

A Pt-Co paired-site nanozyme with a triple-synergistic action enhances periodontitis therapy

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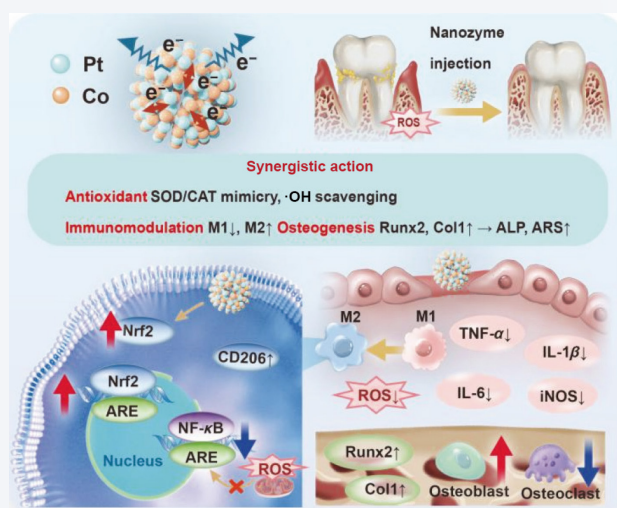
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ABSTRACT: Nanozymes have emerged as promising therapeutic agents, but clinical translation remains hindered by limited catalytic efficiency, structural disorder, and single-function activity. An atomic-level ordered platinum-cobalt (Pt-Co) nanozyme was designed to overcome these limitations, achieving enhanced catalytic performance and multifunctional bioactivity. The highly uniform L1₀-type Pt-Co structure, featuring strong electronic coupling and lattice strain effects between Pt and Co, synergistically lowers reaction energy barriers, thereby significantly enhancing superoxide dismutase (SOD)- and catalase (CAT)-like activities for rapid scavenging of reactive oxygen species (ROS), as evidenced by 82% SOD-like inhibition (vs. 46% for Pt-C catalysts) and a doubled H₂O₂ decomposition rate. *In vitro* and *in vivo* studies demonstrated that the nanozyme attenuated ROS-induced inflammation by shifting macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 (ROS-positive macrophages decreased from 98.1% to 29.5%), reducing inflammatory cytokine production and activating nuclear factor erythroid 2-related factor 2 (Nrf2)/nuclear factor kappa-B (NF-κB) signaling. Moreover, Co endowed the nanozyme with osteogenic capabilities by upregulating osteogenic gene expression, including a twofold increase in *Runx2*, substantially promoting bone regeneration in a mouse model of periodontitis. The dual-metal nanozyme thus serves as a versatile therapeutic platform, simultaneously addressing ROS accumulation, inflammation, and bone resorption, and offers a promising advance in the treatment of periodontitis and other oxidative stress-related diseases.

KEYWORDS: intermetallic nanozymes, reactive oxygen species scavenging, macrophage polarization, osteogenesis, periodontitis



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1 Introduction

Periodontitis, a redox-driven chronic inflammatory disease, affects over one billion individuals worldwide and is characterized by excessive accumulation of ROS, immune dysregulation, and progressive alveolar bone loss [1–3]. Lipopolysaccharide (LPS) from oral bacterial pathogens activates neutrophils and macrophages that then generate large quantities of ROS, which not only aggravate tissue inflammation but also disrupt redox homeostasis by inhibiting the Nrf2 antioxidant pathway and hyperactivating nuclear factor kappa-B (NF- κ B) signaling [4, 5]. Sustained polarization of macrophages toward the pro-inflammatory M1 phenotype amplifies the release of cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), enhances osteoclast-mediated bone resorption, and suppresses the reparative M2 phenotype, further impairing tissue regeneration [6, 7]. However, current clinical strategies, including mechanical debridement, adjunctive antibiotics, host modulation therapies, and regenerative procedures, typically target only one pathological axis at a time (bacterial infection, inflammation, or tissue loss) [8]. Such limited scope does not address the multifactorial etiology of periodontitis well, underscoring the urgent need for integrated therapeutic platforms that simultaneously regulate the microbial load, the immune responses, and tissue regeneration to achieve comprehensive and durable clinical outcomes. Structurally engineered hollow nanocarriers have also advanced local drug delivery through high loading capacity, stimulus-responsive release, and controllable antimicrobial delivery, underscoring the importance of nanoscale structural design in biomedical therapy [9–13].

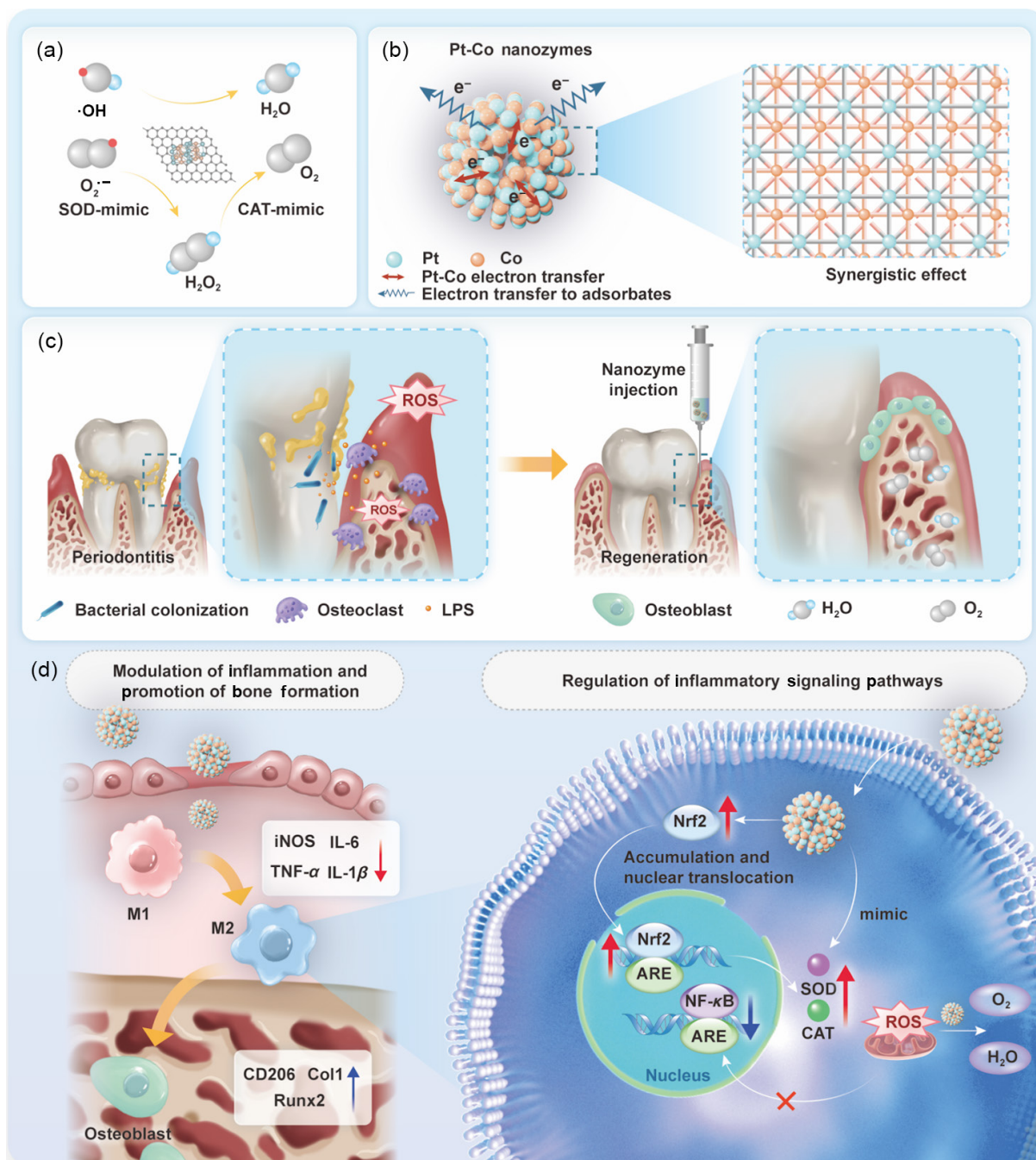
Nanozymes are nanomaterials with intrinsic enzyme-mimicking catalytic activities that have emerged as promising alternatives to natural enzymes given their low cost, high stability, and tunable catalytic performance [14–16]. In periodontitis therapy, nanozymes have recently been recognized as multifunctional platforms capable of antibacterial intervention, inflammation modulation, and tissue repair [17]. Among them, noble-metal-based nanozymes, particularly platinum (Pt)-based systems, have garnered widespread interest because of their excellent biocompatibility and their capacity to mimic superoxide dismutase (SOD) and catalase (CAT) activities, enabling effective ROS scavenging when oxidative stress is in play [18–20]. However, most of these systems improve therapeutic performance through platform-level engineering, such as supported Pt nanoparticles, carrier-assisted active sites, light activation, metal-organic framework (MOF)-mediated oxygen generation, hydrogel delivery, or ion release, rather than through an intrinsically ordered Pt-Co catalytic lattice. In contrast, the present L₁₀ Pt-Co intermetallic compounds (IMCs) use the atomically ordered intermetallic lattice itself as the catalytic core, achieving a more comprehensive trigger-free ROS-scavenging profile, including CAT-like, SOD-like, and $\cdot$ OH-scavenging activities, together with macrophage redox immunoregulation and preservation of osteogenic potential in periodontitis (Table S1 in the Electronic Supplementary Material (ESM)) [21–25]. Despite notable advances, existing nanozyme systems have three major limitations that critically undermine therapeutic efficacy in complex disease environments. First, many exhibit atomic-level disorder in the alloy structures, impairing metal-metal electronic coupling and interface charge transfer [26]. Second, catalytic efficiency remains suboptimal because of inhomogeneous active site distribution and poor ROS adsorption/conversion kinetics [27, 28]. Third, many nanozymes

exhibit principally antioxidant activity; their use to modulate immune responses synergistically or promote tissue regeneration remains underexplored [29–31]. The latter functions are crucial when seeking to improve the therapeutic outcomes of chronic inflammation, in which oxidative stress, immune dysregulation, and impaired repair are all in play.

To overcome these limitations, ordered IMCs have emerged as compelling material platforms for next-generation nanozyme design. In contrast to disordered alloys, IMCs exhibit long-range atomic ordering that enables uniform active site exposure, enhanced metal-metal orbital hybridization, and precisely tunable surface chemistry, all of which are key in terms of catalytic selectivity, efficiency, and stability [32–35]. For example, L₁₀-phase Pt-Co IMCs with a controlled lattice strain enhanced peroxidase-like activity 17.4-fold compared to Pt nanoparticles [36]. Similarly, high-index Pt₃Sn IMCs exhibit exceptional peroxidase-like activity (345.3 U/mg) with superior substrate specificity and minimal oxidase- and catalase-like activities. Density functional theory (DFT) simulations have shown that ordered Pt-based IMCs lower the activation barriers for H₂O₂ decomposition and enhance reaction pathways by modulating the d-band center and charge delocalization at the metal interfaces [37]. These findings highlight the functional advantages afforded by IMCs beyond structural ordering, positioning them as ideal platforms for nanozyme-based treatment of redox-driven diseases such as periodontitis. Thus, the novelty of the present design lies not merely in incorporating Pt and Co, but in organizing them into an L₁₀ intermetallic lattice that provides defined Pt-Co electronic coupling for efficient antioxidant catalysis.

