & Shi, 2019). Especially, PK and PD studies are the key factors for the preclinical translation of novel drugs. Thus, systematic parameters of nanozymes such as clearance, half-time, MED, and MTD should be evaluated in preclinical studies in detail.

Data availability

The data are available upon reasonable request.

Declaration of competing interest

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

Acknowledgments

This work was supported by Jiangsu Provincial Key R&D Program (grant No. BE2022836), National Key R&D Program of China (grant Nos. 2021YFF1200700 and 2019YFA0709200), National Natural Science Foundation of China (grant No. 21874067), PAPD Program, the Innovation Foundation of Nanjing University, and Fundamental Research Funds for the Central Universities (grant No. 021314380195).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.partic.2022.08.009.

References

Alpaslan, E., Yazici, H., Golshan, N. H., Ziemer, K. S., & Webster, T. J. (2015). pH-dependent activity of dextran-coated cerium oxide nanoparticles on prohibiting osteosarcoma cell proliferation. *ACS Biomaterials Science & Engineering, 1*, 1096–1103.

Bao, X., Zhao, J., Sun, J., Hu, M., & Yang, X. (2018). Polydopamine nanoparticles as efficient scavengers for reactive oxygen species in periodontal disease. *ACS Nano, 12*, 8882–8892.

Baron, R., & Hesse, E. (2012). Update on bone anabolics in osteoporosis treatment: Rationale, current status, and perspectives. *Journal of Clinical Endocrinology and Metabolism, 97*, 311–325.

Birt, M. C., Anderson, D. W., Toby, E. B., & Wang, J. (2017). Osteomyelitis: Recent advances in pathophysiology and therapeutic strategies. *Journal of Orthopaedics, 14*, 45–52.

Burmester, G. R., & Pope, J. E. (2017). Novel treatment strategies in rheumatoid arthritis. *Lancet, 389*, 2338–2348.

Cao, F., Zhang, L., You, Y., Zheng, L., Ren, J., & Qu, X. (2020). An enzyme-mimicking single-atom catalyst as an efficient multiple reactive oxygen and nitrogen species scavenger for sepsis management. *Angewandte Chemie International Edition, 59*, 5108–5115.

Chande, S., & Bergwitz, C. (2018). Role of phosphate sensing in bone and mineral metabolism. *Nature Reviews Endocrinology, 14*, 637–655.

