

Bonyzymes: Efficient Anti-Inflammatory, Antibacterial and Osteogenic Agents for Peri-Implantitis Reconstruction Treatment

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Peri-implantitis is an inflammatory disease characterized by progressive bone loss, challenging implant longevity and incurring significant treatment burden. Current reconstruction therapy involves mechanical scaling followed by artificial bone grafting. However, residual bacteria and inflammation memory often lead to recurrence, highlighting the critical need for the artificial bone substitute with sustained anti-inflammatory and antibacterial properties. By utilizing the phosphate groups of mesoporous hydroxyapatites for in situ loading of CeO_2 , followed by a reduction method to fabricate a CeO_2 -Cu heterojunction interface, this study develops Bonyzymes with anti-inflammatory, antibacterial, and osteogenic properties. The incorporation of Cu modulates the electronic environment of the CeO_2 surface, increasing superoxide dismutase and catalase activities of Bonyzymes. In vitro, the Bonyzymes effectively regulated oxidative stress by scavenging excess reactive oxygen species, exerting anti-inflammatory effects. Additionally, it exhibits antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Porphyromonas gingivalis*, and facilitates osteogenic differentiation in bone marrow stem cells. In vivo experiments in rat peri-implantitis models confirm its ability to promote bone regeneration in the infected peri-implant area. Overall, this work develops novel artificial bone substitutes, Bonyzymes, which not only promotes bone regeneration but also exhibits anti-inflammatory and antibacterial properties, offering a promising strategy for peri-implantitis treatment.

1. Introduction

As the global number of dental implants increases, peri-implantitis is a growing and troublesome problem.^[1] It is reported that the prevalence of peri-implantitis is 19.83% at the patient level, with an incidence rate as high as 43.9% within 5 years after final restoration.^[2] Following the onset of peri-implantitis, the peri-implant region develops into a complex microenvironment characterized by bacterial infection, inflammatory infiltration, and a cascade of cellular and molecular event, leading to peri-implant tissue destruction.^[3] Reconstructive therapy is a crucial approach for peri-implantitis management, enabling implant surface decontamination and promoting osseointegration with artificial bone grafting materials.^[4] However, current reconstructive therapy approaches still face significant challenges. First, the current methods for implant surface decontamination demonstrate limited efficacy. Physical decontamination methods fail to completely remove bacteria residing deep within implant threads, while

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chemical agents provide only transient antimicrobial effects and pose the risk of fostering bacterial resistance.^[5] Additionally, widely used artificial bone materials, such as inorganic bovine bone, primarily function through osteoconduction and lack anti-inflammatory and antibacterial properties, limiting their effectiveness in promoting bone regeneration within complex inflammatory microenvironment.^[6]

In the progression of peri-implantitis, oxidative stress is closely related to the development of inflammation as well as the destruction of bone tissue.^[7] It is reported that oxidative stress markers were highly expressed in salivary secretions of patients with peri-implantitis, and oxidative stress-related genes showed high transcript levels in peri-implantitis samples.^[8] Most current research on the treatment or prevention of peri-implantitis focused on surface modifications of implants, such as loading zinc oxide (ZnO) nanoparticles,^[9] silver nanoparticles,^[10] or cerium oxide (CeO₂) nanoparticles^[11] onto the implant surface. While these surface modifications significantly enhanced the antibacterial and anti-inflammatory capabilities of implants by utilizing nanozymes, there may be certain risks to the osseointegration and long-term stability of non-pure titanium surface implants.^[12] Moreover, this approach did not offer a direct solution for treating peri-implantitis in patients with clinically used implants. To treat infectious bone defects, composite bone grafting materials have been developed, such as those using hydroxyapatite^[13] or demineralized bone matrix as carriers,^[14] loaded with antibiotics like vancomycin,^[13b] curcumin,^[14] and melatonin^[13a] to impart antimicrobial properties to the bone grafting materials. However, these materials lack significant anti-inflammatory properties, raising concerns about their effectiveness in treating complex inflammatory conditions such as periodontitis and peri-implantitis.

Recent studies have shown that nanozymes hold promise in treating bacterial infections and controlling inflammation.^[15] Nanozymes are a class of inorganic materials that can exhibit enzyme properties at the nanoscale (1–100 nm) and can efficiently catalyze enzyme substrates under mild conditions.^[16] The catalytic activities of nanozymes mainly include superoxide dismutase (SOD)-like activity and catalase (CAT)-like activity.^[17] This is attribute to the presence of empty *d* or *f* orbital in transition metal and lanthanide metal, which enable the formation of coordinating bonds with substrate molecules.^[18] Consequently, these interactions facilitate the form of transition state with a lower energy barrier.^[18] As a result, nanozymes effectively reduce the activation energy of the overall reaction and accelerate the chemical processes. The catalytic activity of nanozymes is closely related to oxidative stress regulation, and scavenging excessive reactive oxygen species (ROS) to maintain the oxidative stress balance.^[19] The surface modifications of implants mentioned above utilized the properties of nanozymes to enhance the antibacterial and anti-inflammatory capabilities of implants. Meanwhile, nanozymes have become a key focus in the treatment of periodontitis, an inflammatory bone loss disease caused by bacterial infection, which is similar to peri-implantitis.^[20] Currently, nanozymes such as CeO₂,^[21] manganese dioxide (MnO₂),^[22] copper-based nanozymes^[23] have been developed for the treatment of periodontitis. However, current materials for periodontitis as well as peri-implantitis failed to regulate the electronic environment of nanozymes, which plays a critical role in determin-

ing their catalytic efficiency and biological function. Additionally, the tissue regeneration effects of these materials primarily relied on the regulation of osteoblasts by metal ions released, rather than the osteoconductive, osteoinductive, and osteogenic properties typical of conventional bone substitutes.

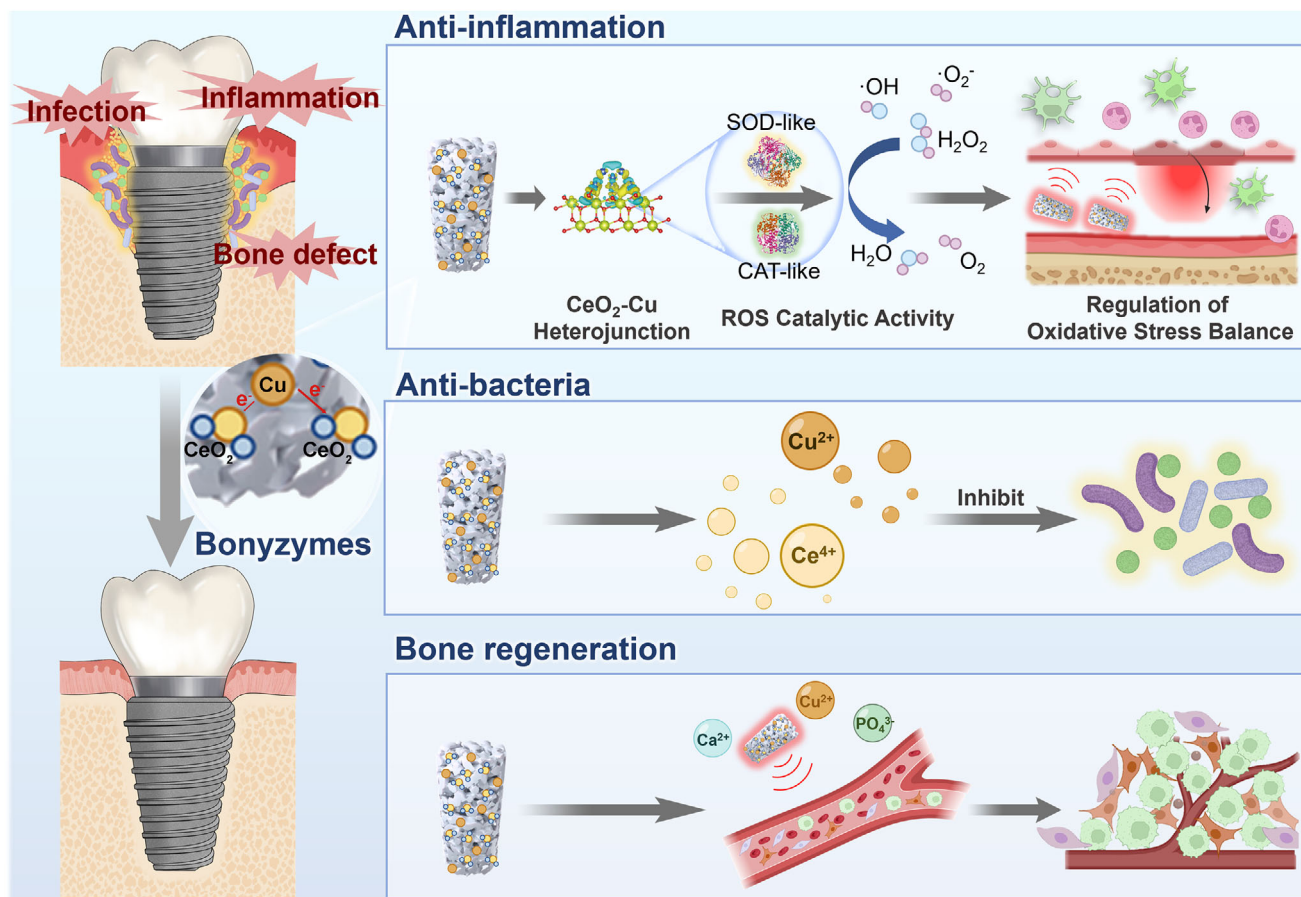
In this study, we developed a novel class of biomaterials termed “Bonyzymes,” mesoporous hydroxyapatite (MHA) scaffolds integrated with CeO₂-Cu nanozymes, as a therapeutic strategy for peri-implantitis reconstruction. The name “Bonyzyme” reflected the material’s dual functionality in bone regeneration, enzyme-like catalytic anti-inflammatory property. MHA, a widely recognized bone substitute, was used as the carrier due to its high surface area, excellent loading capacity, and superior biocompatibility, bioactivity, as well as its osteoconductive and osteoinductive properties.^[24] The Bonyzymes were fabricated by in situ loading of CeO₂ onto MHA via phosphate groups, followed by a reduction process to create a CeO₂-Cu heterojunction interface. As illustrated in **Scheme 1**, this novel artificial bone exhibited enhanced antibacterial, anti-inflammatory, and osteogenic properties. It addressed the complex challenges of peri-implantitis by scavenging excess ROS to restore oxidative stress balance, releasing metal ions for antibacterial effects, and promoting bone tissue regeneration through combined osteoconductive, osteoinductive, and osteogenic effects in the infected peri-implant area.

2. Results

2.1. Synthesis and Characterization of Bonyzymes

For the synthesis of Bonyzymes (MHA-CeO₂-Cu), MHA was synthesized via a hydrothermal reaction method, followed by the sequential loading of CeO₂ and Cu onto MHA through high-temperature calcination and hydration hydrazine reduction, respectively. The detailed synthesis procedure was illustrated in **Figure 1A**. As illustrated by field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) images, MHA formed rod-shaped aggregates, where CeO₂ and Cu were dispersed on the surface of MHA and within its mesoporous structure (Figure 1B,C). High resolution transmission electron microscope (HRTEM) further identified three distinct lattice spacings on MHA-CeO₂-Cu: 0.28 nm (corresponding to the (211) crystal plane of HA),^[25] 0.31 nm (corresponding to the (111) crystal plane of CeO₂),^[26] and 0.21 nm (corresponding to the (111) crystal plane of Cu) (Figure 1D).^[27] The successful loading of Ce and Cu onto MHA was also verified by the corresponding profile of energy-dispersive X-ray spectroscopy (EDS) (Figure 1E and Figure S1, Supporting Information).