Structural design aside, rational element selection can introduce bioactive functionalities that go beyond catalysis [38, 39]. Notably, cobalt ions (Co²⁺) play biological roles in that they mimic hypoxic conditions and activate angiogenic and osteogenic pathways [40]. Recent studies have revealed that Co²⁺ can reprogram macrophage polarization toward the M2 phenotype, facilitate anti-inflammatory cytokine secretion, and stimulate osteogenic differentiation via upregulation of transcription factors such as *Runx2* and signaling axes including mTOR-Sema6d-peroxisome PPAR γ [41]. Moreover, CoCl₂ has been shown to suppress LPS/interferon- γ (IFN- γ)-induced oxidative stress and inflammatory cytokine production by inhibiting NF- κ B activation, underscoring the dual capacity to alleviate the oxidative burden and modulate immune responses [42]. These findings imply that incorporation of Co into bimetallic nanozymes would not only enhance catalytic synergy but also endow the materials with intrinsic immunoregulatory and osteoinductive functions. Given the complementary catalytic and biological properties of Pt and Co, a bimetallic Pt-Co IMC nanozyme platform might simultaneously target ROS overproduction, the immune imbalance, and bone regeneration failure of periodontitis.

Herein, we describe an atomically ordered Pt-Co IMC nanozyme exhibiting enhanced catalytic activity, immunomodulation, and osteogenic stimulation that synergistically treat oxidative-stress-driven periodontitis (Scheme 1). Overall, this work establishes L₁₀ Pt-Co IMCs as a structurally ordered nanozyme platform that integrates Pt-Co electronic modulation, multi-ROS catalytic scavenging, and inflammatory microenvironment reprogramming to support periodontal bone repair. Via rational alloy engineering, we synthesized carbon-supported L₁₀-Pt-Co nanoparticles exhibiting periodic atomic stacking and a compressed lattice strain,



Scheme 1 The mechanism by which Pt-Co IMCs treat periodontitis. (a) Antioxidant activity of Pt-Co IMCs that mimics SOD and CAT activities to scavenge ROS by catalyzing their conversion to H_2O and O_2 . (b) Schematic of Pt-Co IMCs showing the synergistic Pt-Co electronic interaction and electron transfer to adsorbates, collectively enhancing catalytic performance. (c) Schematic of the therapeutic effect of Pt-Co IMCs in terms of periodontitis. Left: Bacterial colonization at the gingival sulcus initiates LPS and ROS production, which subsequently induces osteoclast activation and bone resorption. Right: Injected Pt-Co IMCs scavenge ROS, producing O_2 and H_2O , thereby promoting tissue repair and supporting osteogenesis. (d) Schematic of the therapeutic mechanisms of Pt-Co IMCs. Left: Pt-Co IMCs repolarize macrophages from M1 to M2; lowering iNOS levels and secretion of $\text{TNF-}\alpha$, IL-6 , and interleukin-1 beta ($\text{IL-1}\beta$); but increasing CD206 expression, thereby establishing an anti-inflammatory microenvironment that supports osteogenesis via upregulation of *Runx2* and *Col1*. Right: Via SOD- and CAT-like catalysis ($\text{O}_2^{\cdot-}$ dismutation; H_2O_2 decomposition) with $\cdot\text{OH}$ scavenging, overall ROS neutralization by Pt-Co IMCs yields O_2 and H_2O ; such ROS depletion suppresses NF- κB nuclear translocation and κB -dependent transcription, and Nrf2 accumulates and translocates to the nucleus, therein binding to antioxidant response elements (AREs), in turn enhancing endogenous antioxidant defenses by increasing native SOD and CAT activities, thereby reinforcing the antioxidant and anti-inflammatory responses.

as confirmed by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), X-ray diffraction (XRD), and extended X-ray absorption fine structure (EXAFS) analyses. DFT calculations revealed that the electronic interactions between Pt and Co atoms were optimized, significantly

lowering the reaction energy barriers and enhancing ROS-scavenging catalytic efficiency. Functionally, the Pt-Co IMCs efficiently suppressed ROS-induced inflammation via NF- κB inhibition and Nrf2 activation, repolarized macrophages from the pro-inflammatory M1 to the reparative M2 phenotype, and

markedly upregulated osteogenic differentiation markers such as Runx2 and collagen type I (Col1). Both *in vitro* and *in vivo* experiments demonstrated attenuation of inflammation, restoration of immune homeostasis, and enhanced bone regeneration. This structurally ordered, multifunctional nanozyme thus serves as a versatile and promising therapeutic strategy for periodontitis and other redox-associated inflammatory diseases.

2 Experimental

2.1 Synthesis of the Pt-Co IMCs

$\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ (88.5 mg; Shanghai Clinical Research Center (SCRC)) and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (40.7 mg; SCRC) were dissolved in 15 mL of ultrapure water. Subsequently, 50 mg of the Ketjenblack EC-600JD (Lion Specialty Chemicals, Japan) was added. After sonicating for 30 min, the mixture was stirred overnight. After drying with a rotary evaporator, the precursor was placed in a tube furnace. The temperature of the precursor was increased to 950 °C at a heating rate of 5 °C/min in an atmosphere of 5% H_2/Ar , and held at this temperature for 2 h. Then, it was cooled to 600 °C at an annealing rate of 1 °C/min, and finally cooled to room temperature to obtain the sample.

2.2 Characterization of Pt-Co IMCs

The structural characteristics of the Pt-Co IMCs and Pt-C catalysts were examined utilizing scanning electron microscopy (SEM) and transmission electron microscopy (TEM, Hitachi H-7650). The materials' structural properties were examined via XRD with a Rigaku Miniflex 600, operated at 40 kV and 15 mA, employing Cu $K\alpha$ radiation ($\lambda = 0.15406$ nm) at a scanning rate of 10° per minute. High-resolution transmission electron microscopy (HRTEM) images and associated energy-dispersive X-ray spectroscopy (EDS) maps were obtained using a JEOL JEM-2100F field-emission electron microscope operating at 200 kV. HAADF-STEM images were obtained to examine the material's surface and internal structure, utilizing an FEI Themis Z with a probe corrector at 200 kV. X-ray photoelectron spectroscopy (XPS) was performed using a Thermo K-Alpha system to analyze the surface chemical states and electronic structure of Pt-Co IMCs and Pt-C catalysts.

2.3 CAT-like O_2 generation assay

The ability of the nanozymes to mimic catalase activity was investigated by monitoring the increase in oxygen concentration in a 0.6% hydrogen peroxide solution. This was achieved using a dissolved oxygen meter (JPSJ-606L, Leici China). The reaction mixture consisted of 200 μL of nanozyme solution (40 $\mu\text{g}/\text{mL}$) and 0.6% hydrogen peroxide in phosphate-buffered saline (PBS) at pH 7.4. The oxygen release during the reaction was tracked to evaluate the efficiency of the nanozymes in catalytically decomposing hydrogen peroxide.

2.4 CAT- and SOD-like activity assays

The CAT activity of the Pt-Co IMCs was evaluated with a commercially available Catalase Assay Kit. The reaction commenced with the addition of a 5 mM hydrogen peroxide solution as the substrate. Catalase facilitates the decomposition of hydrogen peroxide into water and oxygen. The residual hydrogen peroxide, in conjunction with peroxidase, oxidizes a colorimetric substrate, yielding a red product with a peak absorbance at 520 nm.

A standard curve derived from known hydrogen peroxide concentrations was employed to measure the catalase activity in the sample. The SOD activity of Pt-Co IMCs was measured using an SOD Assay Kit (Solarbio, Beijing). Superoxide anions (O_2^-) were generated through a xanthine/xanthine oxidase system. The O_2^- reduced nitroblue tetrazolium (NBT) to form a blue formazan product, which absorbs at 560 nm. The SOD enzyme scavenges O_2^- , thereby inhibiting the formation of the blue formazan. The SOD activity was quantified by measuring the decrease in absorbance at 560 nm, where a lower absorbance indicates higher SOD activity.

2.5 $\cdot\text{OH}$ scavenging activity of Pt-Co IMCs

$\cdot\text{OH}$ scavenging capacity was evaluated by the terephthalic acid (TA) assay. In this assay, 500 μL of H_2O_2 (6 mM) and 500 μL of FeSO_4 (60 μM) were mixed for 30 min to generate $\cdot\text{OH}$. Then, 100 μL of Pt-Co IMCs (30–200 $\mu\text{g}/\text{mL}$) were added to the solution and incubated for 1 h. After adding 50 μL TA (30 mM, 0.1 M NaOH) and 2.8 mL of PBS (pH 7.4), the mixture was shaken for 1 h. Following centrifugation to remove Pt-Co IMCs, the fluorescence emission was measured using a fluorescence spectrometer.

2.6 EPR-based evaluation of $\cdot\text{OH}$ scavenging activity

The scavenging ability of nanozymes towards $\cdot\text{OH}$ was evaluated using electron paramagnetic resonance (EPR) spectroscopy on a Bruker E500CW spectrometer. $\cdot\text{OH}$ was generated via a Fenton reaction, where FeCl_2 was used to decompose H_2O_2 in the reaction system. The final concentrations of FeCl_2 , H_2O_2 , and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were fixed at 1, 1, and 10 mM, respectively. DMPO served as the spin trap for $\cdot\text{OH}$, allowing for the detection and quantification of the generated radicals. The EPR spectra were recorded under the following conditions: microwave power of 2.5 mW, modulation field frequency of 100 kHz, amplitude of 0.2 mT, central magnetic field of 336.5 mT, and scanning speed of 20 mT/min. The intensity of the EPR peaks corresponding to the DMPO- $\cdot\text{OH}$ adducts was used to quantitatively assess $\cdot\text{OH}$ scavenging capacity of the nanozymes.

2.7 EPR-based evaluation of superoxide anion scavenging activity

Superoxide anions (O_2^-) were generated using xanthine (0.4 mM) and xanthine oxidase (0.1 U/mL), which catalyzed their synthesis. Following preparation of O_2^- , Pt-Co IMCs or Pt-C catalysts were added to the reaction mixture. DMPO (10 mM) was included prior to initiating superoxide generation to enable *in situ* spin trapping (DMPO-OOH). The variation in peak intensity on the EPR spectrometer was observed to assess the efficacy of the nanozymes in scavenging O_2^- .

2.8 DFT computational details

DFT calculations were conducted utilizing the Vienna *ab initio* simulation package (VASP) software program to examine the adsorption energies, differential charge densities, projected density of states (PDOS), and free energy diagrams of the Pt-Co IMCs and Pt-C catalysts. The generalized gradient approximation (GGA) with the Perdew-Burke-Ernzerhof (PBE) functional was employed to characterize the exchange-correlation potential. The projector augmented-wave (PAW) approach was utilized to address the interaction between ionic cores and valence electrons [43–45]. A

plane-wave cutoff energy of 450 eV was employed, and structural optimizations were performed with force convergence below 0.03 eV/Å and a Gamma-centered k -point grid with a spacing of 0.03 Å⁻¹. Dispersion forces were incorporated with Grimme's DFT-D3 methodology to simulate the interatomic interactions in the adsorption systems [46].