Chen, C., Xie, L., Ren, T., Huang, Y., Xu, J., & Guo, W. (2021). Immunotherapy for osteosarcoma: Fundamental mechanism, rationale, and recent breakthroughs. *Cancer Letters, 500*, 1–10.
Chen, X., Xing, H., Zhou, Z., Hao, Y., Zhang, X., Qi, F., et al. (2021). Nanozymes go oral: Nanocatalytic medicine facilitates dental health. *Journal of Materials Chemistry B, 9*, 1491–1502.
Chen, Z., Yuen, J., Crawford, R., Chang, J., Wu, C., & Xiao, Y. (2015). The effect of osteoimmunomodulation on the osteogenic effects of cobalt incorporated beta-tricalcium phosphate. *Biomaterials, 61*, 126–138.
Choi, S. W., Cha, B. G., & Kim, J. (2020). Therapeutic contact lens for scavenging excessive reactive oxygen species on the ocular surface. *ACS Nano, 14*, 2483–2496.
Compston, J. E., McClung, M. R., & Leslie, W. D. (2019). Osteoporosis. *Lancet, 393*, 364–376.
Dalbeth, N., Gosling, A. L., Gaffo, A., & Abhishek, A. (2021). *Gout. Lancet, 397*, 1843–1855.
Dehlin, M., Jacobsson, L., & Roddy, E. (2020). Global epidemiology of gout: Prevalence, incidence, treatment patterns and risk factors. *Nature Reviews Rheumatology, 16*, 380–390.
Dong, S., Chen, Y., Yu, L., Lin, K., & Wang, X. (2019). Magnetic hyperthermia–synergistic H_2O_2 self-sufficient catalytic suppression of osteosarcoma with enhanced bone-regeneration bioactivity by 3D-printing composite scaffolds. *Advanced Functional Materials, 30*, Article 1907071.
Dong, Y., Chi, Y., Lin, X., Zheng, L., Chen, L., & Chen, G. (2011). Nano-sized platinum as a mimic of uricase catalyzing the oxidative degradation of uric acid. *Physical Chemistry Chemical Physics, 13*, 6319–6324.
Eaton, B. R., Schwarz, R., Vatner, R., Yeh, B., Claude, L., Indelicato, D. J., et al. (2021). Osteosarcoma. *Pediatric Blood and Cancer, 68*, Article e28352.
Fan, K., Xi, J., Fan, L., Wang, P., Zhu, C., Tang, Y., et al. (2018). *In vivo* guiding nitrogen-doped carbon nanozyme for tumor catalytic therapy. *Nature Communications, 9*, 1440.
Gao, L., Zhuang, J., Nie, L., Zhang, J., Zhang, Y., Gu, N., et al. (2007). Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nature Nanotechnology, 2*, 577–583.
Geng, H., Zhou, M., Li, B., Liu, L., Yang, X., Wen, Y., et al. (2021). Metal-drug nanoparticles-mediated osteolytic microenvironment regulation for enhanced radiotherapy of orthotopic osteosarcoma. *Chemical Engineering Journal, 417*, 128103.
George, G., Shyni, G. L., & Raghu, K. G. (2020). Current and novel therapeutic targets in the treatment of rheumatoid arthritis. *Inflammopharmacology, 28*, 1457–1476.
Ghorbani, M., Izadi, Z., Jafari, S., Casals, E., Rezaei, F., Aliabadi, A., et al. (2021). Preclinical studies conducted on nanozyme antioxidants: Shortcomings and challenges based on US FDA regulations. *Nanomedicine, 16*, 1133–1151.
Golchin, J., Golchin, K., Alidadian, N., Ghaderi, S., Eslamkhah, S., Eslamkhah, M., et al. (2017). Nanozyme applications in biology and medicine: An overview. *Artificial Cells, Nanomedicine, and Biotechnology, 45*, 1–8.
Guo, G., Zhang, H., Shen, H., Zhu, C., He, R., Tang, J., et al. (2020). Space-selective chemodynamic therapy of $CuFe_5O_8$ nanocubes for implant-related infections. *ACS Nano, 14*, 13391–13405.
Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N. J., & Xu, J. (2018). Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies. *Bone Research, 6*, 15.
Han, J., Kim, Y. S., Lim, M.-Y., Kim, H. Y., Kong, S., Kang, M., et al. (2018). Dual roles of graphene oxide to attenuate inflammation and elicit timely polarization of macrophage phenotypes for cardiac repair. *ACS Nano, 12*, 1959–1977.
Hao, C., Qu, A., Xu, L., Sun, M., Zhang, H., Xu, C., et al. (2019). Chiral molecule-mediated porous Cu_xO nanoparticle clusters with antioxidation activity for ameliorating Parkinson's disease. *Journal of the American Chemical Society, 141*, 1091–1099.