X-ray diffraction (XRD) analysis was employed to reveal the crystal structure of the samples. As shown in Figure 1F, MHA only displayed diffraction peaks for Ca₅(PO₄)₃(OH).^[28] As for MHA-CeO₂, diffraction peaks for both Ca₅(PO₄)₃(OH) and CeO₂ were present,^[29] while in MHA-CeO₂-Cu, additional peaks representing Cu were detected.^[30] Meanwhile, Figure 1G showed the X-ray photoelectron spectroscopy (XPS) survey spectra of the surface across samples, confirming the presence of Ca, P, O, Ce, and Cu elements on MHA-CeO₂-Cu. The high-resolution XPS spectra qualitatively illustrated shifts in the Ce 3d XPS spectra for MHA-CeO₂ and MHA-CeO₂-Cu, in which MHA-CeO₂-Cu showed higher characteristic peaks of Ce³⁺ (Figure 1H).



Scheme 1. Schematic illustration of the application and bioactive functions of bonyzymes for peri-implantitis treatment.

Quantitative analysis revealed that upon the incorporation of Cu, the surface concentration of Ce^{4+} (CeO_2) decreased from 65.44% to 60.09%, while Ce^{3+} (CeO_2) increased from 34.56% to 39.94% (Figure 1I). High-resolution XPS analysis of the Cu 2p region revealed the presence of two distinct chemical states of Cu: metallic copper (Cu^0) and copper oxide (Cu^{2+}) (Figure S2, Supporting Information). The detection of CuO was attributed to the formation of Cu-O bonds at the CeO_2 -Cu heterojunction interface, where Cu was coordinated to CeO_2 via interfacial oxygen atoms.

To enhance the loading capacity of the Bonyzymes, MHA were synthesized with ordered mesoporous structures. The Brunauer-Emmett-Teller (BET) isotherms were conducted according to the previous study,^[31] suggesting that all three materials exhibited type IV isotherms, which indicated all three samples as mesoporous materials (Figure S3, Supporting Information). Further results revealed that the loading of CeO_2 and Cu onto MHA led to a reduction in its BET surface area (Table S1, Supporting Information). The alteration in the surface area reflected the incorporation of CeO_2 and Cu within the framework of MHA.

Meanwhile, in vitro degradation experiment demonstrated that MHA- CeO_2 -Cu exhibited considerable stability in a 37 °C aqueous environment (Figure S4 and Table S2, Supporting Information). After 14 days of immersion, SEM revealed no significant changes on surface morphology, and the nanozymes remained firmly attached. Correspondingly, ion release analysis showed

that MHA- CeO_2 -Cu exhibited a certain degree of Ca release in the in vitro aqueous environment, and the release of Ce and Cu was negligible after 14 days. These findings suggested that MHA- CeO_2 -Cu can provide sustained structural support and catalytic activity in vivo.

2.2. Antioxidant Performance and Mechanism of Bonyzymes

Figure 2A–D illustrated the catalytic activities of the three kinds of bone grafting materials. The results demonstrated that MHA- CeO_2 -Cu exhibited markedly higher CAT-like activity (Figure 2A), SOD-like activity (Figure 2B), superoxide scavenging ability (Figure 2C), and overall antioxidant capacity (Figure 2D), compared to MHA and MHA- CeO_2 groups.

The experimental data demonstrated that MHA- CeO_2 -Cu exhibited the most efficient CAT-like and SOD-like ROS scavenging capabilities. To elucidate the catalytic mechanism underlying ROS elimination, systematic density functional theory (DFT) calculations were performed by constructing comparative models of pristine CeO_2 (111) surfaces and CeO_2 -Cu heterojunction interfaces (Figure 2E).

Following model construction, the SOD-like and CAT-like reaction pathways were calculated on CeO_2 surface sites and CeO_2 -Cu heterojunction sites. The SOD-like reaction pathway revealed

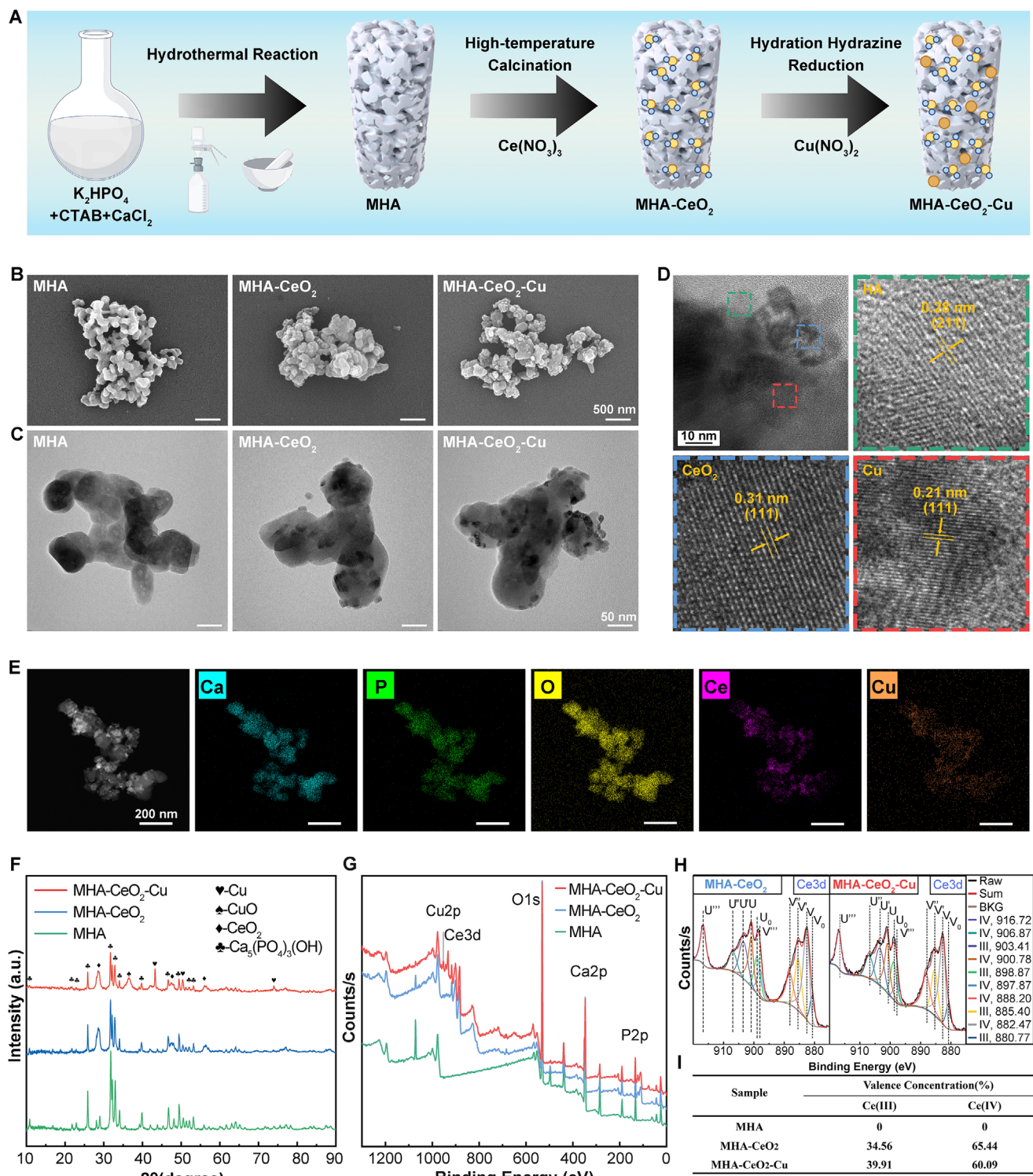


Figure 1. Characterizations of MHA-CeO₂-Cu. A) Schematic illustration of the synthesis process of MHA-CeO₂-Cu. B) SEM images of samples. C) TEM images of samples. D) HRTEM images of MHA-CeO₂-Cu with lattice spacings characteristic of hydroxyapatite, CeO₂, and Cu. E) TEM images of MHA-CeO₂-Cu with elemental distributions of Ca, P, O, Ce and Cu. F) XRD measurement. G) XPS survey spectra. H) Fitted Ce 3d XPS spectra of MHA-CeO₂ and MHA-CeO₂-Cu. I) Analysis of the valence state concentrations of Ce (IV) and Ce (III).