2.9 *In vitro* toxicity evaluation of Pt-Co IMCs

MC3T3-E1 and RAW264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin at 37 °C in a 5% CO₂ atmosphere with 100% humidity, with medium changes every 48 h, using cells from passages 3–5. The effect of Pt-Co IMCs on the viability of RAW264.7 macrophages and MC3T3-E1 preosteoblasts was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Biosharp, China). RAW264.7 cells were plated at a density of 1×10^4 cells/well in a 96-well plate and cultured for 24 h to allow attachment. Fresh culture medium containing 0, 2.5, 5, 7.5, or 10 µg/mL Pt-Co IMCs was then added, and the cells were further incubated for 24 h. After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plate was incubated at 37 °C for 4 h. The supernatant was discarded, and 150 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was measured at 490 nm using a microplate reader (BioTek Synergy H1, USA), and cell viability was calculated. For MC3T3-E1 cells, the seeding density was 5×10^4 cells/well, and the remaining steps were performed as described for RAW264.7 cells. RAW264.7 cells were seeded at a density of 5×10^5 cells/well in a 24-well plate and incubated for 24 h. MC3T3-E1 cells were seeded at a density of 4×10^4 cells/well and cultured for 24 h before incubation with nanozymes for 24 h. Cells were then stained with calcein-AM/PI for 15 min in the dark at 37 °C and observed using an inverted fluorescence microscope.

2.10 *In vitro* and *in vivo* ROS scavenging by nanozymes

RAW264.7 cells were seeded in 24-well plates at a density of 5×10^5 cells per well and incubated for 24 h. After adhesion, the cells were treated with 10 µg/mL LPS for 24 h, followed by exposure to nanozymes for another 24 h. ROS levels were measured using the CM-H₂DCFDA-based ROS Assay Kit (Beyotime, China), and fluorescence intensity was quantified using a flow cytometer. To assess the ROS scavenging effect *in vivo*, LPS-induced periodontitis in C57BL/6 mice was established by injecting LPS (1 mg/mL, 0.02 mL) into the gingival sulcus for 3 days. PBS, Pt-C catalysts, and Pt-Co IMCs (10 µg/mL) were then injected into the gingival sulcus, and the treatment was continued for another 3 days. After the 3-days treatment, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) was administered intravenously via the tail vein at a dose of 2 mg/kg to detect residual ROS. Whole-body fluorescence was recorded using a small animal imaging system ($n = 3$), and fluorescence signal intensity was measured and quantified using ImageJ software (National Institutes of Health, USA).

2.11 Immunofluorescence analysis of LPS-induced RAW264.7 cells with nanozyme treatment

RAW264.7 cells were seeded at a density of 5×10^5 cells per well onto pre-cleaned coverslips in a 24-well plate. After reaching 70%–80% confluence, cells were induced with LPS for 24 h, followed by 24 h of treatment with the materials. Cells were fixed with 4% paraformaldehyde (PFA) for 15 min and washed with PBS.

They were then permeabilized with 0.5% Triton X-100, blocked with 1% bovine serum albumin (BSA), and incubated overnight at 4 °C with primary antibodies for inducible nitric oxide synthase (iNOS), CD206, NF-κB1, and Nrf2 [47, 48]. After washing, secondary antibodies were applied and incubated at room temperature for 1 h. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI), and cells were imaged using a confocal laser scanning microscope (CLSM) (Olympus FV3000). Fluorescence intensity was quantified using ImageJ software (National Institutes of Health, USA).

2.12 Enzyme-linked immunosorbent assay (ELISA) analysis of inflammatory cytokines

RAW264.7 cells (2×10^5 cells/well) were seeded in 48-well plates and incubated overnight. After activation with LPS (10 µg/mL) for 24 h, nanozymes were added for an additional 24 h of incubation. The levels of TNF-α, IL-6, and IL-1β in the supernatant were measured using ELISA. A standard curve was prepared, and 100 µL of the supernatant was incubated at 37 °C for 90 min. After washing, biotinylated detection antibodies, enzyme conjugates, and substrate were added sequentially. The absorbance at 450 nm was measured to assess the regulatory effects of nanozymes on inflammatory cytokines.

2.13 Flow cytometric analysis of macrophage polarization

RAW264.7 cells were first stimulated with LPS (10 µg/mL) to induce an M1 phenotype, then treated with the test materials (Pt-Co IMCs or Pt-C catalysts). After PBS washes, cells were gently detached with a cell scraper to preserve surface antigens, pelleted by centrifugation, and resuspended. Surface staining was performed with a phycoerythrin (PE)-conjugated anti-mouse CD206 antibody, and intracellular iNOS was stained with a fluorescein isothiocyanate (FITC)-conjugated antibody following fixation and permeabilization. Data were acquired on a BD FACS Calibur flow cytometer and analyzed in FlowJo, using appropriate isotype and single-stained controls for accurate gating and compensation.

2.14 *In vitro* osteogenic differentiation assessment

Mouse osteoblast precursor cells (MC3T3-E1) were seeded at a density of 1×10^4 cells per well in osteogenic medium and incubated with nanozymes (10 µg/mL). On day 7, alkaline phosphatase (ALP) activity of MC3T3-E1 cells was assessed using the BCIP/NBT staining kit (Beyotime, China). Subsequently, the cells were lysed in a 24-well plate, and ALP activity was quantified using an ALP assay kit (Beyotime, China) by measuring absorbance at 405 nm. Additionally, after 21 days of culture in osteogenic induction medium, Alizarin Red S (ARS) staining was performed to observe calcium deposition. The calcium deposits were then dissolved in 10% cetylpyridinium chloride (Solarbio, China) and absorbance was measured at 570 nm for ARS quantification. After 21 days of incubation, cells from different treatments were collected, and osteogenic gene expression, including *Col1* and *Runx2*, was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) using primers listed in Table S5 in the ESM.

2.15 Establishment of the periodontal inflammation model

All animal experiments were conducted in accordance with institutional guidelines and were approved by the Peking University

School of Stomatology Research Ethics Committee (Approval No. PUIRB-LA2023136). Twenty-four male C57BL/6 mice (10–12 weeks, 20–25 g) were acclimated to the environment for one week before modeling. The mice were randomly assigned to four groups: no treatment group (Ctrl), LPS-induced periodontitis models treated with PBS (PBS), Pt-C group, and Pt-Co group, with six mice in each group. The second molars (M2) of the mice were ligated using a 5-0 silk suture and a continuous knotting method. After one week of ligation, nanozymes (10 µg/mL) or PBS were injected into the palatal gingival sulcus of the upper second molars every other day for two weeks. After 2 weeks, the animals were sacrificed, and the upper jawbones and major organs were harvested for analysis.

2.16 ICP-OES analysis of Co²⁺ ion release

Co²⁺ release from Pt-Co IMCs was evaluated by inductively coupled plasma optical emission spectrometry (ICP-OES) under physiological and acidic conditions. Briefly, Pt-Co IMCs were dispersed in PBS at a concentration of 10 mg/L and incubated at 37 °C at pH 7.4 or 5.5. At predetermined time points (days 0, 1, 2, 4, 7, and 10), the suspensions were centrifuged at 3000 rpm for 15 min, and the Co²⁺ concentration in the supernatants was quantified by ICP-OES. The net Co²⁺ release was calculated by subtracting the day 0 background value.

2.17 Micro-CT scanning

Micro-computed tomography (Micro-CT) scanning (Bruker SkyScan 1276) was performed on the upper jaw and dental hard tissue samples from mice to obtain three-dimensional imaging data [49]. Reconstruction was performed in NRecon, reorientation in DataViewer, and volumetric/morphometric analysis in CTAn. The vertical distance between the alveolar bone crest (ABC) and the cemento-enamel junction (CEJ) of the upper second molars was measured on two-dimensional sagittal sections in CTAn. Three-dimensional renderings were generated in CTvox for visualization. Key bone tissue parameters were also analyzed, including bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp).

2.18 Histological and immunological evaluation of tissue markers and protein expression

The collected maxillary specimens were first fixed in 4% PFA, followed by decalcification in 10% (w/v) ethylenediaminetetraacetic acid (EDTA) (pH 7.2–7.4) at room temperature with regular changes for one month. The samples were then embedded in paraffin and sectioned into 4 µm thick slices. The sections were stained with hematoxylin and eosin (H&E) to observe tissue structure and then subjected to Masson's trichrome staining to highlight components such as collagen fibers. All stained slides were observed and imaged using an optical microscope. Additionally, immunofluorescence was performed to assess the expression of NF-κB1 and Nrf2, and immunohistochemical staining was used to detect the inflammatory marker IL-1β, in order to evaluate the expression levels and localization of relevant proteins.

2.19 Hemolysis assay of Pt-Co IMCs

1 mL of C57BL/6 mouse blood was suspended in 10 mL of PBS, and red blood cells (RBCs) were isolated by centrifugation at 1000g for 10 min. The RBCs were then washed four times with PBS and resuspended in PBS. Next, 200 µL of Pt-Co IMCs were mixed with 200 µL of the RBC suspension and incubated at 37 °C for 4 h. After

incubation, the supernatant was separated by centrifugation at 450g for 10 min. Finally, the optical density (OD) of the supernatant was measured at 540 nm using a multifunctional microplate reader. The hemolysis ratio was calculated using Eq. (1):

$$\text{Hemolysis ratio (\%)} = \frac{\text{OD}_s - \text{OD}_0}{\text{OD}_w - \text{OD}_0} \times 100\% \quad (1)$$

where OD_s represents the OD of the Pt-Co IMC-treated sample, OD_w represents the OD of the positive control group, and OD₀ represents the OD of the negative control group.

2.20 Statistical analysis

All quantitative data are presented as mean ± standard deviation (SD). In the evaluation of outliers, any data points deviating more than two SDs from the mean were excluded. Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test or Student's *t*-test, as appropriate, using GraphPad Prism 8.0 software. The sample size (*n*) for each statistical analysis is indicated in the corresponding figure legend. A significance level of α = 0.05 was pre-set, and *P*-values < 0.05 were considered statistically significant (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001). Each experiment was conducted in triplicate.

3 Results and discussion

3.1 Synthesis and characterization of Pt-Co IMCs

Pt-Co IMCs were synthesized via high-temperature annealing in a reductive atmosphere and subsequently characterized in terms of the alloy structure and nanoscale uniformity. TEM was used to examine the morphology and dispersion of Pt-C catalysts and Pt-Co IMCs. TEM images revealed that both samples were uniformly anchored onto the carbon support with no apparent aggregation (Fig. 1(a) and Fig. S1 in the ESM). Further characterization revealed well-dispersed Pt-Co nanoparticles of average diameter ~ 9.72 nm (Fig. S2 in the ESM). The HAADF-STEM images in Figs. 1(b) and 1(e) clearly show highly ordered Pt₂Co₁ IMC nanoparticles. The superlattice diffraction spot (001) in the FFT patterns in Figs. 1(c) and 1(f) confirmed that the alloy structure was ordered [50]. The 3D atom-overlapping Gaussian function fitting mapping in Figs. 1(d) and 1(g) confirmed the periodic interatomic arrangement. Figure 1(h) shows the results of EDS used for elemental mapping. The extent of overlap of Pt and Co was extremely high, indicating that both coexist within the particles. This is a characteristic of IMCs, as shown in Fig. 1(h).