He, L., Ni, Q., Mu, J., Fan, W., Liu, L., Wang, Z., et al. (2020). Solvent-assisted self-assembly of a metal-organic framework based biocatalyst for cascade reaction driven photodynamic therapy. *Journal of the American Chemical Society, 142*, 6822–6832.
Heymann, M. F., Lezot, F., & Heymann, D. (2019). The contribution of immune infiltrates and the local microenvironment in the pathogenesis of osteosarcoma. *Cellular Immunology, 343*, 103711.
Hodges, N. A., Sussman, E. M., & Stegemann, J. P. (2021). Aseptic and septic prosthetic joint loosening: Impact of biomaterial wear on immune cell function, inflammation, and infection. *Biomaterials, 278*, 121127.
Hou, W., Ye, C., Chen, M., Gao, W., Xie, X., Wu, J., et al. (2021). Excavating bioactivities of nanozyme to remodel microenvironment for protecting chondrocytes and delaying osteoarthritis. *Bioactive Materials, 6*, 2439–2451.
Huang, Y., Ren, J., & Qu, X. (2019). Nanozymes: Classification, catalytic mechanisms, activity regulation, and applications. *Chemical Reviews, 119*, 4357–4412.
Hunter, D. J., & Bierma-Zeinstra, S. (2019). Osteoarthritis. *Lancet, 393*, 1745–1759.
Jiang, D., Ni, D., Rosenkrans, Z. T., Huang, P., Yan, X., & Cai, W. (2019). Nanozyme: New horizons for responsive biomedical applications. *Chemical Society Reviews, 48*, 3683–3704.
Ji, M.-X., & Yu, Q. (2015). Primary osteoporosis in postmenopausal women. *Chronic diseases and translational medicine, 1*, 9–13.
Kalashnikova, I., Chung, S. J., Nafiujjaman, M., Hill, M. L., Siziba, M. E., Contag, C. H., et al. (2020). Ceria-based nanotheranostic agent for rheumatoid arthritis. *Theranostics, 10*, 11863–11880.
Kanis, J. A., Cooper, C., Rizzoli, R., & Reginster, J. Y. (2019). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis International, 30*, 3–44.
Karakoti, A. S., Tsigkou, O., Yue, S., Lee, P. D., Stevens, M. M., Jones, J. R., et al. (2010). Rare earth oxides as nanoadditives in 3-D nanocomposite scaffolds for bone regeneration. *Journal of Materials Chemistry, 20*, 8912–8919.
Kenkre, J. S., & Bassett, J. (2018). The bone remodelling cycle. *Annals of Clinical Biochemistry, 55*, 308–327.
Kim, S., Kim, M., Jung, S., Kwon, K., Park, J., Kim, S., et al. (2019). Co-delivery of therapeutic protein and catalase-mimic nanoparticle using a biocompatible nanocarrier for enhanced therapeutic effect. *Journal of Controlled Release, 309*, 181–189.
Kim, J., Kim, H. Y., Song, S. Y., Go, S., Sohn, H. S., Baik, S., et al. (2019). Synergistic oxygen generation and reactive oxygen species scavenging by manganese ferrite/ceria co-decorated nanoparticles for rheumatoid arthritis treatment. *ACS Nano, 13*, 3206–3217.
Koons, G. L., Diba, M., & Mikos, A. G. (2020). Materials design for bone-tissue engineering. *Nature Reviews Materials, 5*, 584–603.
Kuchma, M. H., Komanski, C. B., Colon, J., Teblum, A., Masunov, A. E., Alvarado, B., et al. (2010). Phosphate ester hydrolysis of biologically relevant molecules by cerium oxide nanoparticles. *Nanomedicine, 6*, 738–744.
Kumar, S., Adjei, I. M., Brown, S. B., Liseth, O., & Sharma, B. (2019). Manganese dioxide nanoparticles protect cartilage from inflammation-induced oxidative stress. *Biomaterials, 224*, 119467.
Kwon, H. J., Kim, D., Seo, K., Kim, Y. G., Han, S. I., Kang, T., et al. (2018). Ceria nanoparticle systems for selective scavenging of mitochondrial, intracellular, and extracellular reactive oxygen species in Parkinson's disease. *Angewandte Chemie International Edition, 57*, 9408–9412.
Lepetsos, P., & Papavassiliou, A. G. (2016). ROS/oxidative stress signaling in osteoarthritis. *Biochimica et Biophysica Acta, 1862*, 576–591.