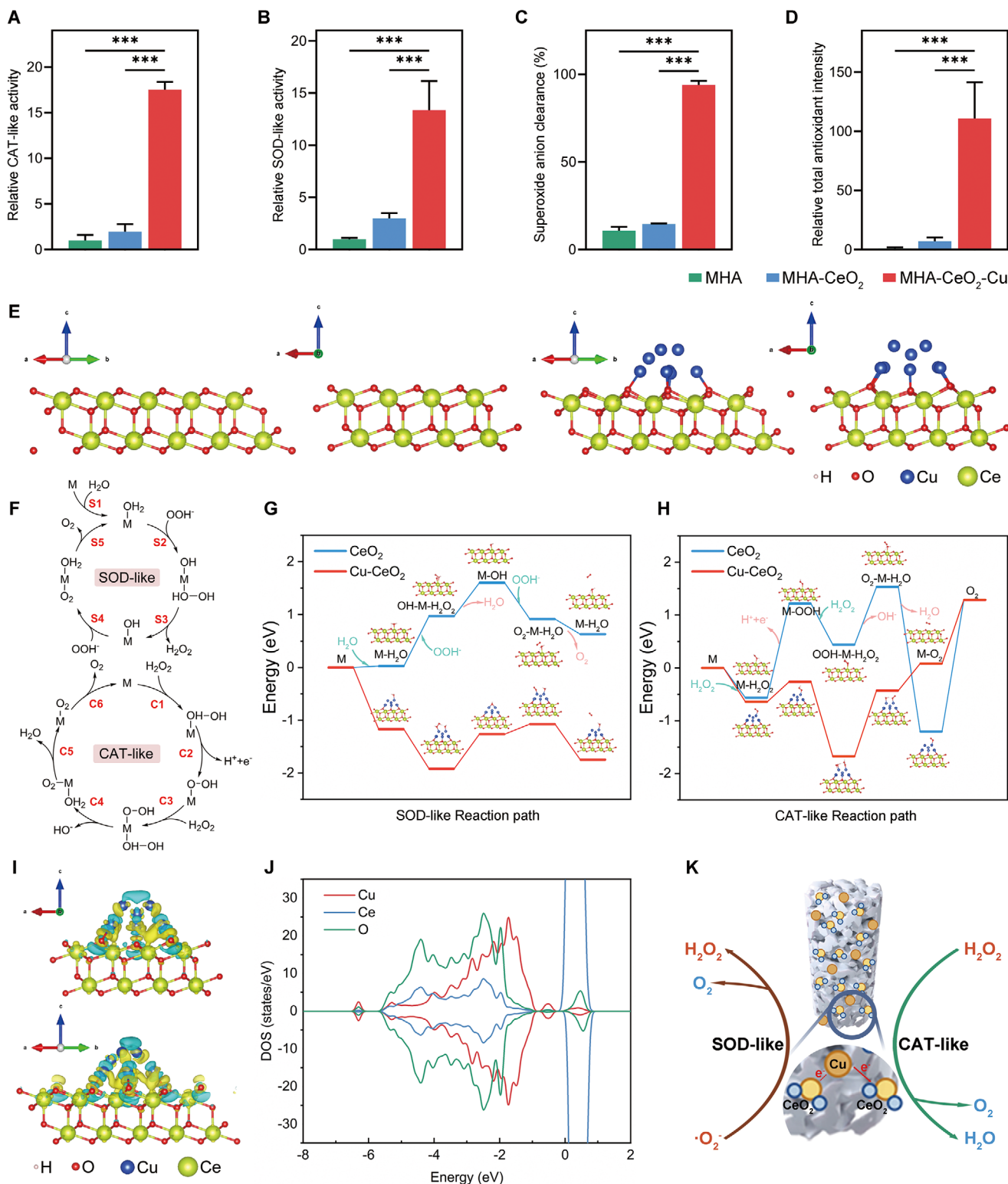


Figure 2. Antioxidant performance and mechanism of MHA-CeO₂-Cu. A) Analysis of CAT-like activity (n = 5). B) Analysis of SOD-like activity (n = 5). C) Analysis of superoxide anion clearance capacity (n = 5). D) Analysis of total antioxidant capacity (n = 5). E) The calculated models of pristine CeO₂ surface (left1, left2) and CeO₂-Cu heterojunction (right1, right2). F) The reaction mechanism of SOD-like (top) and CAT-like (bottom) reaction: M, metal sites. G) The theoretical calculation for SOD-like path of CeO₂ and CeO₂-Cu. H) The theoretical calculation for CAT-like path of CeO₂ and CeO₂-Cu. I) Differential charge density of CeO₂-Cu: cyan, the charge depletion; yellow, the charge accumulation. J) Calculated DOS projected onto Ce, Cu, and O in CeO₂-Cu. K) Schematic representation of the antioxidant mechanism of MHA-CeO₂-Cu. The p-values were determined using one-way ANOVA followed by Dunnett test, ***p < 0.001 compared with the MHA-CeO₂-Cu group.

that H_2O was initially adsorbed to form $\text{M-H}_2\text{O}$, followed by proton from the $\text{M-H}_2\text{O}$ capture by OOH^- to generate H_2O_2 . Subsequently, the remaining M-OH oxidized OOH^- to O_2 , thereby initiating the next catalytic cycle (Figure 2F, top). As shown in Figure 2G and Table S3, Supporting Information, the rate-determining step (RDS) energy of SOD-like process at CeO_2 surface sites was 0.9945 eV, whereas CeO_2 -Cu heterojunction sites exhibited a significantly lower RDS energy of SOD-like process of 0.654 eV. Moreover, the overall activation energy at CeO_2 -Cu heterojunction sites (-2.378 eV) is substantially lower than at CeO_2 surface sites, indicating more prone SOD reactions at CeO_2 -Cu heterojunction sites.

For the CAT-like reaction pathways at CeO_2 surface sites and CeO_2 -Cu heterojunction sites: H_2O_2 was first adsorbed followed by an H^+ abstraction. After another H_2O_2 reacted with the residual M-OOH at the active site, $\text{OOH-M-H}_2\text{O}_2$ intermediate formed and ultimately converted to O_2 (Figure 2F, bottom). In the CAT pathway, the RDS energy at CeO_2 -Cu heterojunction sites (1.24 513 eV) is markedly lower than at CeO_2 surface sites (2.49 172 eV) (Figure 2H and Table S4, Supporting Information).

To further clarify the mechanism of efficient ROS scavenging at CeO_2 -Cu heterojunction sites in MHA-CeO_2 -Cu, the differential charge density maps was simulated. The results revealed significant charge transfer at the CeO_2 -Cu heterojunction interface, with noticeable electron depletion around Cu atoms in the Cu cluster (Figure 2I). Bader charge calculation suggested 1.27 electrons transferring from Cu cluster to CeO_2 surface. Further density of states (DOS) analysis demonstrated strong participation of Cu-3d orbital electronic states near the Fermi level, thereby influencing the catalytic activity and electron transport capacity of MHA-CeO_2 -Cu (Figure 2J). Furthermore, the DOS of Ce showed exceptionally high values near the Fermi level, attributable to Ce-4f electrons, suggesting oxygen vacancy formation around Ce (Figure 2J). The simultaneous non-zero DOS values for both Cu and Ce indicated electronic hybridization between them, further confirming charge transfer results and enhancing interface stability (Figure 2J).

Therefore, the CeO_2 -Cu heterojunction interface facilitated substantial electron transfer, which significantly reduced the RDS energy of both SOD-like and CAT-like reactions, thereby synergistically enhancing the SOD-like and CAT-like activities of MHA-CeO_2 -Cu (Figure 2K).

2.3. Biocompatibility of Bonyzymes

To verify the biocompatibility of Bonyzymes, rat bone marrow mesenchymal stem cells (BMSCs) were incubated for 5 days with MHA, MHA-CeO_2 , and MHA-CeO_2 -Cu at varying concentrations (ranging from 0.1 to 0.4 mg mL^{-1}). Cytoskeletal staining of BMSCs revealed that none of the materials induced notable changes in cell morphology at concentrations of 0.1 and 0.2 mg mL^{-1} (Figure 3A, Figures S5 and S6, Supporting Information). However, at a concentration of 0.4 mg mL^{-1} , some BMSCs in the MHA-CeO_2 and MHA-CeO_2 -Cu groups exhibited abnormal cytoskeletal structures, deviating from the typical star-shaped or spindle-shaped morphology (Figure S6, Supporting Information). As demonstrated by the CCK-8 assay, there was no significant difference in cell viability observed across groups

(Figure 3B and Figure S7, Supporting Information). However, the 0.2 mg mL^{-1} MHA-CeO_2 -Cu group demonstrated slightly higher cell viability compared to the 0.1 and 0.4 mg mL^{-1} MHA-CeO_2 -Cu group.

2.4. In Vitro Osteogenic Effect of Bonyzymes

Prior to osteogenic differentiation induction of BMSCs, the osteogenic differentiation potential of BMSCs cultured at various concentrations was initially evaluated by ALP activity assay. The result demonstrated that BMSCs in the 0.2 mg mL^{-1} MHA-CeO_2 -Cu group exhibited the strongest osteogenic differentiation potential (Figure S8, Supporting Information). In conjunction with the findings on the biocompatibility of Bonyzymes, the concentration of 0.2 mg mL^{-1} was selected for subsequent in vitro cell experiments. Following this, to assess the early-stage osteogenic potential of Bonyzymes, ALP staining and ALP activity assay were conducted on day 7 of osteogenic induction. As shown in Figure 3C,D, MHA-CeO_2 -Cu significantly enhanced ALP activity in BMSCs compared with the other groups, suggesting a higher osteogenic differentiation potential. Similarly, on day 21 of osteogenic induction, Alizarin Red staining and quantification indicated that BMSCs influenced by MHA-CeO_2 -Cu formed more abundant and pronounced calcium nodules (Figure 3E,F).

On day 7 of osteogenic induction, quantitative real-time polymerase chain reaction (qRT-PCR) analysis revealed that the expression of *Alp*, *Bsp* and *Ocn* was significantly elevated in the MHA-CeO_2 -Cu group (Figure 3G–I). Western blots result further evidenced that MHA-CeO_2 -Cu enhanced the expression of ALPL, BSP, and OCN in BMSCs (Figure 3J and Figure S9, Supporting Information). On day 14 of osteogenic induction, both qRT-PCR and western blots analysis demonstrated that MHA-CeO_2 -Cu significantly promoted the expression of osteogenic markers as well (Figure 3K–N and Figure S9, Supporting Information).

2.5. In Vitro Antibacterial, and Immunomodulatory Function of Bonyzymes

Plate count assays confirmed MHA-CeO_2 -Cu showed inhibitory effect on *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) (Figure 4A–D). Remarkably, MHA-CeO_2 -Cu demonstrated exceptionally antibacterial activity against *Porphyromonas gingivalis* (*P. gingivalis*), with nearly undetectable proliferation of *P. gingivalis* observed in the MHA-CeO_2 -Cu group (Figure 4E,F).

To investigate the in vitro immunomodulatory function of Bonyzymes, qRT-PCR and enzyme-linked immunosorbent assay (ELISA) were conducted to evaluate the effect of MHA-CeO_2 -Cu on the inflammatory response of mononuclear macrophages to *P. gingivalis* lipopolysaccharide (LPS). As indicated by qRT-PCR analysis, LPS-treated RAW 264.7 cells cocultured with MHA-CeO_2 -Cu ((LPS + MHA-CeO_2 -Cu) group) exhibited significantly lower expression of *Il-1 β* and *Tnf- α* , even compared to untreated RAW 264.7 cells (Figure 4G,H). Additionally, compared with LPS-treated RAW 264.7 and the (LPS + MHA) group, the (LPS + MHA-CeO_2 -Cu) group showed a significant reduction in *Il-6* expression (Figure 4I). For *Il-10*, the (LPS + MHA-CeO_2 -Cu) group demonstrated a significant increase compared to LPS-treated