Ordered IMCs typically exhibit shorter interatomic distances than disordered alloys due to long-range atomic ordering. The periodic Pt-Co arrangement along the *c*-direction further indicates anisotropic lattice distortion [51]. Furthermore, the periodic Pt-Co-Pt-Co structure along the *c*-direction indicated that the lattice experienced more compression in this than in other directions. Such structural transformation reduces the crystallographic symmetry. Specifically, long-range atomic ordering breaks the original cubic symmetry by eliminating the threefold rotational axis along the body diagonal ([111] direction) of the cubic cell. The structure transforms from a high-symmetry cubic phase into a lower-symmetry tetragonal phase. This alters the diffraction extinction rules, associated with emergence of new superlattice reflections in the powder XRD (PXRD) pattern. For comparison,

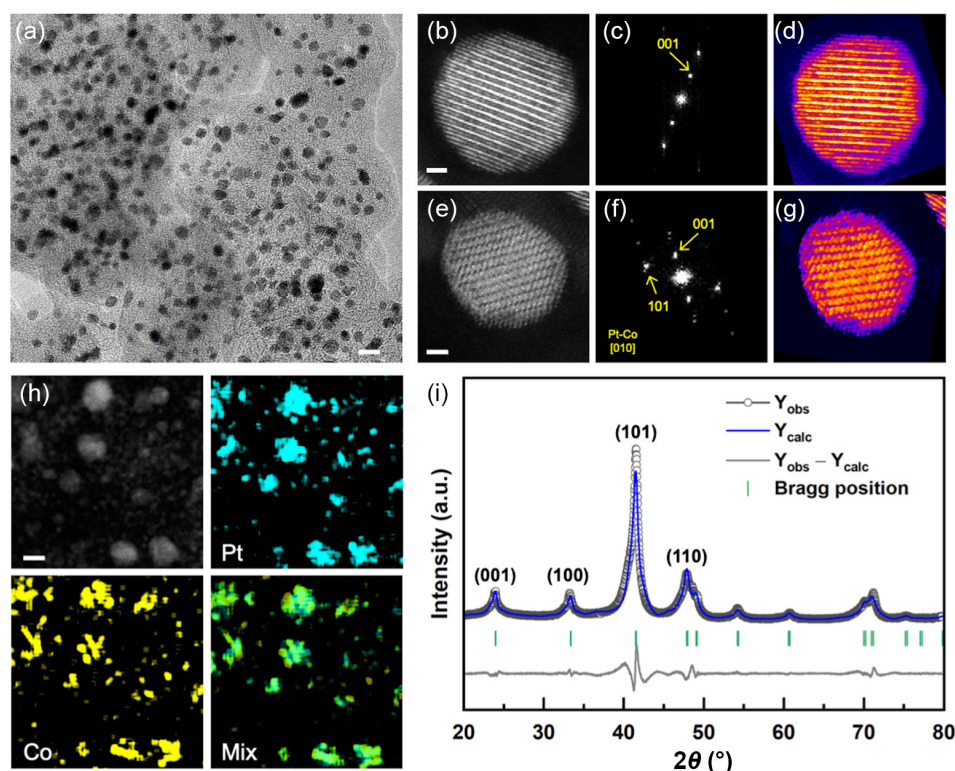


Figure 1 Atomic-scale structural and compositional characterization of Pt-Co IMCs. (a) TEM image of Pt-Co IMCs (scale bar: 10 nm). (b) and (e) Atomic-resolution HAADF-STEM images of Pt-Co IMCs (scale bars: 1 nm). (c) and (f) Fast Fourier transform (FFT) patterns of Pt-Co IMCs. (d) and (g) The three-dimensional (3D) atom-overlapping Gaussian function fitting mapping of the Pt-Co IMCs of panel (b). (h) EDS elemental mappings of Pt-Co IMCs (scale bar: 25 nm). (i) Rietveld refinement of the XRD pattern of Pt-Co IMCs.

the Pt-C control exhibited the characteristic face-centered cubic Pt diffraction peaks assigned to the (111), (200), (220), and (311) planes, without the superlattice reflections characteristic of ordered L_{10} Pt-Co IMCs (Fig. S3 in the ESM). By contrast, Fig. 1(i) shows the XRD pattern, refined using the Rietveld method, of Pt-Co IMCs. Distinct superlattice reflections are apparent at 24.2° and 33.4° , corresponding to the (001) and (100) planes, respectively. These peaks are characteristic of the ordered L_{10} -Pt-Co IMC phase with a tetragonal structure of the $P4/mmm$ space group (Table S2 in the ESM), confirming formation of a highly crystalline and compositionally ordered alloy [52]. The diffraction peak at $\sim 41.7^\circ$, corresponding to the (101) plane of the tetragonal L_{10} -Pt-Co phase, exhibited the highest XRD pattern intensity, indicative of strong atomic ordering and a preferred orientation. Notably, such dominant diffraction intensity behavior is reminiscent of the intense (111) reflection commonly observed in face-centered cubic (fcc) metals. Importantly, this comparison is relevant only in the context of the relative intensity prominences of XRD profiles and does not imply any crystallographic similarity or any analogous atomic stacking between the (101) and (111) planes. In addition, the crystallite size of Pt-Co IMCs estimated from the XRD peak broadening using the Scherrer equation was approximately 9.9 nm, consistent with the nanoscale crystalline nature of the ordered L_{10} intermetallic structure (Fig. 1(i)).

X-ray absorption fine structure (XAFS), including X-ray absorption near-edge structure (XANES) and EXAFS analyses, was next employed to investigate the local atomic structure and chemical states of Pt-Co. In the Pt L_3 -edge XANES spectra (Fig. 2(a)), the white-line intensity of Pt-Co IMCs was higher than that of Pt foil, indicating a higher valence of Pt in Pt-Co IMCs. In

the R-space Pt L_3 -edge EXAFS spectra (Fig. 2(b)), the scattering peak attributable to the first shell of Pt-Co IMCs shifted to a shorter bond distance compared to that of Pt foil, implying formation of Pt-Co bonds with shorter bond distance. The detailed coordination environments of the samples were obtained by fitting the EXAFS spectra (Figs. 2(c)–2(f), Figs. S4(a) and S4(b), and Table S3 in the ESM). The corresponding structural parameters derived from the EXAFS fitting are further summarized in Table S4 in the ESM. The Pt-Co IMC coordination number was 3.2 and the bond distance was 2.62 Å, confirming formation of Pt-Co IMCs. Similar results were apparent in the Co K-edge XANES spectra. As shown in Fig. 2(d), the white-line intensity of Pt-Co IMCs was higher than that of Co foil, implying a higher valence of Co in Pt-Co IMCs. The Co-Pt bond distance determined via EXAFS fitting at both the Pt L_3 -edge and Co K-edge was consistently 2.62 Å, confirming formation of Pt-Co bonds (Figs. 2(c) and 2(f)).

The Co-Co bond length within the Pt-Co structure (2.57 Å) was significantly longer than that of metallic Co foil (2.49 Å), indicative of lattice distortion and strain induced by alloy formation (Fig. 2(f)). The longer Co-Co bond distance of Pt-Co IMCs is attributable to Pt-Co IMC formation. On wavelet transform analysis (Fig. S4(c) in the ESM), the maximum intensities of Pt-Co IMCs (Pt L_3 -edge and Co K-edge) were from the 2 to 3 Å range, implying that the main chemical states of Pt and Co in Pt-Co are Pt-Co IMCs, although a few Pt-C and Co-C/O bonds remained evident. The XPS spectrum of Pt-Co IMCs confirmed the presence of Pt, Co, C, and O (Fig. S5 in the ESM). As shown in the high-resolution Pt 4f and Co 2p spectra, Pt existed in both the metallic and oxidized states, whereas Co exhibited mixed valence states corresponding to Co^{2+} and Co^{3+} . These results indicate that the Pt-Co system features the coexistence

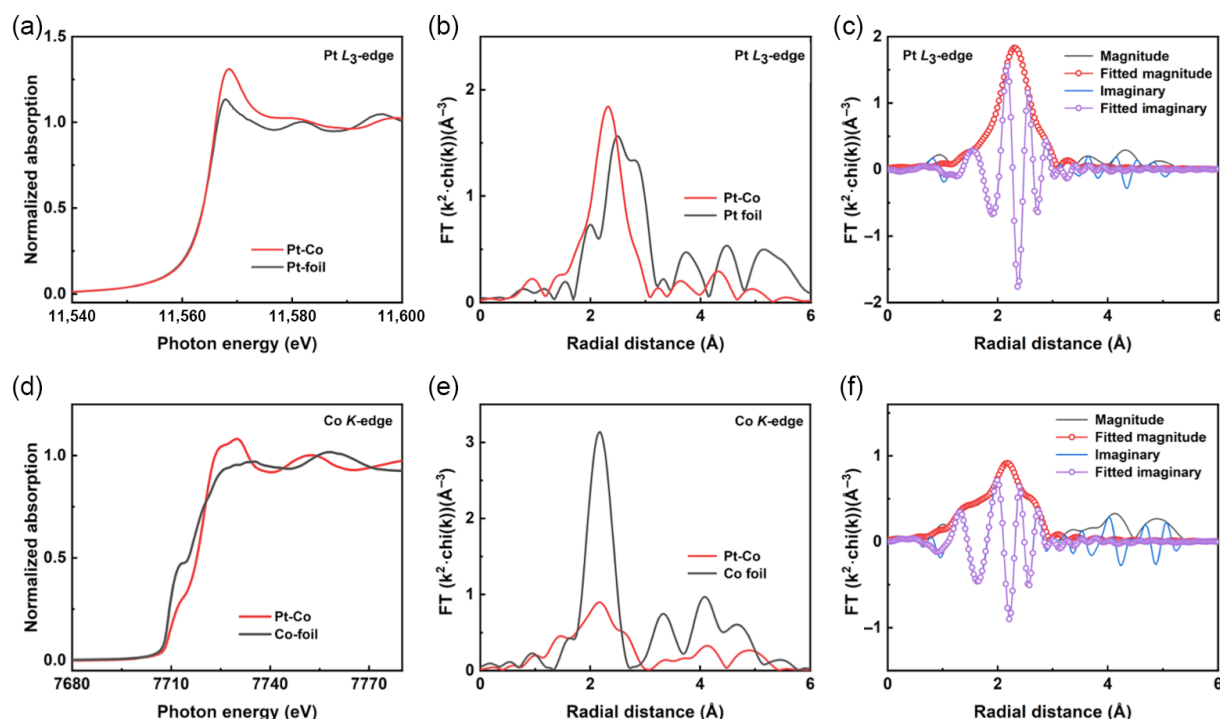


Figure 2 X-ray absorption spectroscopy (XAS) of Pt-Co IMCs at the Pt L_3 and Co K edges. (a) XANES spectra at the Pt L_3 -edge for Pt foil and Pt-Co IMCs. (b) k^2 -weighted R -space EXAFS at the Pt L_3 -edge for Pt foil and Pt-Co IMCs. (c) R -space EXAFS fitting of Pt-Co IMCs at the Pt L_3 -edge. (d) XANES spectra at the Co K-edge for Pt-Co IMCs and Co foil. (e) k^2 -weighted R -space EXAFS at the Co K-edge for Pt-Co IMCs and Co foil. (f) R -space EXAFS fitting of Pt-Co IMCs at the Co K-edge.

of multiple surface oxidation states. Collectively, the XPS core-level signatures were consistent with interfacial electronic interaction within the Pt-Co system.