Lew, D. P., & Waldvogel, F. A. (2004). Osteomyelitis. *Lancet, 364*, 369–379.
Li, J., Han, F., Ma, J., Wang, H., Pan, J., Yang, G., et al. (2021a). Targeting endogenous hydrogen peroxide at bone defects promotes bone repair. *Advanced Functional Materials, 32*, 2111208.
Li, J., Kang, F., Gong, X., Bai, Y., Dai, J., Zhao, C., et al. (2019). Ceria nanoparticles enhance endochondral ossification–based critical-sized bone defect regeneration by promoting the hypertrophic differentiation of BMSCs via DHX15 activation. *The FASEB Journal, 33*, 6378–6389.
Li, J., Liu, W., Wu, X., & Gao, X. (2015). Mechanism of pH-switchable peroxidase and catalase-like activities of gold, silver, platinum and palladium. *Biomaterials, 48*, 37–44.
Lin, Y., Ren, J., & Qu, X. (2014). Nano-gold as artificial enzymes: Hidden talents. *Advanced Materials, 26*, 4200–4217.
Liao, J., Han, R., Wu, Y., & Qian, Z. (2021). Review of a new bone tumor therapy strategy based on bifunctional biomaterials. *Bone Research, 9*, 18.
Lin, A., Sun, Z., Xu, X., Zhao, S., Li, J., Sun, H., et al. (2022). Self-cascade uricase/catalase mimics alleviate acute gout. *Nano Letters, 22*, 508–516.
Li, X., Qi, M., Sun, X., Weir, M. D., Tay, F. R., Oates, T. W., et al. (2019). Surface treatments on titanium implants via nanostructured ceria for antibacterial and anti-inflammatory capabilities. *Acta Biomaterialia, 94*, 627–643.
Li, W., Song, Y., Liang, X., Zhou, Y., Xu, M., Lu, Q., et al. (2021b). Mutual-reinforcing sonodynamic therapy against rheumatoid arthritis based on sparfloxacin sonosensitizer doped concave-cubic rhodium nanozyme. *Biomaterials, 276*, 121063.
Liu, Y., Ai, K., Ji, X., Askhatova, D., Du, R., Lu, L., et al. (2017). Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury in ischemic stroke. *Journal of the American Chemical Society, 139*, 856–862.
Liu, X., Wu, J., Liu, Q., Lin, A., Li, S., Zhang, Y., et al. (2021). Synthesis-temperature-regulated multi-enzyme-mimicking activities of ceria nanozymes. *Journal of Materials Chemistry B, 9*, 7238–7245.
Liu, X., Zhang, Z., Zhang, Y., Guan, Y., Liu, Z., Ren, J., et al. (2016). Artificial metalloenzyme-based enzyme replacement therapy for the treatment of hyperuricemia. *Advanced Functional Materials, 26*, 7921–7928.
Liu, Y., Cheng, Y., Zhang, H., Zhou, M., Yu, Y., Lin, S., et al. (2020). Integrated cascade nanozyme catalyzes *in vivo* ROS scavenging for anti-inflammatory therapy. *Science Advances, 6*. eabb2695.
Liu, Y.-C., Zou, X.-B., Chai, Y.-F., & Yao, Y.-M. (2014). Macrophage polarization in inflammatory diseases. *International Journal of Biological Sciences, 10*, 520–529.
Li, H., Xiao, Z., Quarles, L. D., & Li, W. (2021). Osteoporosis: Mechanism, molecular target and current status on drug development. *Current Medicinal Chemistry, 28*, 1489–1507.
Li, K., Xie, Y., You, M., Huang, L., & Zheng, X. (2016). Plasma sprayed cerium oxide coating inhibits H_2O_2-induced oxidative stress and supports cell viability. *Journal of Materials Science: Materials in Medicine, 27*, 100.
Li, S., Zhou, Z., Tie, Z., Wang, B., Ye, M., Du, L., et al. (2022). Data-informed discovery of hydrolytic nanozymes. *Nature Communications, 13*, 827.
Li, N., Zhou, L., Xie, W., Zeng, D., Cai, D., Wang, H., et al. (2019). Alkaline phosphatase enzyme-induced biomineralization of chitosan scaffolds with enhanced osteogenesis for bone tissue engineering. *Chemical Engineering Journal, 371*, 618–630.
Lu, X., Chen, R., Lv, J., Xu, W., Chen, H., Ma, Z., et al. (2019). High-resolution bimodal imaging and potent antibiotic/photodynamic synergistic therapy for osteomyelitis with a bacterial inflammation-specific versatile agent. *Acta Biomaterialia, 99*, 363–372.