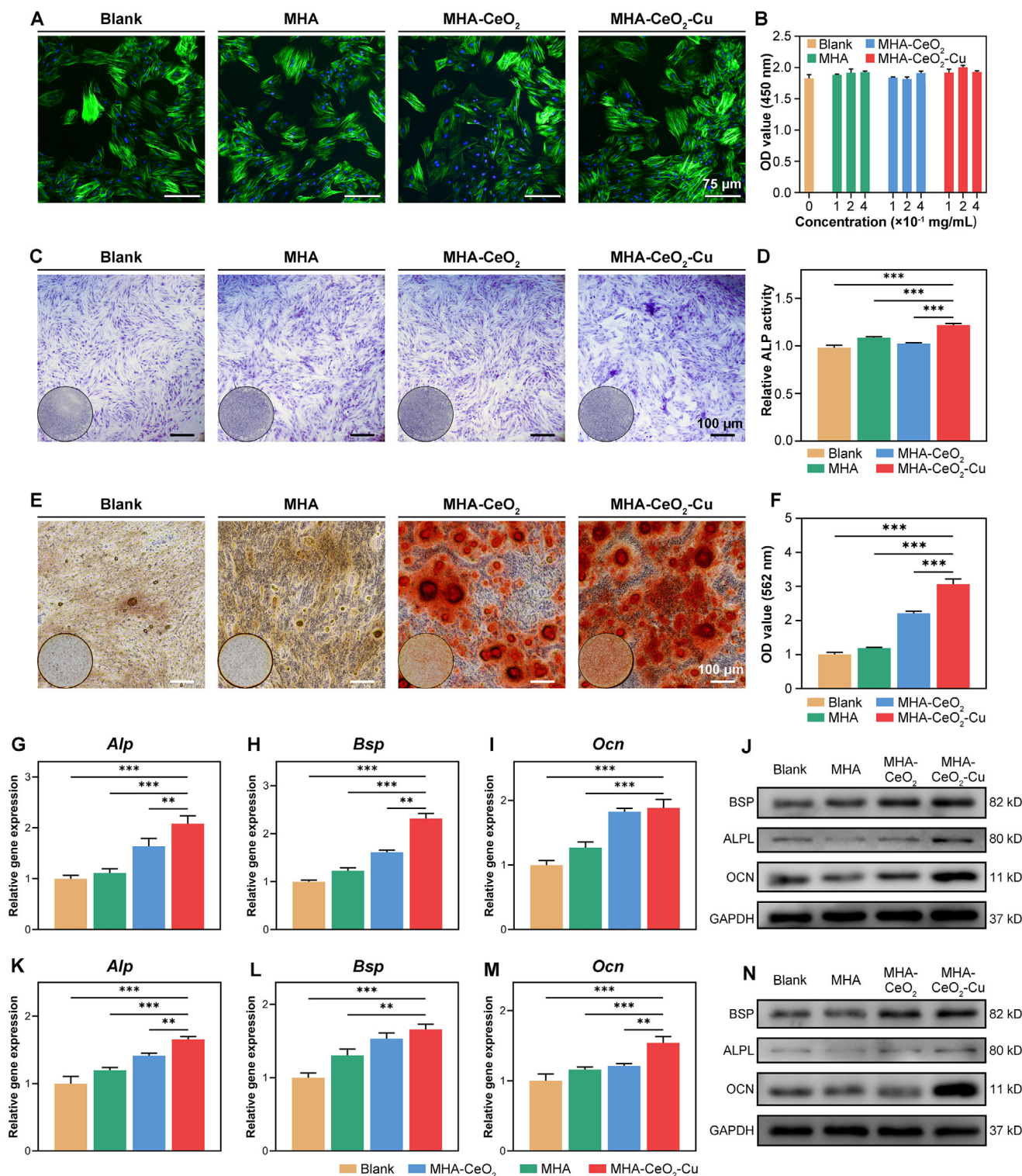
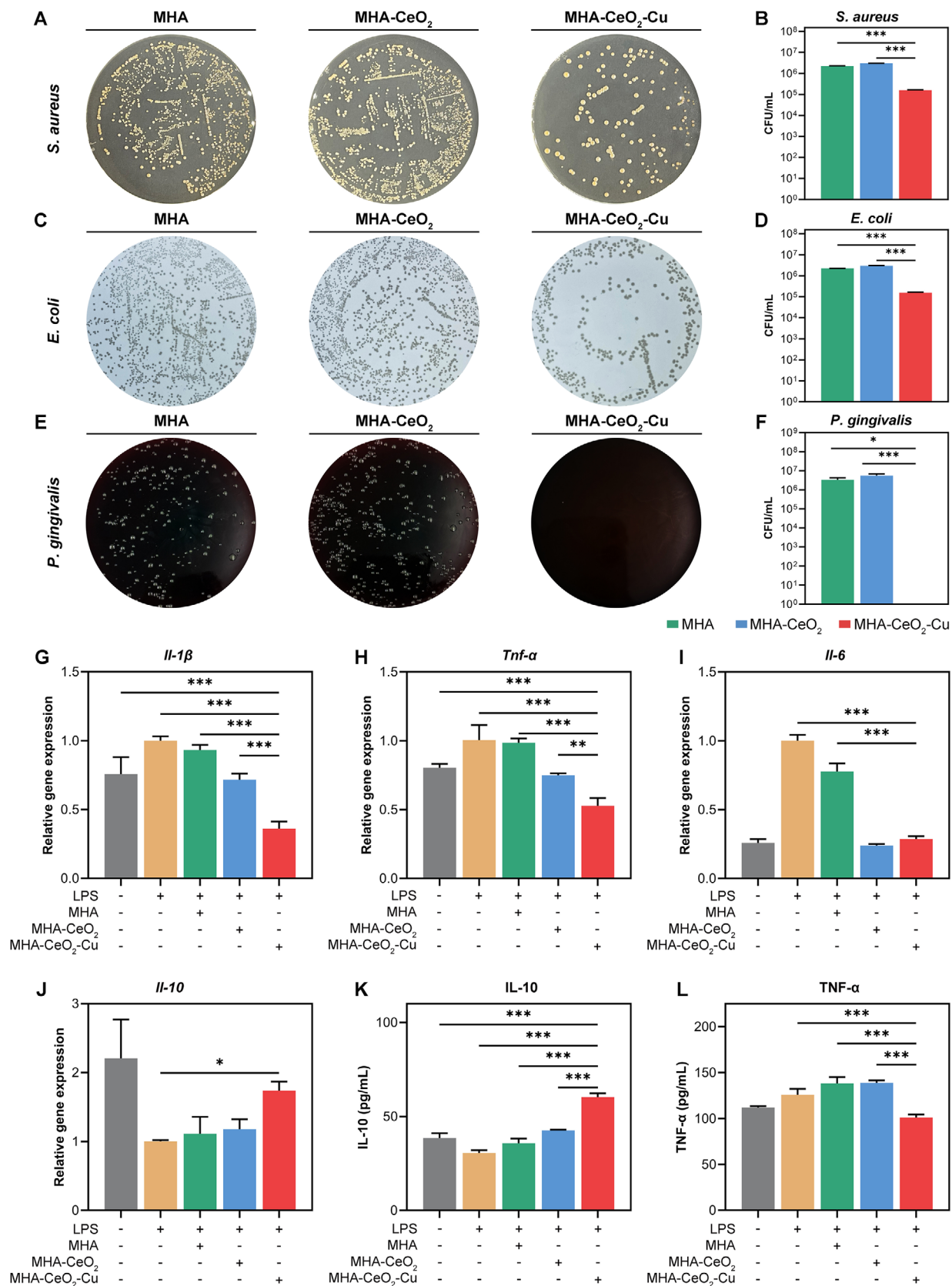


Figure 3. Biocompatibility assays and in vitro osteogenic function of MHA-CeO₂-Cu. A) Fluorescent images of BMSCs cytoskeleton: green, phalloidin; blue, DAPI. B) CCK-8 results for BMSCs at day 5 ($n = 5$). C) Representative ALP staining images of BMSCs. D) Quantitative analysis of ALP activity in BMSCs ($n = 3$). E) Representative Alizarin red staining images of BMSCs. F) Semi-quantitative analysis of Alizarin red staining in BMSCs ($n = 3$). G–I) At day 7, gene expression levels of *Alp*, *Bsp* and *Ocn* in BMSCs as detected by qRT-PCR ($n = 3$). J) At day 7, representative western blot images of ALPL, BSP, and OCN in BMSCs. K–M) At day 14, gene expression levels of *Alp*, *Bsp* and *Ocn* in BMSCs as detected by qRT-PCR ($n = 3$). N) At day 14, representative western blot images of ALPL, BSP, and OCN in BMSCs. The p -values were determined using one-way ANOVA followed by Dunnett test, $**p < 0.01$, $***p < 0.001$, compared with the MHA-CeO₂-Cu group.



RAW 264.7 cells (Figure 4J). Meanwhile, ELISA results confirmed a significant elevation of IL-10 expression in the (LPS + MHA-CeO₂-Cu) group compared to all other groups, accompanied by a marked decrease in TNF- α expression among all LPS-treated groups (Figure 4K,L).

To further elucidate the potential mechanisms by which Bonyzymes regulated anti-inflammatory and antioxidant stress responses in mononuclear macrophages, RNA-Seq was conducted to assess changes of transcriptional profiles among untreated RAW 264.7 cells (none LPS group), LPS-treated RAW 264.7 cells (LPS group), and LPS-treated RAW 264.7 cells co-cultured with MHA-CeO₂-Cu ((LPS + MHA-CeO₂-Cu) group). Principal component analysis (PCA) analysis (Figure 5A) and heatmap of overall transcriptional profiles (Figure S10, Supporting Information) indicated that the (LPS + MHA-CeO₂-Cu) group exhibited distinct transcriptional profiles compared to the other two groups, suggesting a specific polarization trend of RAW 264.7 cells in response to MHA-CeO₂-Cu. The volcano plot revealed that, compared to LPS-treated RAW 264.7 cells, there were 352 genes upregulated and 148 genes downregulated in the (LPS + MHA-CeO₂-Cu) group (Figure 5B). Among them, the expression of *Mt1*, *Slc30a1*, *Hmox1*, and *Mt2* was notably increased, while *Slc39a10* expression was significantly reduced. In the transcriptional profile of the (LPS + MHA-CeO₂-Cu) group, the most significantly altered genes predominantly exhibited changes associated with anti-inflammatory and antioxidant responses (Figure 5C),^[32] further highlighting the potential role of the Bonyzymes in modulating inflammation and oxidative stress.

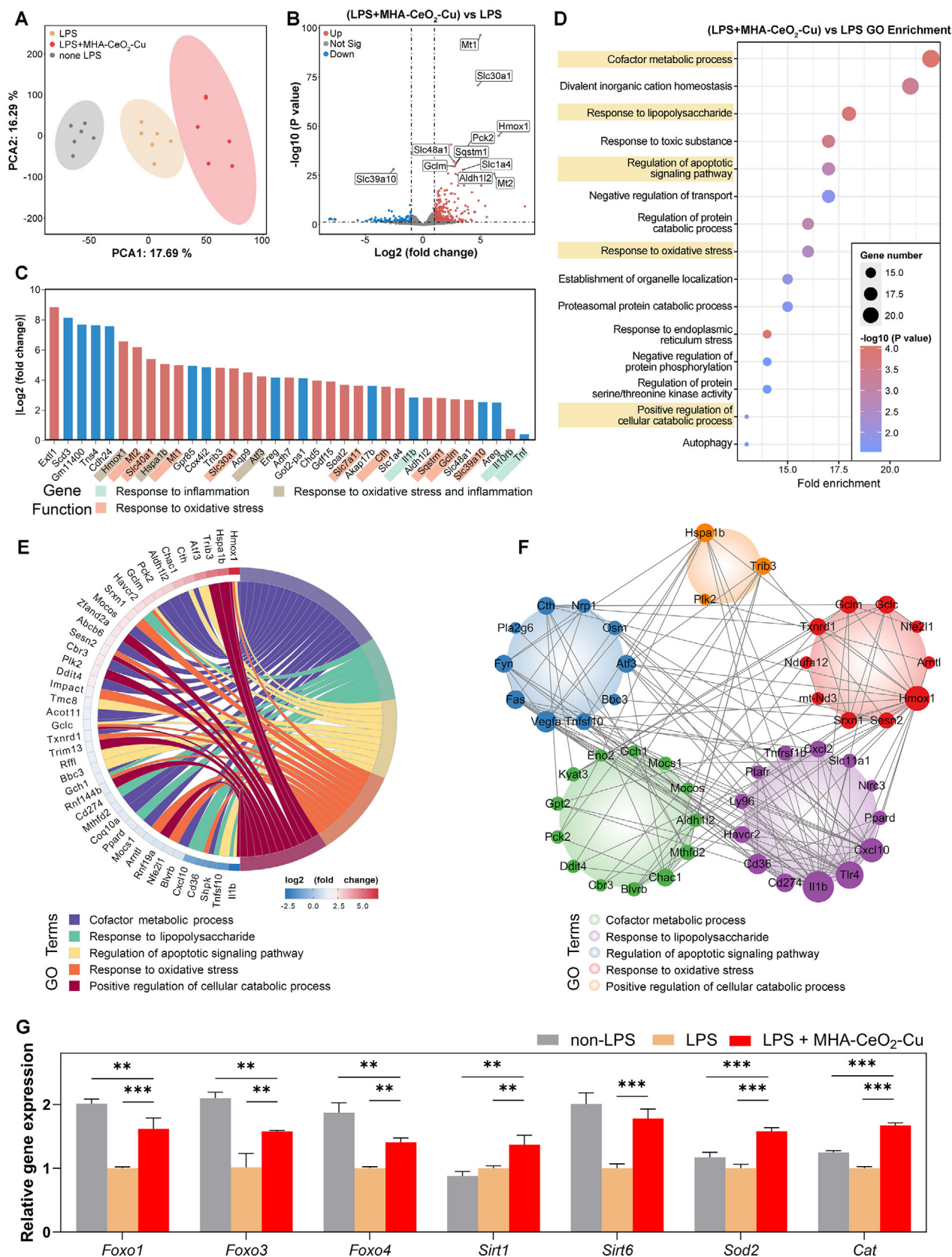
Further enrichment analysis of the differentially expressed genes was conducted. Gene ontology (GO) enrichment analysis revealed that, in comparison to LPS-treated RAW 264.7 cells, the (LPS + MHA-CeO₂-Cu) group significantly changed processes related to cofactor metabolic processes, response to oxidative stress, and the positive regulation of cellular catabolic process (Figure 5D). The chord diagram illustrated the classification of GO terms associated with significantly altered genes, along with the magnitude and direction of these changes (Figure 5E). Meanwhile, the protein-protein interaction (PPI) network highlighted the interconnections among significantly altered gene (Figure 5F). Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment results indicated that, compared to LPS-treated RAW 264.7 cells, the (LPS + MHA-CeO₂-Cu) group exhibited significant change of the MAPK, AMPK and FoxO (Figures S11–S13, Supporting Information), which was then validated by qRT-PCR (Figure 5G and Figure S14, Supporting Information). These findings collectively demonstrated that the Bonyzymes effectively reprogrammed the transcriptional profile of LPS-stimulated RAW 264.7 cells, promoting anti-inflammatory and antioxidant responses through the modulation of key genes, biological processes, and signaling pathways.