3.2 Evaluation of the enzyme-mimicking activity of Pt-Co IMCs

We systematically evaluated the enzyme-mimicking activities of Pt-Co IMCs, including CAT-like, SOD-like, and hydroxyl radical ($\cdot\text{OH}$)-scavenging actions. In terms of CAT-like activity, H_2O_2 decomposition and O_2 generation were monitored. Figure 3(a) shows that Pt-Co IMCs exhibited a significantly greater catalytic rate than Pt-C catalysts, with the O_2 concentration attaining 16 mg/L by 800 s, compared to 8.34 mg/L for Pt-C catalysts. At a nanozyme concentration of 40 $\mu\text{g}/\text{mL}$, the CAT-like activity of Pt-Co IMCs was 63.3 U/mL, 1.48-fold greater than that of Pt-C catalysts (Fig. 3(b)). Both Fig. 3(b) and Fig. S6 in the ESM reveal clear concentration-dependent catalytic performance, with increasing nanozyme concentrations associated with more CAT-like activity. Such activity of Pt-Co IMCs was superior to that of Pt-C catalysts and increased in a concentration-dependent manner. Next, SOD-like activity was evaluated by measuring inhibition of superoxide radical ($\text{O}_2^{\cdot-}$) production at different Pt-Co IMC concentrations. Figure S7 in the ESM further shows the concentration-dependent $\text{O}_2^{\cdot-}$ inhibition rates of Pt-C catalysts and Pt-Co IMCs. Figure 3(c) shows that Pt-Co IMCs afforded significantly higher inhibition than did Pt-C catalysts, with a maximum of 82% at 80 $\mu\text{g}/\text{mL}$ compared to 46% for Pt-C. These results indicate that Pt-Co IMCs exhibited enhanced SOD-like activity attributable to a synergistic interaction between Pt and Co that both facilitated electron transfer and promoted ROS scavenging. Next, the $\cdot\text{OH}$ antioxidant capacity (HORAC) of Pt-Co IMCs was evaluated, because $\cdot\text{OH}$ is one of the most

reactive/damaging ROS species, and no specific enzymatic detoxification pathway is known. $\cdot\text{OH}$ was generated via the Fenton reaction. TA serves as a probe for $\cdot\text{OH}$, given that it reacts with the latter, generating a fluorescent signal. Effective $\cdot\text{OH}$ scavenging decreases the fluorescence intensity. Figure S8 in the ESM further demonstrates a progressive decrease in fluorescence intensity with increasing Pt-Co IMC concentration, confirming a concentration-dependent radical-scavenging effect. Figure S9 in the ESM shows that Pt-Co IMCs reduced the fluorescence to an extent greater than afforded by Pt-C catalysts at the same concentration, indicating that the HORAC of Pt-Co IMCs was superior. These results suggest that Pt-Co IMCs may alleviate oxidative stress. To assess the overall ROS-scavenging performance further, the DCFH-DA assay was used to explore the action of Pt-Co IMCs under H_2O_2 -induced oxidative stress. Figure 3(d) shows that Pt-Co IMCs markedly reduced the fluorescence intensity compared to Pt-C catalysts, indicating more efficient elimination of intracellular ROS. EPR spectroscopy further confirmed the superior radical-scavenging capacity of Pt-Co IMCs. Both the $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ signals were weaker than when Pt-C catalysts were employed (Figs. 3(e) and 3(f)), consistent with the results described above. Collectively, these findings support the proposed catalytic mechanism. Pt-Co IMCs synergistically scavenge key ROS more effectively than monometallic Pt-C catalysts do.

3.3 DFT study of Pt-Co IMC catalytic activity

DFT calculations were performed to elucidate the origin of the enhanced CAT- and SOD-like catalytic activities of Pt-Co IMCs relative to Pt-C catalysts [53]. Although $\cdot\text{OH}$ scavenging was confirmed experimentally by HORAC and EPR assays, its ultrashort lifetime, nonselective reactivity, and rapid quenching make a well-defined surface-scavenging pathway difficult to model reliably. Therefore, the DFT calculations focused on the longer-

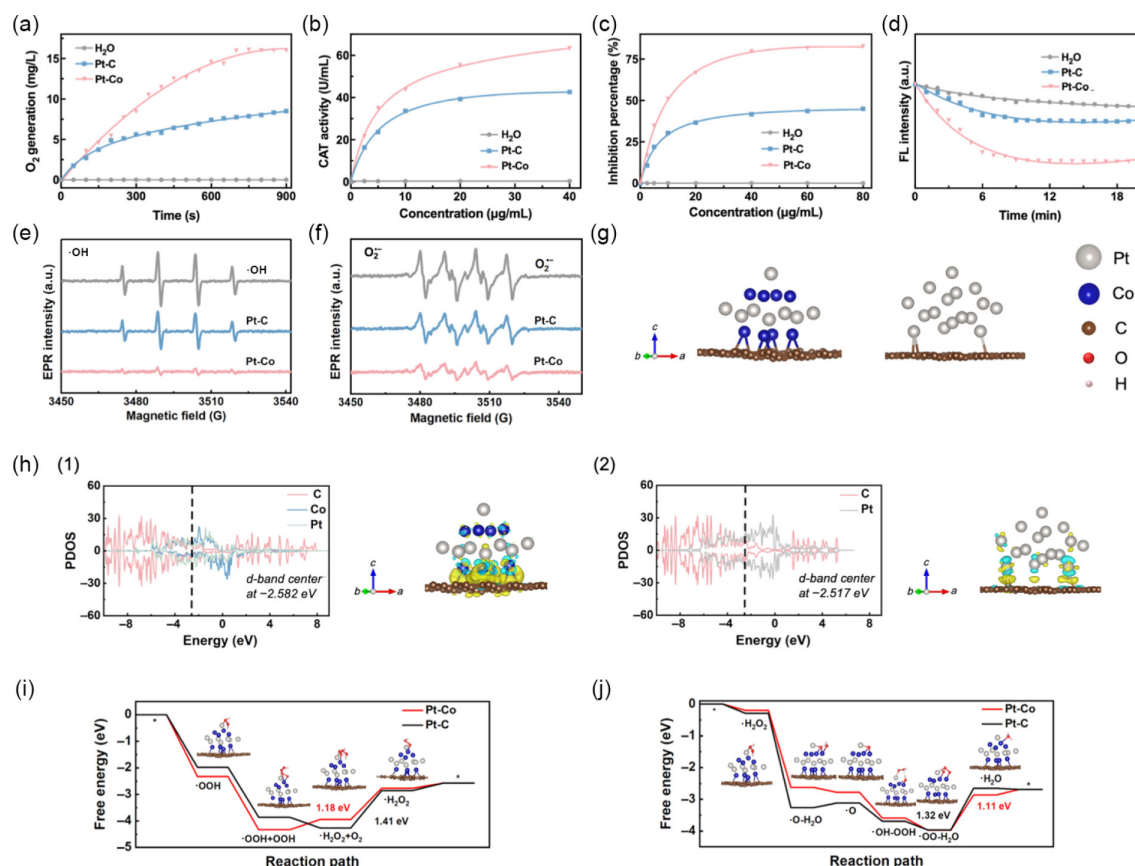


Figure 3 *In vitro* ROS-scavenging and mechanistic insights afforded by DFT of Pt-Co IMCs compared to Pt-C catalysts. (a) O_2 production over time during H_2O_2 decomposition by Pt-C catalysts and Pt-Co IMCs. (b) CAT-like activities of Pt-C catalysts and Pt-Co IMCs. (c) Percentage inhibition of O_2^- production by Pt-C catalysts and Pt-Co IMCs. (d) Time-dependent DCFH fluorescence intensities in the HORAC reactions. EPR assessment of $\cdot\text{OH}$ scavenging by (e) Pt-C catalysts and (f) Pt-Co IMCs. (g) DFT-optimized structures of Pt-Co IMCs and Pt-C catalysts supported on carbon (front view). (h) PDOS and differential charge density distributions (isosurface = 0.001 e/Bohr³) of (1) Pt-Co IMCs and (2) Pt-C catalysts. Yellow and cyan represent electron accumulation and depletion, respectively. The corresponding d-band centers, derived from the PDOS relative to the Fermi level, are indicated. (i) Free-energy diagram of the SOD-like catalytic pathway. Adsorbed H_2O_2 is explicitly distinguished as H_2O_2 (product) formed from O_2^- disproportionation. (j) Free-energy diagram of the CAT-like catalytic pathway. Adsorbed H_2O_2 is denoted as H_2O_2 (reactant), which undergoes stepwise decomposition into H_2O and O_2 .

lived $\text{O}_2^-/\text{H}_2\text{O}_2$ -related SOD- and CAT-like pathways as representative antioxidant reactions [54]. The optimized structural models (Fig. 3(g)) indicate that both Pt-Co IMCs and Pt-C catalysts exhibit favorable interfacial binding with the carbon support.

The differential charge density maps (Fig. 3(h)) show more pronounced interfacial charge redistribution in Pt-Co IMCs than in Pt-C catalysts, with clearer electron accumulation and depletion around the cluster-support contact, indicating stronger charge transfer and electronic coupling in the Pt-Co system.

Further insights into the electronic structure were obtained from PDOS analysis (Fig. 3(h)). PDOS analysis reveals that alloying with Co induces a clear downshift of the Pt d-band center from -2.517 eV for Pt-C catalysts to -2.582 eV for Pt-Co IMCs. This shift reflects an alloy-induced modulation of the Pt electronic structure, accompanied by a redistribution of d states relative to the Fermi level (E_F). Notably, the Pt-Co IMCs exhibit the emergence of Co-derived electronic states in the vicinity of E_F , which are absent in Pt-C catalysts. This leads to a distinctly modified electronic structure with altered d-state distribution near E_F , indicative of strong electronic interaction within the alloy framework. Such changes are expected to directly influence the surface electronic properties relevant to catalytic processes involving oxygen-containing intermediates [21, 55].

The kinetic implications of this electronic modulation were further evaluated by calculating free-energy profiles for both SOD-like and CAT-like reaction pathways (Figs. 3(i) and 3(j)). The corresponding DFT-optimized intermediate configurations for the SOD-like and CAT-like reaction pathways are shown in Figs. S10 and S11 in the ESM, respectively. In the SOD-like pathway (Fig. 3(i)), the largest uphill free-energy barrier is moderately reduced from 1.41 eV on Pt-C catalysts to 1.18 eV on Pt-Co IMCs. A similar trend is observed for the CAT-like pathway (Fig. 3(j)), where the corresponding value decreases from 1.32 to 1.11 eV. These reductions consistently demonstrate that the most energy-demanding steps are substantially facilitated on Pt-Co IMCs.