Luo, G., Li, Z., Wang, Y., Wang, H., Zhang, Z., Chen, W., et al. (2016). Resveratrol protects against titanium particle-induced aseptic loosening through reduction of oxidative stress and inactivation of NF-κB. *Inflammation, 39*, 775–785.
Ma, M., Liu, Z., Gao, N., Pi, Z., Du, X., Ren, J., et al. (2020). Self-protecting biomimetic nanozyme for selective and synergistic clearance of peripheral amyloid-beta in an Alzheimer's disease model. *Journal of the American Chemical Society, 142*, 21702–21711.
Mandal, A. K., & Mount, D. B. (2015). The molecular physiology of uric acid homeostasis. *Annual Review of Physiology, 77*, 323–345.
Mandoli, C., Pagliari, F., Pagliari, S., Forte, G., Nardo, P. D., Licoccia, S., et al. (2010). Stem cell aligned growth induced by CeO_2 nanoparticles in PLGA scaffolds with improved bioactivity for regenerative medicine. *Advanced Functional Materials, 20*, 1617–1624.
Manto, M. J., Xie, P., & Wang, C. (2017). Catalytic dephosphorylation using ceria nanocrystals. *ACS Catalysis, 7*, 1931–1938.
Mehmood, R., Wang, X., Koshy, P., Yang, J., & Sorrell, C. C. (2018). Engineering oxygen vacancies through construction of morphology maps for bio-responsive nanoceria for osteosarcoma therapy. *CrystEngComm, 20*, 1536–1545.
Miao, Z., Jiang, S., Ding, M., Sun, S., Ma, Y., Younis, M. R., et al. (2020). Ultrasmall rhodium nanozyme with RONS scavenging and photothermal activities for anti-inflammation and antitumor theranostics of colon diseases. *Nano Letters, 20*, 3079–3089.
Mirza, F., & Canalis, E. (2015). Management of endocrine disease: Secondary osteoporosis: Pathophysiology and management. *European Journal of Endocrinology, 173*, R131–R151.
Morita, K., Miyamoto, T., Fujita, N., Kubota, Y., Ito, K., Takubo, K., et al. (2007). Reactive oxygen species induce chondrocyte hypertrophy in endochondral ossification. *Journal of Experimental Medicine, 204*, 1613–1623.
Mu, X., He, H., Wang, J., Long, W., Li, Q., Liu, H., et al. (2019). Carbogenic nanozyme with ultrahigh reactive nitrogen species selectivity for traumatic brain injury. *Nano Letters, 19*, 4527–4534.
Naganuma, T. (2016). Shape design of cerium oxide nanoparticles for enhancement of enzyme mimetic activity in therapeutic applications. *Nano Research, 10*, 199–217.
Nath, I., Chakraborty, J., & Verpoort, F. (2016). Metal organic frameworks mimicking natural enzymes: A structural and functional analogy. *Chemical Society Reviews, 45*, 4127–4170.
Neogi, T. (2013). The epidemiology and impact of pain in osteoarthritis. *Osteoarthritis and Cartilage, 21*, 1145–1153.
Ni, D., Jiang, D., Kutyreff, C. J., Lai, J., Yan, Y., Barnhart, T. E., et al. (2018). Molybdenum-based nanoclusters act as antioxidants and ameliorate acute kidney injury in mice. *Nature Communications, 9*, 5421.
Oryan, A., Alidadi, S., Moshiri, A., & Maffulli, N. (2014). Bone regenerative medicine: Classic options, novel strategies, and future directions. *Journal of Orthopaedic Surgery and Research, 9*, 18.
Pillinger, M. H., & Mandell, B. F. (2020). Therapeutic approaches in the treatment of gout. *Seminars in Arthritis and Rheumatism, 50*, S24–S30.
Pinna, A., Baghbaderani, M. T., Hernández, V. V., Naruphontjirakul, P., Li, S., McFarlane, T., et al. (2021). Nanoceria provides antioxidant and osteogenic properties to mesoporous silica nanoparticles for osteoporosis treatment. *Acta Biomaterialia, 122*, 365–376.
Portal-Nunez, S., Esbrit, P., Alcaraz, M. J., & Largo, R. (2016). Oxidative stress, autophagy, epigenetic changes and regulation by miRNAs as potential therapeutic targets in osteoarthritis. *Biochemical Pharmacology, 108*, 1–10.
Rahmati, M., Nalesso, G., Mobasheri, A., & Mozafari, M. (2017). Aging and osteoarthritis: Central role of the extracellular matrix. *Ageing Research Reviews, 40*, 20–30.
Redlich, K., & Smolen, J. S. (2012). Inflammatory bone loss: Pathogenesis and therapeutic intervention. *Nature Reviews Drug Discovery, 11*, 234–250.