2.6. In Vivo Therapeutic Effects of Bonyzymes on Peri-implantitis

A rat model of peri-implantitis was first established following a strict clinical protocol. The construction of the model involved several steps: tooth extraction, followed by the placement of an implant in the first stage surgery and the placement of a healing abutment in the second stage surgery. Next, peri-implantitis was induced via silk ligature combined with *S. aureus* and *P. gingivalis* inoculation. The implant procedure, peri-implantitis induction, and treatment protocol were illustrated in Figure 6A,B and Figure S15, Supporting Information. In order to assess the therapeutic effects of Bonyzymes, maxillary bone samples were scanned by micro-computed tomography (micro-CT) before and 8 weeks after the reconstructive surgery (Figure 6C,D and Figure S16, Supporting Information). Bone loss around implants was quantified by measuring the vertical distance between the implant platform and the bone-implant interface. The CT images showed marked vertical bone resorption around the implants in peri-implantitis-induced rats (Figure S16, Supporting Information, untreated group in Figure 6C,D). As illustrated in the bucco-palatal (Figure 6C) and mesio-distal (Figure 6D) section images, there was significant bone loss at mesial, distal, buccal, and palatal sites in the untreated, Scaling, and Bio-Oss groups. Quantitative analysis further demonstrated the reduction in bone height around implants in these three groups compared to baseline (8 weeks prior). In contrast, MHA-CeO₂-Cu exhibited a notable capacity for restoring bone height around implants in peri-implantitis rats, especially at mesial and distal sites (Figure 6E,F). At the buccal site, the MHA-CeO₂-Cu group showed significant restorative capability compared to the scaling and Bio-Oss groups, whereas at the palatal site, there was no significant difference between MHA-CeO₂-Cu and the scaling group (Figure 6G,H). Compared to the untreated and Bio-Oss groups, the MHA-CeO₂-Cu group exhibited a significantly higher BV/TV, indicating an improved bone quality (Figure 6I).

Further histological evaluation was conducted to assess the therapeutic impact of Bonyzymes on peri-implantitis. Hematoxylin-eosin (H&E) staining revealed substantial bone loss and extensive inflammatory cell infiltration, including inflammatory cell clusters, in the untreated and Bio-Oss groups (Figure 7A). At the same time, a certain number of osteoclasts were observed on the surface of the remaining bone in the Bio-Oss group, indicating ongoing bone resorption (Figure 7A). Interestingly, the Scaling group exhibited reduced inflammatory cells around the implant (Figure 7A). In the MHA-CeO₂-Cu group, partial restoration of bone height and a certain amount of residual implanted material was observed (Figure 7A). Toluidine blue staining indicated ongoing bone destruction around the implant in the untreated group, while remaining bone tissue around the implants in the scaling and Bio-Oss group appeared relatively intact (Figure 7B). In the MHA-CeO₂-Cu group, a deeply stained area along the bone edge suggested active bone formation (Figure 7B).

Figure 4. Antibacterial, and immunomodulatory abilities of MHA-CeO₂-Cu. A) Representative agar plate images of *S. aureus*. B) Live bacterial count of *S. aureus* based on CFU enumeration ($n = 3$). C) Representative agar plate images of *E. coli*. D) Live bacterial count of *E. coli* based on CFU enumeration ($n = 3$). E) Representative agar plate images of *P. gingivalis*. F) Live bacterial count of *P. gingivalis* based on CFU enumeration ($n = 3$). G–J) Gene expression levels of *Il-1 β* , *Il-6*, *Il-10*, and *Tnf- α* in RAW264.7 cells as detected by qRT-PCR ($n = 3$). K,L) Protein expression levels of IL-10 and TNF- α in RAW264.7 cells as detected by ELISA ($n = 3$). The p -values were determined using one-way ANOVA followed by Dunnett test, $*p < 0.05$, $***p < 0.001$, compared with the MHA-CeO₂-Cu group (B, D, and F); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with the (LPS + MHA-CeO₂-Cu) group (G–L).



To further monitor bone remodeling and mineralization during the repair process, calcein was injected at the time of reconstructive surgery, followed by xylenol orange injection at week 7 after surgery, enabling dynamic evaluation of the repair effect of Bonyzymes. The results of double fluorescent labeling showed no significant difference in mineral deposition rates among the three groups at the start of treatment (Figure 7C,D). However, after 2 months of treatment, the MHA-CeO₂-Cu group exhibited a higher mineral deposition rate, which evidenced the ability of MHA-CeO₂-Cu to promote the regeneration of peri-implant bone tissue (Figure 7C,E).

2.7. In Vivo Biocompatibility Assessment

To evaluate the in vivo biocompatibility of Bonyzymes, histological analysis of major organs, including the heart, liver, spleen, lungs, and kidneys, was performed using H&E staining. As shown in Figure S17, Supporting Information, no pathological necrosis or inflammatory lesions were observed in any of the groups.

3. Discussion

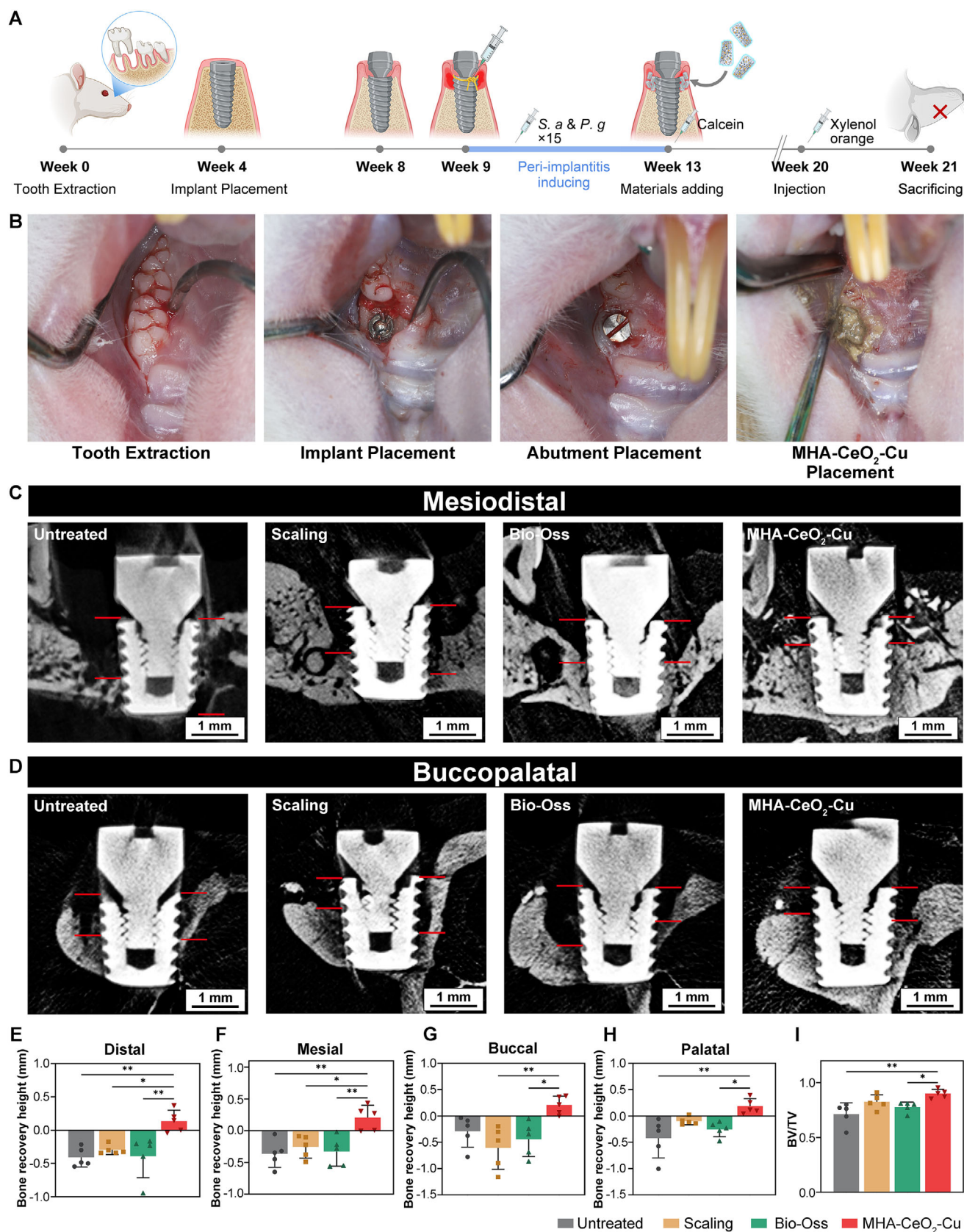
Peri-implantitis leads to progressive loss of peri-implant bone, with a reported prevalence of 19.83%.^[2] Developing an effective treatment for peri-implantitis is therefore critical for the long-term success of dental implants. In recent years, nanozymes have shown significant potential in treating bacterial infections and controlling inflammation, with applications reported in conditions such as hepatitis,^[33] enteritis,^[34] and periodontitis.^[35] Among these, CeO₂ nanozymes have attracted extensive attention due to their superior catalytic activity.^[36] The remarkable catalytic performance of CeO₂ nanozymes arises from surface catalysis enabled by the reversible electron shuttle between Ce³⁺ and Ce⁴⁺.^[36,37] Recent studies have demonstrated that the free electrons of Cu⁰ can effectively modulate the surface charge distribution of CeO₂ nanozymes, thereby synergistically regulating their catalytic activity.^[38] Given its potential for application in bone defect environments, and with MHA established as an effective carrier for drug release and bone defect repair,^[39] we designed novel MHA-based materials loaded with CeO₂-Cu nanozymes, named Bonyzymes. These composite materials were designed to not only control inflammation and inhibit bacteria but also to promote bone defect repair in peri-implantitis.