Importantly, both catalysts follow comparable reaction pathways, indicating that Co incorporation preserves the fundamental reaction mechanism while markedly improving the energetic landscape. The consistently lower energy requirements observed for Pt-Co IMCs across both reaction pathways highlight a robust and pathway-independent enhancement in catalytic efficiency.

Overall, the DFT results demonstrate that Co incorporation induces a pronounced modulation of the Pt electronic structure, as evidenced by the systematic downshift of the d-band center and the reconstruction of electronic states near E_F . This electronic modulation directly correlates with the substantially reduced free-

energy barriers in both SOD-like and CAT-like reactions, providing strong mechanistic support for the superior catalytic performance of Pt-Co IMCs.

3.4 Evaluation of ROS scavenging efficiency of Pt-Co IMCs in models of LPS-induced oxidative stress

Neutralization of excessive ROS produced by classically activated (M1) macrophages in periodontal lesions is essential for mitigating periodontitis progression [56]. Upon stimulation with bacterial LPS or oxidative stressors such as H_2O_2 , RAW264.7 macrophages polarize toward the pro-inflammatory M1 phenotype and generate abundant ROS together with inflammatory cytokines. These events lead to overactivation of NF- κ B signaling and suppression of Nrf2-mediated antioxidant defenses, thereby amplifying inflammation and aggravating periodontal tissue damage [57, 58]. First, we verified the biocompatibility of Pt-Co IMCs. Concentrations up to 10 μ g/mL caused no significant cytotoxicity in murine macrophage RAW264.7 cells, mouse pre-osteoblast MC3T3-E1 cells, human gingival fibroblasts (HGF), or mouse L929 fibroblasts, with all cell viabilities exceeding 90%; notably, at the working concentration of 10 μ g/mL, cell viabilities remained approximately 97% in all tested cell types (Figs. S12 and S13 in the ESM). Given this favorable cytocompatibility and the high catalytic efficiency of Pt-Co IMCs, 10 μ g/mL was selected as an effective working concentration for subsequent ROS-scavenging experiments. The reduced cell viability observed at higher concentrations may be attributed to increased cellular exposure to the metal-rich Pt/Co nanozyme, whereas higher-dose exposure was not required to achieve the desired antioxidative effect. A time-dependent increase in intracellular Cy5 fluorescence was observed from 1 to 4 h (Fig. S14 in the ESM), confirming efficient uptake of Pt-Co IMCs by RAW264.7 macrophages. This uptake enables Pt-Co IMCs to access intracellular ROS-rich microenvironments, providing a cellular basis for their strong ROS-scavenging activity at the low working concentration of 10 μ g/mL. Next, to assess whether Pt-Co IMCs could effectively suppress intracellular ROS accumulation, we analyzed ROS levels in macrophages using immunofluorescence imaging and flow cytometry. As shown in Figs. 4(a) and 4(b), LPS stimulation markedly elevated intracellular ROS levels in RAW264.7 macrophages, whereas treatment with Pt-C or Pt-Co IMCs (10 μ g/mL) effectively reduced ROS accumulation. Notably, Pt-Co IMCs exhibited a markedly stronger ROS-scavenging effect than monometallic Pt-C catalysts, substantially lowering intracellular ROS levels toward those of untreated controls. Consistently, flow cytometry analysis revealed that the proportion of ROS-positive cells decreased from 98.1% in the LPS-stimulated group to 85.5% after Pt-C treatment and 29.5% following Pt-Co IMC treatment (Fig. 4(c)). These results demonstrate that Pt-Co IMCs possess strong and efficient ROS-scavenging capability. Compared with Pt-C, the enhanced ROS-scavenging performance of Pt-Co IMCs is likely attributable to the synergistic interactions between the two metal sites, which promote electron transfer and optimize radical adsorption, thereby improving their overall catalytic efficiency [21, 59].

Building on these promising *in vitro* results, the antioxidative efficacy of Pt-Co IMCs was next evaluated *in vivo*. An oxidative stress model was established by injecting LPS into the gingival sulcus to induce a localized inflammatory environment (Fig. 4(d)). After Pt-Co IMC administration, gingival fluorescence markedly decreased to near-baseline levels, indicating efficient ROS

neutralization within the complex tissue environment (Figs. 4(e) and 4(f)). These *in vivo* outcomes were consistent with the cellular results, further confirming that Pt-Co IMCs effectively scavenge ROS and mitigate oxidative stress under physiological conditions. Mechanistically, the dual active centers of Pt-Co IMCs are proposed to work synergistically, lowering the reaction energy barrier for radical elimination and thereby enhancing antioxidative efficiency. Collectively, these results demonstrate that Pt-Co IMCs exhibit robust ROS-scavenging capacity both *in vitro* and *in vivo*, significantly outperforming Pt-C catalysts. Their strong antioxidative performance underscores the therapeutic potential for managing inflammation associated with oxidative stress.

3.5 Dual-pathway regulation and inflammatory resolution by Pt-Co IMCs

The NF- κ B and Nrf2 pathways are pivotal in terms of regulating inflammation and the antioxidant responses of periodontitis. Excessive ROS production drives NF- κ B hyperactivation and impairs Nrf2-mediated detoxification. To evaluate the therapeutic advantage afforded by Pt-Co IMCs compared to conventional Pt-C catalysts, we examined their regulatory effects on these two signaling pathways in LPS-stimulated RAW264.7 macrophages.

Immunofluorescence imaging showed that LPS stimulation induced strong nuclear accumulation of NF- κ B and weak cytoplasmic retention of Nrf2. In contrast, Pt-Co treatment markedly reduced NF- κ B nuclear translocation while promoting Nrf2 nuclear localization, as evidenced by decreased red fluorescence in the NF- κ B channel and intensified nuclear red signals in the Nrf2 channel (Figs. 4(g) and 4(h)). Quantitative analysis further revealed that Pt-Co IMCs reduced the LPS-induced NF- κ B fluorescence intensity by 63.6%, significantly outperforming Pt-C catalysts (49.4% reduction). Pt-Co IMCs markedly increased Nrf2 nuclear retention (4.1-fold relative to the LPS group) whereas Pt-C induced only a 1.9-fold increase (Figs. 4(i) and 4(j)). Such dual modulation highlights the superior capacity of Pt-Co IMCs in terms of suppressing pro-inflammatory signaling and enhancing the antioxidant defense.

To further elucidate the anti-inflammatory efficacy of Pt-Co IMCs, the expression levels of key pro-inflammatory cytokines, IL-1 β , IL-6, and TNF- α , were quantified by ELISA (Figs. 4(k)–4(m)) [60, 61]. After LPS stimulation, cytokine secretion was markedly elevated compared to that of the control, with IL-1 β , IL-6, and TNF- α levels increasing 3.94-, 2.91- and 2.19-fold, respectively. Pt-Co treatment significantly attenuated this inflammatory surge, reducing the IL-1 β , IL-6, and TNF- α levels by 61.5%, 42.3%, and 27.3%, respectively, relative to those of the LPS group. In contrast, Pt-C treatment yielded modest reductions of 17.1%, 18.8%, and 28.5%, respectively. Notably, Pt-Co IMCs exerted a 2.2-fold stronger inhibitory effect on IL-1 β secretion than Pt-C catalysts, underscoring the superior capacity of the former to suppress inflammasome-associated cytokine production. The pronounced reduction in IL-1 β is particularly significant, as recent studies have identified IL-1 β inhibition as a critical strategy for mitigating periodontal inflammation and preventing tissue destruction. We further compared the performance of Pt-Co IMCs and the commercial non-steroidal anti-inflammatory drug (NSAID) diclofenac sodium (DS) in reducing the expression of inflammatory factors. It was found that when treated at the same concentration of 10 μ g/mL, DS showed no significant anti-inflammatory effect, and the anti-inflammatory performance of Pt-Co IMCs was comparable

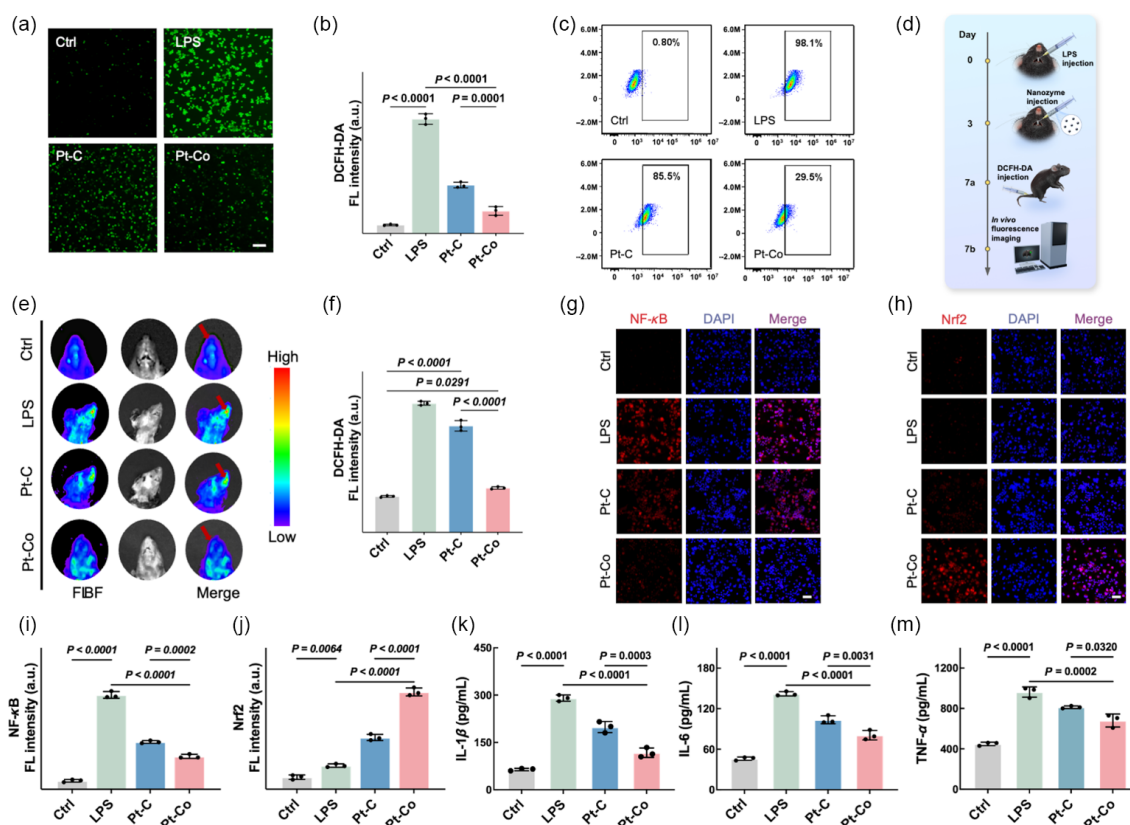


Figure 4 Antioxidant and anti-inflammatory effects of Pt-Co IMCs in LPS-induced oxidative stress models *in vitro* and *in vivo*. (a) Fluorescence microscopy images of DCFH-DA-stained RAW264.7 cells showing intracellular ROS levels after LPS stimulation and nanozyme treatment (scale bar: 50 μ m). (b) Quantification of intracellular ROS fluorescence intensity. (c) Flow cytometry analysis of intracellular ROS levels in RAW264.7 cells after LPS stimulation. (d) Schematic of LPS-induced periodontitis model and experimental timeline: LPS induction (day 0), nanozyme treatment (day 3), DCFH-DA probe injection at day 7 (day 7a), and subsequent *in vivo* fluorescence imaging at day 7 (day 7b). (e) *In vivo* fluorescence imaging of ROS levels in periodontal tissues following different treatments. (f) Quantitative analysis of *in vivo* fluorescence intensity. (g) CLSM images showing NF- κ B expression in RAW264.7 cells (scale bar: 100 μ m). (h) CLSM images showing Nrf2 expression in RAW264.7 cells (scale bar: 100 μ m). Quantitative analysis of (i) NF- κ B and (j) Nrf2 fluorescence intensities. (k) IL-1 β , (l) IL-6, and (m) TNF- α levels in RAW264.7 cells following LPS induction and nanozyme treatment, measured by ELISA. Data are presented as mean $\pm$ SD. All experiments were repeated at least three times. Statistical significance was determined by one-way ANOVA.

to that of DS at a concentration 100 times higher (Fig. S15 in the ESM). The results are consistent with the enhanced ROS scavenging and dual-pathway (NF- κ B/Nrf2) modulation of Pt-Co IMCs, which together reduce pro-inflammatory signaling and cytokine release. Mechanistically, the enhanced ROS scavenging by Pt-Co IMCs disrupts I κ B kinase beta (I κ K β)-mediated NF- κ B activation and stabilizes Nrf2, facilitating transcription of cytoprotective genes [62]. In contrast, the weaker ROS clearance capacity of the Pt-C group resulted in only partial NF- κ B inhibition and inadequate Nrf2 activation.