Reginster, J. Y., Neuprez, A., Dardenne, N., Beaudart, C., Emonts, P., & Bruyere, O. (2014). Efficacy and safety of currently marketed anti-osteoporosis medications. *Best Practice & Research Clinical Endocrinology & Metabolism, 28*, 809–834.
Sang, Y., Cao, F., Li, W., Zhang, L., You, Y., Deng, Q., et al. (2020). Bioinspired construction of a nanozyme-based H_2O_2 homeostasis disruptor for intensive chemodynamic therapy. *Journal of the American Chemical Society, 142*, 5177–5183.
Shao, D., Li, K., Hu, T., Wang, S., Xu, H., Zhang, S., et al. (2021). Titania nanotube array supported nanoceria with redox cycling stability ameliorates oxidative stress-inhibited osteogenesis. *Chemical Engineering Journal, 415*, Article 128913.
Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S. A., Mardani, F., et al. (2018). Macrophage plasticity, polarization, and function in health and disease. *Journal of Cellular Physiology, 233*, 6425–6440.
Shen, X., Liu, W., Gao, X., Lu, Z., Wu, X., & Gao, X. (2015). Mechanisms of oxidase and superoxide dismutation-like activities of gold, silver, platinum, and palladium, and their alloys: A general way to the activation of molecular oxygen. *Journal of the American Chemical Society, 137*, 15882–15891.
Singh, N., NaveenKumar, S. K., Geethika, M., & Mugesh, G. (2021). A cerium vanadate nanozyme with specific superoxide dismutase activity regulates mitochondrial function and ATP synthesis in neuronal cells. *Angewandte Chemie International Edition, 60*, 3121–3130.
Smolen, J. S., Aletaha, D., & McInnes, I. B. (2016). Rheumatoid arthritis. *Lancet, 388*, 2023–2038.
Soh, M., Kang, D.-W., Jeong, H.-G., Kim, D., Kim, D. Y., Yang, W., et al. (2017). Ceria-zirconia nanoparticles as an enhanced multi-antioxidant for sepsis treatment. *Angewandte Chemie International Edition, 56*, 11399–11403.
Song, Z., Borgwardt, L., Hoiby, N., Wu, H., Sorensen, T. S., & Borgwardt, A. (2013). Prosthesis infections after orthopedic joint replacement: The possible role of bacterial biofilms. *Orthopedic Reviews, 5*, 65–71.
Sozen, T., Ozisik, L., & Basaran, N. C. (2017). An overview and management of osteoporosis. *European Journal of Rheumatology, 4*, 46–56.
Sun, H., Zhou, Y., Ren, J., & Qu, X. (2018). Carbon nanozymes: Enzymatic properties, catalytic mechanism, and applications. *Angewandte Chemie International Edition, 57*, 9224–9237.
Tang, G., He, J., Liu, J., Yan, X., & Fan, K. (2021). Nanozyme for tumor therapy: Surface modification matters. *Explorations, 1*, 75–89.
Taylor, W. J., Fransen, J., Jansen, T. L., Dalbeth, N., Schumacher, H. R., Brown, M., et al. (2015). Study for updated gout classification criteria: Identification of features to classify gout. *Arthritis Care & Research, 67*, 1304–1315.
Thanindratarn, P., Dean, D. C., Nelson, S. D., Hornicek, F. J., & Duan, Z. (2019). Advances in immune checkpoint inhibitors for bone sarcoma therapy. *Journal of Bone Oncology, 15*, Article 100221.
Udalova, I. A., Mantovani, A., & Feldmann, M. (2016). Macrophage heterogeneity in the context of rheumatoid arthritis. *Nature Reviews Rheumatology, 12*, 472–485.
Varela-Eirin, M., Loureiro, J., Fonseca, E., Corrochano, S., Caeiro, J. R., Collado, M., et al. (2018). Cartilage regeneration and ageing: Targeting cellular plasticity in osteoarthritis. *Ageing Research Reviews, 42*, 56–71.
Vernekar, A. A., Sinha, D., Srivastava, S., Paramasivam, P. U., D'Silva, P., & Mugesh, G. (2014). An antioxidant nanozyme that uncovers the cytoprotective potential of vanadia nanowires. *Nature Communications, 5*, Article 5301.
Verrier, S., Alini, M., Alsberg, E., Buchman, S. R., Kelly, D., Laschke, M. W., et al. (2016). Tissue engineering and regenerative approaches to improving the healing of large bone defects. *European Cells and Materials, 32*, 87–110.