Compared to pure CeO₂ nanozymes, the MHA-CeO₂-Cu synthesized in this study exhibited enhanced nanozymes catalytic activity. This result aligned with the finding that Cu acted as an electron donor, thereby boosting the catalytic activity of CeO₂.^[40] Meanwhile, the catalytic role of copper-based nanozymes should

also be considered. Xi et al. developed a Copper/Carbon Hybrid Nanozyme based on copper-based nanozymes, which demonstrated certain CAT-like and SOD-like activities.^[41] The XPS analysis revealed that MHA-CeO₂-Cu exhibited an $\approx 5\%$ increase in Ce³⁺ content compared to MHA-CeO₂, partially explaining the observed enhancement effect.^[42] However, this magnitude of increase cannot fully account for the more than tenfold enhancement in SOD-like and CAT-like activities or the nearly 100-fold improvement in total antioxidant activity. Furthermore, Cheng et al. calculated the catalytic rates and turnover number of various nanozymes and found that cerium oxide nanozymes and copper-based nanozymes exhibited similar catalytic performance.^[43] Therefore, the enhanced performance cannot be attributed solely to the presence of individual CeO₂ or Cu species. To further elucidate the enhanced catalytic efficiency of Bonyzymes, we performed DFT calculations. Based on HRTEM and XRD characterization results, we constructed an atomic model using the CeO₂ (111) crystal plane as the substrate, upon which Cu nanocluster were computationally simulated. Cu was coordinated to CeO₂ via interfacial oxygen atoms. DFT calculations revealed that the incorporation of Cu nanocluster not only introduced new active sites but also significantly modified the surface charge distribution of CeO₂. Due to differences in electronegativity, substantial charge transfer from Cu nanocluster to the CeO₂ surface was calculated, which altered the interfacial charge environment of the CeO₂-Cu heterojunction. This electronic modulation dramatically reduced the RDS energy for both CAT-like and SOD-like reactions, and furthermore decreased the overall activation energy for the SOD-like reaction. SOD-like activity is able to convert the toxic $\cdot\text{O}_2^-$ into H₂O₂,^[44] while CAT-like activity further decomposes H₂O₂, a typical inflammatory molecule, into water and oxygen.^[45] In this work, Bonyzymes integrated the enzymatic functions of CAT-like and SOD-like activities, while the CeO₂-Cu heterojunction served to dramatically enhance these activities. This synergistic configuration enabled highly efficient cascade catalysis that completely converts toxic $\cdot\text{O}_2^-$ into O₂ and H₂O through sequential disproportionation. The optimized catalytic system effectively eliminates ROS accumulation at target sites, thereby preventing subsequent inflammatory damage.^[46] Thus, the inhibition of bone reconstruction by ROS accumulation was avoided.^[47]

IL-1 β , IL-6, and TNF- α are typical pro-inflammatory cytokines, while IL-10 is a typical anti-inflammatory cytokine.^[48] In vitro experiments showed downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory cytokine, indicating that MHA-CeO₂-Cu was able to modulate macrophages to shift toward the anti-inflammatory phenotype under LPS stimulation.^[49] RNA-Seq analysis further revealed the potential mechanisms by which MHA-CeO₂-Cu regulated the anti-inflammatory response in macrophages, showing a significant

Figure 5. RNA-Seq analysis of LPS-treated RAW264.7 cells following MHA-CeO₂-Cu treatment. A) PCA plot illustrating sample clusters of none LPS group, LPS group, and (LPS + MHA-CeO₂-Cu) group. B) Volcano plot illustrating significant gene fold changes in the (LPS + MHA-CeO₂-Cu)/LPS comparison, highlighting the top 10 most significantly changed genes. C) The top 27 significantly changed genes and representative inflammatory genes in the (LPS + MHA-CeO₂-Cu)/LPS comparison: red, upregulated; blue, downregulated. D) GO enrichment analysis of differential genes in the (LPS + MHA-CeO₂-Cu)/LPS comparison. E) Chord diagram of GO functions closely related to inflammation and oxidative stress. F) PPI network of GO functions closely associated with inflammation and oxidative stress. G) Gene expression levels of *Foxo1*, *Foxo3*, *Foxo4*, *Sirt1*, *Sirt6*, *Sod2* and *Cat* in RAW264.7 cells as detected by qRT-PCR ($n = 3$). The p -values were determined using one-way ANOVA followed by Dunnett test, $**p < 0.01$, $***p < 0.001$, compared with the (LPS + MHA-CeO₂-Cu) group.



upregulation of antioxidant-related genes. Among them, *Hmox1* showed a significant upregulation in (LPS + MHA-CeO₂-Cu) group, which encodes Heme Oxygenase-1 (HO-1), a well-known stress protein and metabolic enzyme with anti-inflammatory, cytoprotective, and immunoregulatory properties.^[50] HO-1 can reduce the bioavailability of free heme, thereby playing a critical role in antioxidant and anti-inflammatory functions during disease.^[51] Additionally, the upregulation of the Metallothionein (MT) family, recognized as the guardians of metal and redox homeostasis,^[52] specifically *Mt1* and *Mt2*, was highly significant. These proteins are reported to possess strong antioxidant activity through their thiol groups, playing a crucial role in antioxidant defense and the regulation of cellular redox homeostasis.^[53] Furthermore, the MT family has been shown to be involved in inflammation and defense against infection.^[54] The upregulation of these genes enhances cellular antioxidant capacity and reduces LPS-induced oxidative stress. GO analysis revealed that MHA-CeO₂-Cu enhanced the cofactor metabolic process and response to oxidative stress in LPS-stimulated macrophages, which was closely associated with the upregulation of anti-inflammatory and antioxidant genes such as *Hmox1* according to the chord diagram. KEGG analysis further highlighted that several inflammation-related signaling pathways, including MAPK, FoxO, and HIF-1 signaling pathways, were involved in the regulation of LPS-stimulated macrophages by MHA-CeO₂-Cu. Notably, the activation of the HIF-1 signaling pathway can neutralize ROS by modulating the expression of endogenous SOD and CAT, thereby reducing oxidative stress and protecting cells from ROS-induced damage.^[55]

Meanwhile, the Bonyzymes also demonstrated antibacterial activity in the in vitro experiments. Numerous studies have indicated that nano-scale pure CeO₂ lacked considerable antimicrobial activity,^[56] which was consistent with the results observed in the MHA-CeO₂ group. However, the incorporation of copper significantly enhanced the antibacterial ability of Bonyzymes. This improvement could be attributed to the fact that Cu²⁺ is an effective antibacterial agent, which is primarily achieved by damaging the bacterial membrane, inducing lipid peroxidation, and degrading DNA.^[41] Meanwhile, the incorporation of Cu led to the formation of ceria with more defects, which is believed to make the surface more reactive and exhibit increased bacterial toxicity.^[57] Surprisingly, compared to its anti-*S. aureus* and anti-*E. coli* effects, Bonyzymes exhibited particularly pronounced antibacterial efficacy against *P. gingivalis*. This observation aligned with the graphdiyne-iron nanozymes reported by Wu et al., which similarly demonstrated dual CAT-like and SOD-like activities.^[58] The enhanced suppression of *P. gingivalis* may be attributed to oxygen generation during the CAT-like and SOD-like reactions of Bonyzymes, which disrupted the tricarboxylic acid cycle, a critical metabolic pathway for energy supply in *P. gingivalis*, thereby inhibiting its proliferation.^[59] Concurrently, the aerobic microen-

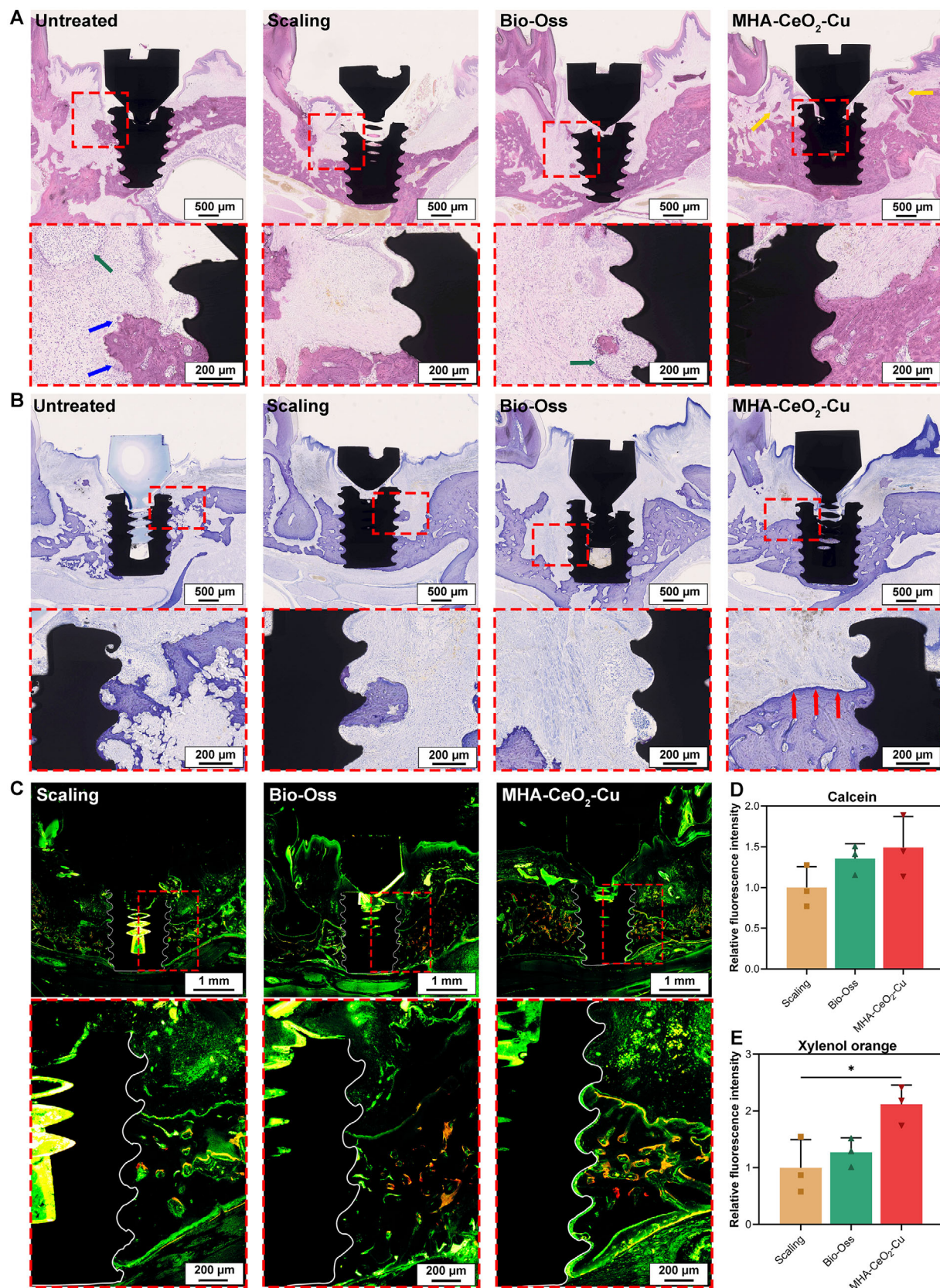
vironment could suppress anaerobic fermentation-derived butyrate production, a key short-chain fatty acid, further impairing cytotoxic effects of *P. gingivalis* and its capacity to induce tissue inflammation.^[60]