Collectively, these findings underscore the unique ability of Pt-Co IMCs to coordinately inhibit inflammation and restore redox homeostasis, positioning Pt-Co IMCs as a promising nanotherapeutic platform for oxidative-stress-related inflammatory diseases such as periodontitis.

3.6 Dual immunomodulatory and osteoinductive functions of Pt-Co IMCs under oxidative stress

Macrophage polarization plays a key role in the pathogenesis and resolution of periodontitis. M1 polarization exacerbates inflammation and tissue destruction, whereas M2 polarization promotes tissue repair and is anti-inflammatory [63, 64]. Modulation of polarization may therapeutically combat periodontal

inflammation and promote regeneration [65]. To investigate the immunomodulatory effects of Pt-Co IMCs in the context of inflammation, we first assessed macrophage polarization status using immunofluorescence staining. LPS-stimulated RAW264.7 cells exhibited strong red fluorescence corresponding to iNOS and weak green fluorescence for CD206, indicating predominantly M1 polarization. After Pt-Co treatment, iNOS fluorescence markedly decreased, whereas CD206 fluorescence was strongly enhanced, implying a phenotypic shift from the pro-inflammatory M1 to the anti-inflammatory M2 phenotype. Pt-C treatment induced only modest changes in marker expression, indicating limited regulatory capacity (Figs. 5(a)–5(d)). Flow cytometry further confirmed these observations. Pt-Co IMC treatment significantly reduced the proportion of iNOS⁺ macrophages and increased the CD206⁺ population (Figs. 5(e) and 5(f)). These findings demonstrate that Pt-Co IMCs effectively reprogram macrophage polarization toward an anti-inflammatory phenotype. Mechanistically, this effect is likely mediated by inhibition of NF- κ B activation together with Nrf2 stabilization, which cooperatively facilitates M2 differentiation. These results underscore the ability of Pt-Co IMCs to modulate innate immune responses and establish a favorable microenvironment for the resolution of inflammation and subsequent tissue regeneration.

Given the critical role of osteogenesis in periodontal repair and

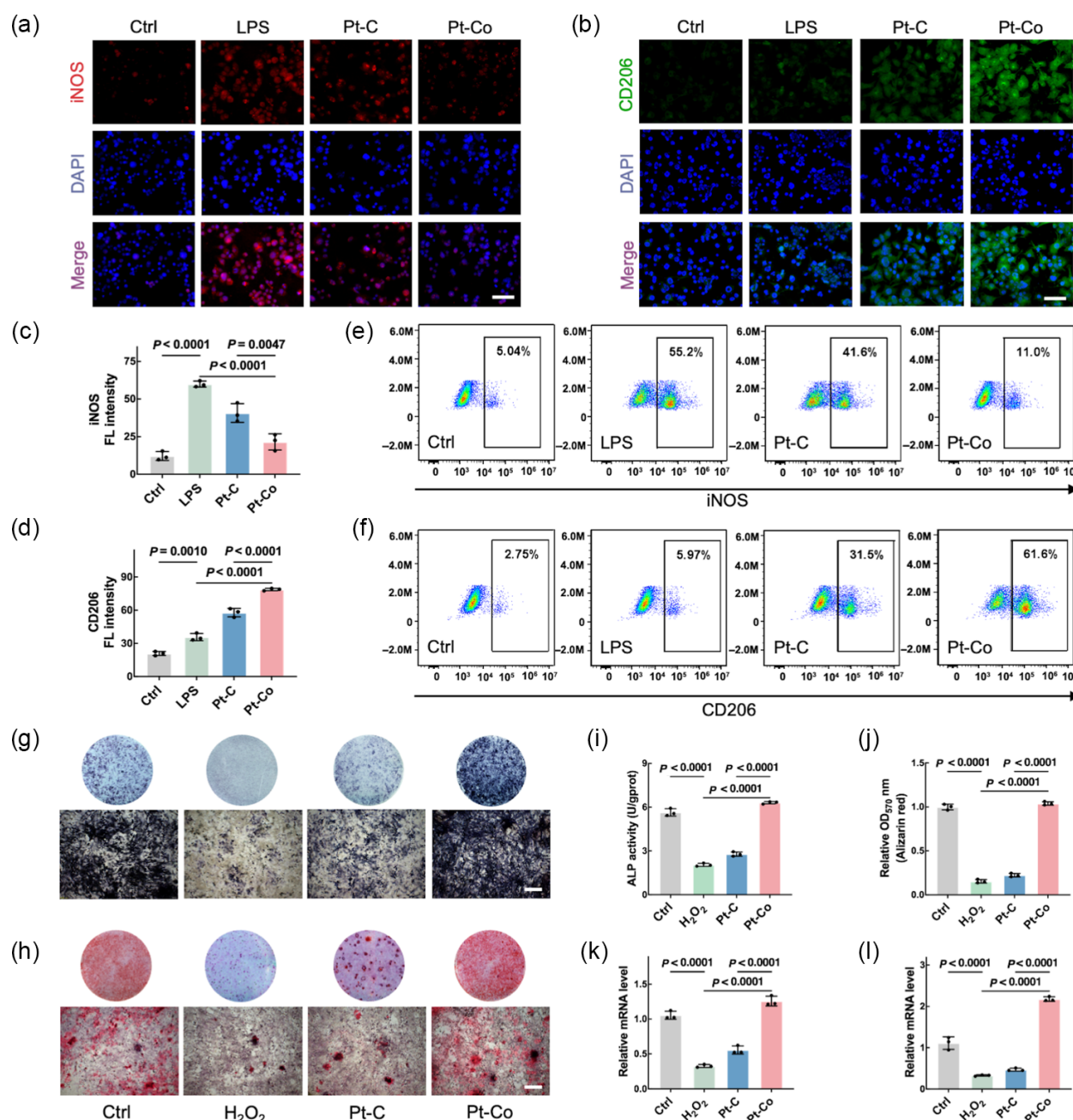


Figure 5 Pt-Co IMCs promoted macrophage polarization from M1 to M2 phenotype and enhanced osteogenic activity. (a) CLSM images showing iNOS immunofluorescence in macrophages (scale bar: 100 μ m). (b) CLSM images showing CD206 immunofluorescence in macrophages. (c) Quantified fluorescence intensity of iNOS. (d) Quantified fluorescence intensity of CD206. Flow cytometry analysis of (e) iNOS⁺ (M1) and (f) CD206⁺ (M2) macrophage populations. (g) ALP staining of MC3T3-E1 preosteoblasts incubated with Pt-C catalysts or Pt-Co IMCs on day 7 to assess osteoblast differentiation (scale bar: 500 μ m). (h) ARS staining of MC3T3-E1 preosteoblasts incubated with Pt-C catalysts or Pt-Co IMCs on day 21 to evaluate mineralized nodule formation (scale bar: 500 μ m). Quantitative analysis of (i) ALP activity and (j) calcium deposition determined by ARS staining. Relative mRNA levels of the osteogenic markers (k) *Runx2* and (l) *Col1* in MC3T3-E1 preosteoblasts incubated with Pt-C catalysts or Pt-Co IMCs on day 21. Data are expressed as mean $\pm$ SD ($n = 3$). Statistical significance was determined by one-way ANOVA.

the beneficial effect of M2 macrophage polarization on bone regeneration, we next assessed the osteogenic potential of Pt-Co IMCs under oxidative stress. MC3T3-E1 preosteoblasts were incubated with Pt-C catalysts or Pt-Co IMCs and subsequently subjected to ALP and ARS staining to evaluate osteogenic differentiation and matrix mineralization. ALP activity, an early marker of osteoblast differentiation, was significantly elevated in Pt-Co-treated cells compared with both the control and Pt-C groups, indicating enhanced osteogenic activity (Figs. 5(g) and 5(i)).

Similarly, ARS staining revealed increased mineralized nodule formation in the Pt-Co group, further supporting its superior osteoinductive capacity (Figs. 5(h) and 5(j)). qRT-PCR analysis confirmed these findings by showing significant upregulation of key osteogenic marker genes, including *Runx2* and *Col1*, in the Pt-Co-treated group (Figs. 5(k) and 5(l)) [66]. The primer sequences used for qRT-PCR are listed in Table S5 in the ESM. Specifically, the *Runx2* expression level was approximately 1.96-fold higher in the Pt-Co group than in the control group, and *Col1* expression also

exhibited a significant increase, reflecting enhanced osteogenic differentiation and collagen production. Together, these results demonstrate that Pt-Co IMCs effectively promoted bone mineralization and osteogenesis, particularly under oxidative stress, highlighting their potential as a multifunctional nanotherapeutic for bone regeneration in inflammatory microenvironments.