Vidal, M., Thibodaux, R. J., Neira, L. F. V., & Messina, O. D. (2019). Osteoporosis: A clinical and pharmacological update. *Clinical Rheumatology, 38*, 385–395.
Wang, X., Gao, X. J., Qin, L., Wang, C., Song, L., Zhou, Y. N., et al. (2019). Eg occupancy as an effective descriptor for the catalytic activity of perovskite oxide-based peroxidase mimics. *Nature Communications, 10*, Article 704.
Wang, H., Wan, K., & Shi, X. (2019). Recent advances in nanozyme research. *Advanced Materials, 31*, Article e1805368.
Wang, Z., Wu, J., Zheng, J. J., Shen, X., Yan, L., Wei, H., et al. (2021a). Accelerated discovery of superoxide-dismutase nanozymes via high-throughput computational screening. *Nature Communications, 12*, 6866.
Wang, L., Yang, Q., Huo, M., Lu, D., Gao, Y., Chen, Y., et al. (2021b). Engineering single-atomic iron-catalyst-integrated 3D-printed bioscaffolds for osteosarcoma destruction with antibacterial and bone defect regeneration bioactivity. *Advanced Materials, 33*, Article e2100150.
Wang, W., Zhou, H., & Liu, L. (2018). Side effects of methotrexate therapy for rheumatoid arthritis: A systematic review. *European Journal of Medicinal Chemistry, 158*, 502–516.
Wei, H., Gao, L., Fan, K., Liu, J., He, J., Qu, X., et al. (2021a). Nanozymes: A clear definition with fuzzy edges. *Nano Today, 40*, Article 101269.
Wei, F., Neal, C. J., Sakthivel, T. S., Kean, T., Seal, S., & Coathup, M. J. (2021b). Multifunctional cerium oxide nanoparticles regulate inflammation and enhance osteogenesis. *Materials Science and Engineering: C, 124*, 112041.
Wei, S., Qiao, Y., Wu, Z., Liu, X., Li, Y., Cui, Z., et al. (2021). Na^+ inserted metal-organic framework for rapid therapy of bacteria-infected osteomyelitis through microwave strengthened Fenton reaction and thermal effects. *Nano Today, 37*, Article 101090.
Wei, H., & Wang, E. (2013). Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes. *Chemical Society Reviews, 42*, 6060–6093.
Wu, J., Wang, Z., Jin, X., Zhang, S., Li, T., Zhang, Y., et al. (2021). Hammett relationship in oxidase-mimicking metal-organic frameworks revealed through a protein-engineering-inspired strategy. *Advanced Materials, 33*, Article e2005024.
Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., et al. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). *Chemical Society Reviews, 48*, 1004–1076.
Xi, J., Zhang, R., Wang, L., Xu, W., Liang, Q., Li, J., et al. (2020). A nanozyme-based artificial peroxisome ameliorates hyperuricemia and ischemic stroke. *Advanced Functional Materials, 31*, 2007130.
Xu, B., Wang, H., Wang, W., Gao, L., Li, S., Pan, X., et al. (2019). A single-atom nanozyme for wound disinfection applications. *Angewandte Chemie International Edition, 58*, 4911–4916.
Yang, S., Ji, J., Luo, M., Li, H., & Gao, Z. (2021). Poly(tannic acid) nanocoating based surface modification for construction of multifunctional composite CeO_2NZs to enhance cell proliferation and antioxidative viability of preosteoblasts. *Nanoscale, 13*, 16349–16361.
Yang, J., Liang, J., Zhu, Y., Hu, M., Deng, L., Cui, W., et al. (2021). Fullerol-hydrogel microfluidic spheres for in situ redox regulation of stem cell fate and refractory bone healing. *Bioactive Materials, 6*, 4801–4815.
Yang, B., Yao, H., Yang, J., Chen, C., Guo, Y., Fu, H., et al. (2022). In situ synthesis of natural antioxidase mimics for catalytic anti-inflammatory treatments: Rheumatoid arthritis as an example. *Journal of the American Chemical Society, 144*, 314–330.
Yang, B., Yao, H., Yang, J., Chen, C., & Shi, J. (2022). Construction of a two-dimensional artificial antioxidase for nanocatalytic rheumatoid arthritis treatment. *Nature Communications, 13*, Article 1988.
Yin, W., Yu, J., Lv, F., Yan, L., Zheng, L. R., Gu, Z., et al. (2016). Functionalized nano-MoS_2 with peroxidase catalytic and near-infrared photothermal activities for safe and synergetic wound antibacterial applications. *ACS Nano, 10*, 11000–11011.