Peri-implantitis, being a complex pathological condition, demands materials with not only anti-inflammatory and antibacterial properties but also enhanced osteogenic capabilities. Li et al. reported that an increased Ce⁴⁺/Ce³⁺ ratio promoted new bone formation and mineralization around the material, which was contrary to catalytic activity.^[42] Therefore, achieving a balance between enhancing osteogenesis and maintaining catalytic activity is crucial. In this study, Cu was incorporated into MHA-CeO₂ at an optimized ratio, which not only stabilized intermediate binding to enhance the material's catalytic efficiency but also largely preserved its osteogenic properties by avoiding an excessive increase in the Ce⁴⁺/Ce³⁺ ratio. Additionally, MHA-CeO₂-Cu released Cu²⁺ in a physiological environment, which had been reported to promote cell proliferation, osteogenic differentiation, and angiogenesis, thereby accelerating wound healing.^[61] Meanwhile, microscale hydroxyapatite materials have been shown to efficiently release calcium ions, significantly reducing apoptotic cell death and thereby promoting cell proliferation and activity essential for bone growth.^[62] While traditional hydroxyapatite materials are inherently brittle with limited loading capacity,^[63] we modified the hydroxyapatite by introducing mesopores, which further enhanced its surface area and nanozymes loading efficiency. Moreover, Bonyzymes exhibited good stability, which enabled them to exert long-lasting effects in ROS scavenging, antibacterial activity, and osteogenesis promotion. Ultimately, as demonstrated by the in vivo experiment, the integration of composite nanozymes with MHA endowed Bonyzymes with effective osteoconductive, osteoinductive, and osteogenic properties, finally achieving robust tissue regeneration for peri-implantitis.

4. Conclusion

This study demonstrated the promising therapeutic potential of Bonyzymes, novel materials that integrates MHA with CeO₂-Cu nanozymes, serving as an artificial bone graft for peri-implantitis treatment. By alleviating oxidative stress, Bonyzymes effectively regulate inflammatory pathways, which are pivotal to the pathogenesis of peri-implantitis. In addition to its anti-inflammatory effects, Bonyzymes demonstrated antimicrobial activity, further enhancing its clinical relevance. Furthermore, Bonyzymes exhibited excellent osteogenic promotion effects, highlighting its potential in facilitating bone regeneration in the peri-implant regions. These multifaceted properties position Bonyzymes as promising candidates for peri-implantitis treatment, offering a comprehensive approach to the inflammatory, infectious and bone repair challenges associated with this condition.

Figure 6. In vivo therapeutic effects of MHA-CeO₂-Cu on Peri-implantitis. A) Schematic workflow of the animal experiment, including the establishment and treatment of the peri-implantitis models. B) Photos illustrating the process of peri-implantitis model establishment and treatment in rats. C) Mesiodistal view of Micro-CT images of peri-implant alveolar bone after 8 weeks of treatment. D) Buccolingual view of Micro-CT images of peri-implant alveolar bone after 8 weeks of treatment. E–H) Quantitative analysis of peri-implant bone height recovery between pre-treatment and 8 weeks post-treatment (*n* = 5). I) BV/TV analysis of peri-implant alveolar bone after 8 weeks of treatment (*n* = 5). The *p*-values were determined using one-way ANOVA followed by Dunnett test, **p* < 0.05, ***p* < 0.01, compared with the MHA-CeO₂-Cu group.



5. Experimental Section

Materials and Reagents: Potassium hydrogen phosphate (K_2HPO_4 , Sinopharm, China), Cetyltrimethylammonium Bromide (CTAB, Sinopharm, China), hydrogen peroxide (NaOH, Sinopharm, China), calcium chloride ($CaCl_2$, Sinopharm, China), ethanol (Sinopharm, China), cerous nitrate ($Ce(NO_3)_3$, Aladdin, China), cupric nitrate ($Cu(NO_3)_2$, Sinopharm, China), hydrazine hydrate ($N_2H_4 \cdot H_2O$, Sinopharm, China).

Preparation of MHA: K_2HPO_4 (5.5 g) and CTAB (8.7 g) was dissolved in ddH_2O (100 mL) at 60 °C, with magnetic stirring for 0.5 h. Then, 1.1 M NaOH solution (40 mL) was added to adjust the pH to 12. Subsequently, 0.4 M $CaCl_2$ solution (60 mL) was gradually added to the above solution, and the mixture was stirred at 120 °C for 24 h. After the reaction, the white precipitate was filtered and washed with ddH_2O and ethanol, then dried overnight at 60 °C under vacuum. Finally, the product was sintered at 600 °C for 6 h to remove residual CTAB, resulting in MHA.

Preparation of MHA- CeO_2 : MHA (1 g) was added to 1 M $Ce(NO_3)_3$ solution (10 mL). The resulting suspension was sonicated for 15 min, followed by magnetic stirring for 3 h. Then, the mixture was then centrifuged at 12 000 rpm for 5 min, washed with ddH_2O and ethanol, and then centrifuged at 12 000 rpm for 15 min. After centrifugation, the sample was vacuum-dried at 60 °C overnight. Finally, the product was calcined in a tube furnace by heating at a rate of 2 °C min^{-1} to 650 °C for 5 h, resulting in MHA- CeO_2 .

Preparation of MHA- CeO_2 -Cu: MHA- CeO_2 (0.15 g) and 0.1 M $Cu(NO_3)_2$ (1 mL) were added to ddH_2O (10 mL), and stirred for 3 h. Subsequently, 85% $N_2H_4 \cdot H_2O$ (44 μ L) was added to the suspension, and stirred continuously for an additional 3 min. The mixture was then centrifuged at 12 000 rpm for 5 min, followed by washing with ddH_2O and ethanol. Afterward, the suspension was centrifuged again at 12 000 rpm for 15 min. The sample was vacuum-dried at 60 °C overnight, resulting in MHA- CeO_2 -Cu.

Characterization: The surface morphology of the samples was observed using SEM (Tescan, MIRA, Czech) combined with EDS. TEM (JEOL, JEM-F200, Japan) with EDS was used to observe the morphology of the samples, while HRTEM (JEOL, JEM-F200, Japan) was utilized to measure the lattice spacings of the samples. XRD (Rigaku, SmartLab SE, Japan) was performed to analyze the X-ray diffraction patterns of the samples. XPS was conducted using an ESCALAB XI+ spectrometer (Thermo Scientific, USA). A surface area and porosity analyzer (Micromeritics, TriStar II 3020, USA) was used for BET test.

In Vitro Degradation Study: To evaluate the stability of materials under physiological conditions, samples were immersed in phosphate-buffered saline (PBS) at 37 °C for 14 days. After immersion, the supernatant was collected and analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Analytik Jena, PQ-MS, Germany) to quantify ion release. The samples were then gently rinsed with ddH_2O , vacuum-dried at 60 °C overnight, and their surface morphology was examined using SEM (Tescan, MIRA).

Evaluation of ROS Clearance Abilities: The CAT-like activity of the samples was measured using the catalase assay kit (Leagene, China) colored by ammonium molybdate. The SOD-like activity was determined using the superoxide dismutase assay kit (Solarbio, China) containing xanthine and xanthine oxidase reaction system. The superoxide anion clearance ability of the samples was evaluated using the superoxide anion clearance assay kit (Solarbio, China) containing AP-TEMED system. The total antioxidant intensity of the samples was measured using the total antioxidant activity assay kit (Beyotime, China) containing ferric-tripyridyltriazine. Above assay kits were used according to the manufacturer's instructions. Above experiments were performed in triplicates.

Theoretical Calculations: All DFT calculations were carried out employing the Vienna ab initio simulation package (VASP),^[64] based on the projector augmented wave (PAW) formalism.^[65] The electron-ion interactions were modeled using standard PAW pseudopotentials. Wavefunction expansions utilized a plane-wave basis set with an energy cutoff of 450 eV for the smooth components. The Electron-electron exchange and correlation interactions were treated with the Perdew-Burke-Ernzerhof (PBE),^[66] a form of the general gradient approximation (GGA). The Van der Waals interaction was simulated by Grimme's DFT-D3 dispersion corrections.^[67] Brillouin zone sampling was performed using a $2 \times 2 \times 1$ grid.^[68] Electronic convergence was achieved when successive iterations reached an energy difference below 10^{-5} eV. Structural optimizations proceeded until all atomic forces were reduced below 0.05 eV $\text{\AA}^{-1}$. Post-processing of charge density data was conducted using the VASPKIT toolkit.^[69]

Cell Culture: RAW264.7 cells were cultured in DMEM high-glucose medium (Cytiva, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) in 5% CO_2 at 37 °C. Rat BMSCs were obtained from femurs and tibias of 8-week-old male Sprague-Dawley (SD) rats. Briefly, after the gathering of femurs and tibias, their ends were removed to expose the marrow cavity. The contents were flushed out by culture medium. The cells were then cultured in α -MEM medium (Cytiva, USA) supplemented with 10% FBS and 1% penicillin-streptomycin (PS, Hyclone, USA) in 5% CO_2 at 37 °C. BMSCs were isolated by selecting adherent cell colonies. BMSCs at passages 2 to 4 (P2-P4) were used for subsequent experiments.

Cytoskeleton Staining: BMSCs were seeded into 12-well plates at a density of 10 000 cells mL^{-1} . After 12 h of incubation, the original culture medium was replaced with the following medium: α -MEM containing 10% FBS and 1% PS, or 0.2 mg mL^{-1} MHA, MHA- CeO_2 or MHA- CeO_2 -Cu in α -MEM (containing 10% FBS and 1% PS). After culturing for 1, 3, and 5 days, cells were fixed with 4% paraformaldehyde (Servicebio, China) for 15 min, permeabilized with 0.5% Triton X-100 (biosharp, China) for 15 min, and blocked with 4% bovine serum albumin (Sigma, USA) for 1 h. Then, the cytoskeletal staining was performed. Briefly, cells were stained with FITC-phalloidin (Servicebio, China) for 30 min to visualize F-actin, and DAPI (Beyotime, China) was used for 5 min to stain the nuclei. Finally, cells were sealed with an anti-fluorescence-quenching agent (Beyotime, China), and observed under a fluorescence microscope (IX83, Olympus, Japan) for imaging. Above experiment was performed in triplicates.

Cell Proliferation: BMSCs were seeded into 96-well plates at a density of 10 000 cells mL^{-1} . After 12 h of incubation, the original culture medium was replaced with the following medium: α -MEM containing 10% FBS and 1% PS, or 0.1–0.4 mg mL^{-1} MHA, MHA- CeO_2 or MHA- CeO_2 -Cu in α -MEM (containing 10% FBS and 1% PS). Cell viability was assessed on day 5 using a cell counting kit-8 (CCK-8, Biosharp, China). A microplate reader (BioTek, PowerWave XS2, China) was used to detect cell viability with absorbance at 450 nm. Above experiment was performed in five times.