3.7 *In vivo* effects of Pt-Co IMCs on bone regeneration and periodontitis-associated inflammation

To further evaluate the therapeutic potential of Pt-Co IMCs *in vivo*, a periodontitis model was established by placing ligatures around the second maxillary molars of mice [67]. The mice then received intralingual injections of either Pt-C catalysts, Pt-Co IMCs (both 10 $\mu\text{g}/\text{mL}$), or saline over 2 weeks. Given the acidic characteristics of the inflammatory periodontal microenvironment, Co^{2+} release from Pt-Co IMCs was further quantified by ICP-OES in PBS at pH 7.4 and 5.5. Greater Co^{2+} release was observed at pH 5.5, suggesting that the therapeutic effect may involve an integrated mechanism combining surface-catalyzed redox regulation with acid-facilitated Co^{2+} contribution to periodontal bone repair (Fig. S16 in the ESM). After treatment, the maxillae and major organs were harvested for analysis (Fig. 6(a)). Micro-CT and histological staining were employed to assess the therapeutic efficacy of Pt-Co IMCs *in vivo*. The vertical distance between the ABC and CEJ serves as a critical parameter when evaluating bone regeneration and tissue repair. As shown in Figs. 6(b) and 6(c), three-dimensional reconstruction of the micro-CT images revealed that the ligation group exhibited a significantly increased ABC-CEJ distance, indicating substantial bone loss and confirming successful establishment of the periodontitis model. Consistently, quantitative bone morphometric parameters, including BV/TV, Tb.Th, Tb.N, and Tb.Sp, showed the typical pattern of bone degradation, with reduced BV/TV, Tb.Th, and Tb.N and elevated Tb.Sp values (Figs. 6(d)–6(g)). In contrast, the Pt-Co group exhibited significant improvements, with a notable reduction in the ABC-CEJ distance; increases in BV/TV, Tb.Th, and Tb.N; and a decrease in Tb.Sp, indicating enhanced bone regeneration and repair. These results imply that Pt-Co IMCs effectively prevent alveolar bone loss and promote new bone formation. Histological examination (H&E and Masson's trichrome staining) revealed substantial collagen loss and inflammatory cells in the ligation group, whereas Pt-Co treatment promoted collagen deposition and alleviated inflammation, consistent with the improved bone parameters (Figs. 6(h) and 6(i)). These findings collectively support the conclusion that Pt-Co IMCs significantly promote bone regeneration and tissue repair, and may serve as a useful therapeutic for periodontitis and other inflammatory bone disorders.

Immunohistochemical staining for IL-1 β was performed on periodontal tissue sections to evaluate the *in vivo* anti-inflammatory effects of Pt-Co IMCs (Fig. S17(a) in the ESM). In the ligation group, IL-1 β expression was markedly elevated, indicating significant inflammation. In contrast, Pt-Co treatment markedly reduced IL-1 β expression compared with both the ligation and Pt-C groups, demonstrating a pronounced attenuation of the inflammatory response. These findings further confirm the potent anti-inflammatory capacity of Pt-Co IMCs and suggest that they mitigate the tissue damage typically associated with periodontitis. To investigate the underlying molecular mechanisms, immunofluorescence staining for Nrf2 and NF- κB 1 was next performed to assess the status of key inflammatory pathways (Figs. S17(b) and S17(c) in the ESM). In the ligation group, NF- κB 1

expression was substantially upregulated, reflecting activation of the pro-inflammatory NF- κB pathway. Pt-Co treatment notably suppressed NF- κB 1 fluorescence, indicating effective inhibition of NF- κB activation. Meanwhile, Nrf2 expression was strongly enhanced in the Pt-Co treatment group, suggesting that Pt-Co IMCs promoted antioxidant responses via Nrf2 upregulation. This coordinated suppression of NF- κB and activation of Nrf2 by Pt-Co IMCs is consistent with the dual-pathway regulation previously observed *in vitro*, supporting the role of Pt-Co IMCs in alleviating inflammation and promoting tissue regeneration by improving cellular antioxidant defenses.

The *in vivo* biocompatibility of Pt-Co IMCs was assessed by H&E staining of major organs, including the heart, liver, spleen, lungs, and kidneys (Fig. S18 in the ESM). No significant abnormality or inflammation was apparent in any of the treatment groups, indicating that Pt-Co IMCs were biocompatible *in vivo*. In addition, Pt-Co IMCs did not induce significant hemolysis at concentrations up to 15 $\mu\text{g}/\text{mL}$ (Fig. S19 in the ESM). Collectively, these results demonstrate that Pt-Co IMCs possess a favorable safety profile, with no detectable adverse effects on major organs or red blood cells. The combination of potent therapeutic efficacy and favorable biocompatibility underscores their promise as a nanotherapeutic platform for inflammation control and bone regeneration.

However, the current administration strategy involving repeated gingival injections should be considered a proof-of-concept approach rather than a clinically practical protocol, as such repeated local injections may cause patient discomfort and poor compliance. For future clinical translation, however, the delivery format of free Pt-Co IMCs should be further optimized to improve local retention and dosing convenience in the dynamic periodontal microenvironment. Incorporating Pt-Co IMCs into mucoadhesive hydrogels, periodontal pocket inserts, or sustained-release scaffolds may help reduce administration frequency and enhance patient acceptability while preserving their low-dose catalytic efficacy [68].

4 Conclusions

In this study, we developed a Pt-Co bimetallic nanozyme (Pt-Co IMCs) with synergistic antioxidant, anti-inflammatory, and osteogenic properties for the treatment of periodontitis. By leveraging its atomic-level ordered alloy structure and the inherent synergistic effects of the bimetallic system, the nanozyme enhanced free radical adsorption and reduced the activation energies of catalytic reactions, thereby accelerating SOD- and CAT-like catalytic processes. This endowed Pt-Co IMCs with exceptional ROS-scavenging capability *in vitro*. Cobalt may further contribute to osteogenic recovery by supporting osteogenesis-related gene expression. Pt-Co IMCs effectively modulate the Nrf2/NF- κB signaling pathway, facilitating polarization of macrophages from the pro-inflammatory M1 to the anti-inflammatory M2 phenotype. This dual regulation not only reduces the secretion of inflammatory cytokines but also creates an environment favorable for tissue regeneration, thereby enhancing periodontal healing. Consistently, *in vivo*, Pt-Co treatment significantly alleviated periodontal inflammation and promoted bone regeneration, validating its therapeutic efficacy. Overall, this multifunctional nanozyme enables precise regulation of inflammation and bone healing, offering an innovative approach for the treatment of periodontitis and other redox-related diseases, and represents a promising candidate for future clinical translation.

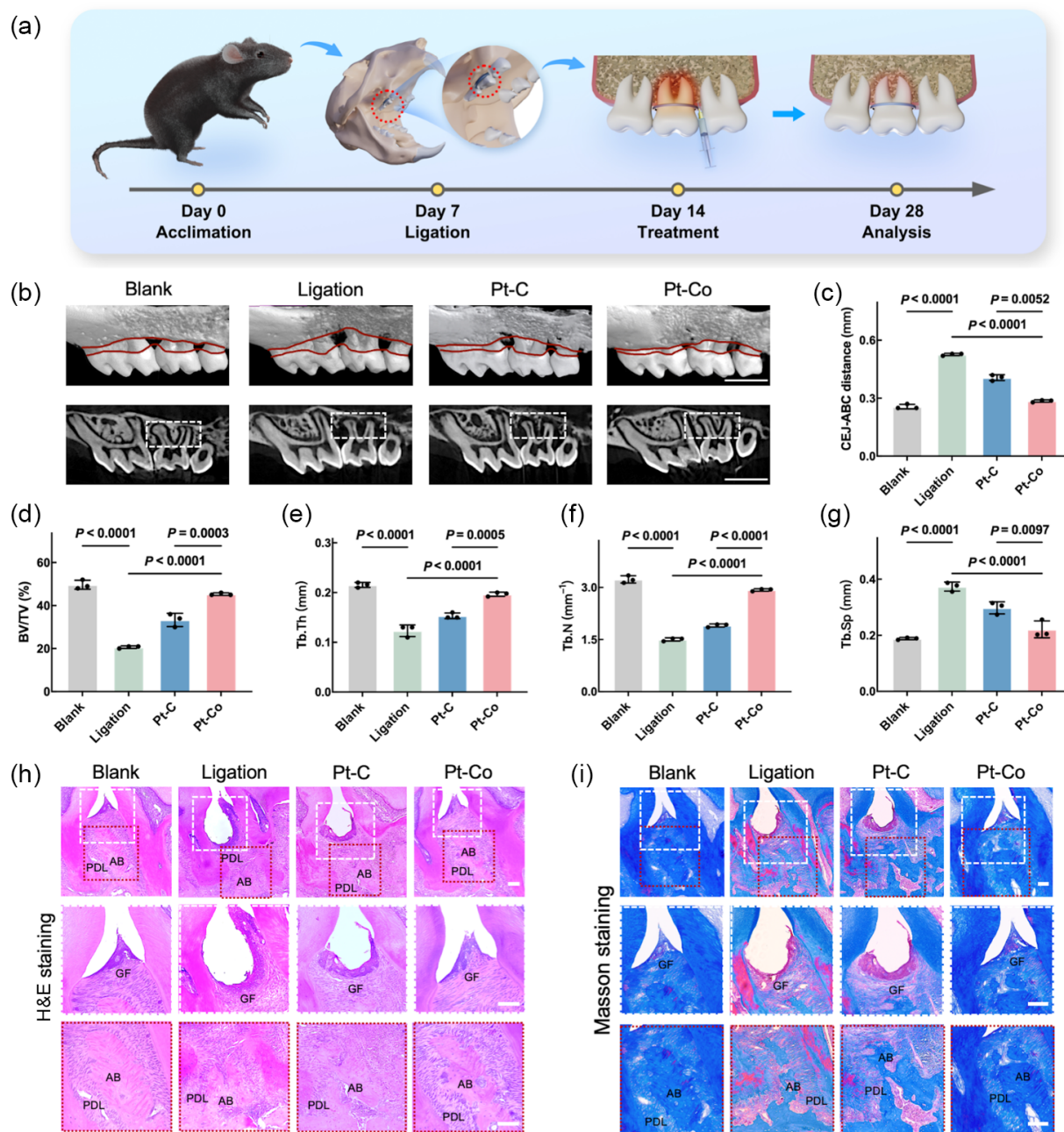


Figure 6 *In vivo* bone regeneration and histological evaluation of the periodontium following 2 weeks of nanozyme treatment. (a) Schematic illustration of the establishment and treatment procedure of the mouse periodontitis model. (b) Micro-CT three-dimensional reconstructions and buccopalatal section images of maxillary molars undergoing different treatments. Red lines indicate the distances between the ABC and CEJ (scale bar: 1 mm). (c) Quantification of the ABC-CEJ distance. Quantitative analysis of alveolar bone parameters, including (d) BV/TV, (e) Tb.Th, (f) Tb.N, and (g) Tb.Sp. (h) H&E and (i) Masson's trichrome-stained images of periodontium tissue showing collagen deposition and bone architecture. PDL: periodontal ligament (scale bar: 50 μ m), AB: alveolar bone, GF: gingival fiber. Data are expressed as mean $\pm$ SD ($n = 3$).

Electronic Supplementary Material: Supplementary material (further details of TEM imaging, XRD pattern, EXAFS fitting spectrum, XPS spectrum, catalytic performances, DFT calculations, and biosafety assessments) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908895>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and/or the ESM. Additional data

related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

Z. H.: Data curation, formal analysis, investigation, writing – original draft. Y. Z.: Data curation, investigation. Y. K. L.: Data curation, investigation, writing – review & editing. Y. P.: Investigation. Y. P. Q.: Investigation. X. B. M.: Resources. T. G.: Resources. Y. K. C.: Investigation. Z. H. W.: Investigation. Y. G. W.: Investigation, funding acquisition. T. H. D.: Resources. L. D. Z.: Conceptualization, supervision, funding acquisition, writing – review & editing. P. J.: Conceptualization, supervision, writing – review & editing. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

All animal experiments were carried out in accordance with the ethical guidelines of the Peking University School of Stomatology Research Ethics Committee and were approved by the Committee (approval number: PUIRB-LA2023136).

Use of AI statement

None.

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