Yudoh, K., Nguyen v, T., Nakamura, H., Hongo-Masuko, K., Kato, T., & Nishioka, K. (2005). Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: Oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Research and Therapy, 7*, R380–R391.

Yu, Y., Zhao, S., Gu, D., Zhu, B., Liu, H., Wu, W., et al. (2022). Cerium oxide nanozyme attenuates periodontal bone destruction by inhibiting the ROS–NFκB pathway. *Nanoscale, 14*, 2628–2637.

Zhang, Z., Gu, Y., Liu, Q., Zheng, C., Xu, L., An, Y., et al. (2018). Spatial confined synergistic enzymes with enhanced uricolytic performance and reduced toxicity for effective gout treatment. *Small, 14*, Article e1801865.

Zhang, Y., Wang, P., Mao, H., Zhang, Y., Zheng, L., Yu, P., et al. (2021a). PEGylated gold nanoparticles promote osteogenic differentiation in *in vitro* and *in vivo* systems. *Materials and Design, 197*, 109231.

Zhang, R., Yan, X., & Fan, K. (2021b). Nanozymes inspired by natural enzymes. *Accounts of Materials Research, 2*, 534–547.

Zheng, L., Zhuang, Z., Li, Y., Shi, T., Fu, K., Yan, W., et al. (2022). Bone targeting antioxidative nano-iron oxide for treating postmenopausal osteoporosis. *Bioactive Materials, 14*, 250–261.

Zimmerli, W., & Sendi, P. (2017). Orthopaedic biofilm infections. *Acta Pathologica, Microbiologica et Immunologica Scandinavica, 125*, 353–364.