Osteogenic Differentiation of BMSCs: BMSCs were seeded into 12-well plates. Once the cell density reached over 80%, the original culture medium was replaced with the following medium: α -MEM containing 10% FBS, 1% PS, 10 mM β -glycerophosphate (Sigma-Aldrich, USA), 0.1 μ M dexamethasone (Sigma-Aldrich, USA), and 50 μ g mL^{-1} ascorbic acid (Sigma-Aldrich, USA), or 0.2 mg mL^{-1} MHA, MHA- CeO_2 , or MHA- CeO_2 -Cu in α -MEM (containing 10% FBS, 1% PS, 10 mM β -glycerophosphate, 0.1 μ M dexamethasone, and 50 μ g mL^{-1} ascorbic acid). The mineralization-inducing medium was refreshed every 2 days. On day 7, cells were fixed with 4% paraformaldehyde and rinsed three times with ddH_2O . An ALP staining kit (Beyotime, China) was then used to stain the cells according to the manufacturer's instructions. Macro images were captured using a camera (Nikon, D7200, Japan), and micrographs were taken using a microscope (Olympus, CKX53, Japan). In the meantime, total protein was harvested using radio immunoprecipitation assay

Figure 7. Histological analysis of the in vivo therapeutic effects of MHA- CeO_2 -Cu on peri-implantitis. A) H&E staining images of peri-implant alveolar bone: green arrows, inflammatory cells clusters; blue arrows, osteoclasts; yellow arrows, residual material. B) Toluidine blue staining images of peri-implant alveolar bone: red arrows, regions with intense toluidine blue staining. C) Double fluorescent labeling images of peri-implant alveolar bone: green, calcein; red, xylenol orange. D) Quantification of green fluorescence intensity ($n = 3$). E) Quantification of red fluorescence intensity ($n = 3$). The p -values were determined using one-way ANOVA followed by Dunnett test, $*p < 0.05$, compared with the MHA- CeO_2 -Cu group.

(RIPA) buffer containing a protease inhibitor (Beyotime, China), and the protein concentration was measured using a BCA Protein Assay Kit (Beyotime, China). ALP activity was determined with an ALP activity kit (Beyotime, China) following the manufacturer's instructions. Absorbance at 405 nm was measured using a microplate reader. The data were normalized to the total protein concentration of each sample.

On Day 7 and 14, total RNA was harvested from BMSCs using the TM Total RNA Kit I (OMEGA, USA) following the manufacturer's instructions. The RNA was reverse-transcribed into cDNA using HiScript II Q RT SuperMix for qPCR (Vazyme, China). The cDNA was amplified using ChamQ SYBR qPCR Master Mix (Vazyme, China) on the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, USA). Gene expression was analyzed according to the $2^{-\Delta\Delta C_t}$ method, which was normalized to *Gapdh*. The sequences of primers were listed in Table S5, Supporting Information.

Simultaneously, on Day 7 and 14, total protein was harvested from BMSCs using RIPA buffer with protease inhibitors. Protein concentration was determined using a BCA protein assay kit. The protein samples were mixed with SDS-PAGE sample loading buffer (Biosharp, China), denatured at 95 °C for 10 min, and separated by 10% SDS-PAGE. The proteins were transferred onto PVDF membranes, blocked with blocking buffer for western blotting (Beyotime, China), and incubated overnight at 4 °C with primary antibodies. After incubation with HRP-conjugated secondary antibodies (AS014, ABclonal, China), protein bands were visualized using the Western Blot ECL HRP Substrate Kit (Advansta, USA). Quantitative analysis of protein bands was performed using ImageJ (NIH, USA). GAPDH was used as a loading control. The primary antibodies included anti-ALPL (A0514, ABclonal, China), anti-BSP (A16220, ABclonal, China), anti-OCN (A20800, ABclonal, China), and anti-GAPDH (AC035, ABclonal, China).

On Day 21, cells were fixed with 4% paraformaldehyde and rinsed three times with ddH₂O. Cells were then stained with Alizarin red solution (Servicebio, China) to assess mineralization. Macro images were captured with a Nikon D7200 camera, and micrographs were taken with an Olympus CKX53 microscope. After recoding, the Alizarin red dye was solubilized by 10% hexadecylpyridinium chloride monohydrate (Servicebio, China) for 2 h. The absorption at 562 nm was measured using a PowerWave XS2 microplate reader. Above experiments were performed in triplicates.

Antibacterial Experiment: The *S. aureus* and *E. coli* strains were cultured in lysogeny broth (LB, BD, USA) liquid and solid medium at 37 °C. And, the *P. gingivalis* strain was cultured in brain heart infusion (BHI, Hopebio, China) liquid medium and sheep blood agar solid medium at 37 °C, which both were supplemented with vitamin K1 (1 mg L⁻¹), and hemin (5 mg L⁻¹). *P. gingivalis* was cultured in an anaerobic atmosphere containing 21% CO₂ and 78.9% N₂. The antibacterial ability of materials was assessed using the colony-forming unit (CFU) method. Briefly, 1×10^4 CFU mL⁻¹ suspensions of *S. aureus*, *E. coli* and *P. gingivalis* were mixed in a 1:1 ratio with liquid medium or suspensions of 0.4 mg mL⁻¹ MHA, MHA-CeO₂, or MHA-CeO₂-Cu (prepared in liquid medium). The tubes were incubated at 37 °C for 24 h. After incubation, 100 µL of the bacterial suspension was plated on solid agar plates. Bacterial colonies were recorded in detail. Above experiment was performed in triplicates.

Macrophage Polarization Assay: RAW264.7 cells were seeded into 12-well plates. After the adhesion of cells, the culture medium was replaced with one of the following: DMEM high-glucose medium containing 10% FBS, DMEM high-glucose medium containing 10% FBS and *P. gingivalis* LPS (1 µg mL⁻¹), or 0.2 mg mL⁻¹ suspensions of MHA, MHA-CeO₂, or MHA-CeO₂-Cu (prepared in DMEM high-glucose medium containing 10% FBS and *P. gingivalis* LPS (1 µg mL⁻¹)). After 6 h of incubation, the polarization state of the cells was assessed using RT-qPCR and ELISA.

RNA extraction, reverse transcription, and RT-qPCR were performed as previously described, with the primers listed in Table S6, Supporting Information.

The supernatants from each group were collected, and IL-10 and TNF-α secretion levels were measured using mouse IL-10 ELISA kit (EMCC005, NeoBioscience, China) and mouse TNF-α ELISA kit (EMC102a, NeoBioscience, China), following the manufacturer's instructions. Above experiments were performed in triplicates.

In Vitro Immunomodulatory Mechanism by Transcriptome Sequencing: After 6 h of treatment, total RNA was isolated from RAW264.7 cells

using TRIzol and quantified with the RNA Nano 6000 assay kit on the Bioanalyzer 2100 system (Agilent Technologies, USA). mRNA was enriched using poly-T oligo-attached magnetic beads and transcribed into cDNA with M-MuLV reverse transcriptase. The second strand of cDNA was synthesized using DNA Polymerase I and RNase H, followed by adaptor ligation. The cDNA library was purified using the AMPure XP system (Beckman Coulter, USA) to obtain fragments of 370–420 bp. PCR amplification was performed, and the products were purified and verified using the Agilent Bioanalyzer 2100 system. Sequencing was conducted on an Illumina Novaseq platform (paired-end, 150 bp depth).

Clean RNA-seq data were processed using FastQC, and the reads were aligned to the mouse reference genome (GRCm38) using Hisat2 v2.0.5. Gene-level read counts were obtained with FeatureCounts v1.5.0-p. Differential expression analysis between groups, based on fragments per FPKM, was performed using the DESeq2 R package (1.42.1) with six biological replicates per group. Visualization of the results, including volcano plots and heatmaps, was carried out using R v4.3.2. Differentially expressed genes were further analyzed using the DAVID platform for GO and KEGG pathway enrichment. PPI networks were constructed using STRING.

Animal Model and Therapeutic Methods: The animal experiments in this study were approved by the Ethics Committee for Animal Research, School and Hospital of Stomatology, Wuhan University (No. S07922080D). All in vivo experiments were conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines. Male SD rats were purchased from the Hubei Research Center of Laboratory Animals (China) and randomly divided into four groups. The rats were housed in specific pathogen-free (SPF) conditions and underwent a 1-week acclimatization period prior to the experiments. Anesthesia was administered using pentobarbital for long procedures or isoflurane for short procedures.

At 5 weeks of age, the maxillary first molars of rats were extracted using a dental probe. After 1 month of alveolar socket healing, an incision was made along the alveolar ridge crest at the site of the maxillary first molars, and the mucoperiosteum was reflected. The implant site was gradually prepared using a dental implant system (Nobel Biocare, Osseoset 200, Switzerland) with drills speed of 800 rpm, while saline irrigation was used to prevent bone overheating. Once the socket reached the predetermined depth, the implant was placed and sutured. After 1 month of osseointegration, an incision was made at the coronal aspect of the implant, and abutments were installed. 1 week after the restoration, 5-0 silk ligatures were placed around the abutments, and 100 µL of bacterial suspension containing *P. gingivalis* and *S. aureus* (each 5×10^8 CFU mL⁻¹) was injected into the peri-implant subgingival area every 2 days for 1 month to induce experimental peri-implantitis.

1 month later, the baseline condition of the peri-implant alveolar bone was assessed using micro-CT scanner (Bruker, Skyscan 1276, Germany). Subsequently, different treatments were applied to the peri-implant tissues: untreated, scaling alone, scaling followed by Bio-Oss placement, or scaling followed by placement of a 1:1 mixture of Bio-Oss and MHA-CeO₂-Cu. Concurrently, 0.002 mL g⁻¹ calcein (5 mg mL⁻¹, Sigma, USA) was administered via intraperitoneal injection. 7 weeks post-surgery, rats received an intraperitoneal injection of 0.002 mL g⁻¹ xylene orange (5 mg mL⁻¹, Vokai, China). 8 weeks post-surgery, the rats were euthanized by CO₂, and peri-implant tissues were harvested for further analysis.

Micro-CT Analysis: The collected peri-implant tissues were fixed in 4% paraformaldehyde for 48 h and subsequently scanned by a Micro-CT scanner (Bruker, Skyscan 1276, Germany). 2D imaging of the samples was performed by DataViewer software. The scan data were analyzed with CT Analyzer software for quantitative assessment.

Histological Assessment: After washing the peri-implant tissues fixed in 4% paraformaldehyde, the samples were then dehydrated in graded ethanol and embedded in methyl methacrylate. The embedded samples were sectioned mesiodistally into slices 200 µm thick, which were subsequently ground to a final thickness of 20 µm. Fluorescence scanning was performed to capture calcein/xylene orange double labeling, followed H&E and toluidine blue staining to evaluate peri-implantitis and tissue repair.

Statistical Assessment: All experiments were replicated at least three times. Statistical analysis was conducted by GraphPad Prism version 8.0.2 (GraphPad Software), with One-way analysis of variance (ANOVA) used among groups, followed by Dunnett's test. The results were presented as the mean $\pm$ standard deviation (S.D) for each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ were considered statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

infection control, inflammation, osteogenesis, oxidative stress, peri-implantitis

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