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Enhanced piezoelectricity and nanotopography resolve the infection-regeneration conflict in implant-associated osteomyelitis

Changxu Chen^{1,2,4}, Jianfeng Sun¹, Zijian Wang^{1,5}, Jiaxuan Li^{1,4}, Xiaozhou Wen^{1,4}, Xuqiao Zhao^{1,5}, Jinxi Liu⁶, Yong Chen^{1,7}, Yilin Qi^{1,4}, Shuncheng Yao², Zhipeng Qu⁴, Engui Wang⁴, Han Ouyang⁴, Swee Hin Teoh^{8,9*}, Xi Cui^{2*}, Zhou Li^{2,3*}

¹ Beijing Institute of Nanoenergy and Nanosystems, Chinese Academy of Sciences, Beijing 101400, China.

² Vita Tech Innovation Center, Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing 100084, China.

³ School of Biomedical Engineering, Tsinghua University, Beijing 100084, China.

⁴ School of Nanoscience and Engineering, School of Chemical Sciences, University of Chinese Academy of Sciences, Beijing 101408, China.

⁵ School of Medical Instrumentation, Shenyang Pharmaceutical University, Shenyang 110016, China.

⁶ Institute of Biophysics, State Key Laboratory of Cognitive Science and Mental Health, Center for Excellence in Biomacromolecules, Chinese Academy of Sciences, Beijing 100101, China.

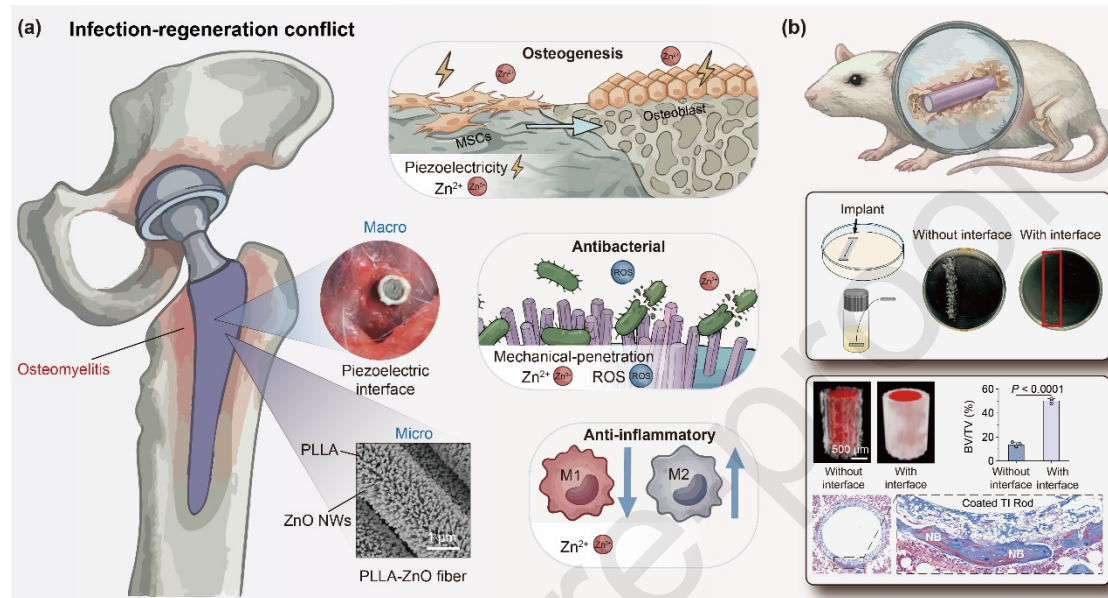
⁷ Department of Plastic Surgery, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China.

⁸ School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore.

⁹ College of Materials Science and Engineering, Hunan University, Changsha 410000, China.

*Corresponding authors. Email addresses: Teohsh@hnu.edu.cn (S. H. Teoh), cuixi@mail.tsinghua.edu.cn (X. Cui), li_zhou@tsinghua.edu.cn (Z. Li).

Graphical abstract



This study developed a PLLA-ZnO piezoelectric interface featuring surface-exposed ZnO nanowires, integrating enhanced piezoelectric response, nanotopography, and microenvironment-responsive Zn^{2+} release within a single material system. The interface combines early-stage antibacterial and immunomodulatory effects with subsequent promotion of osteogenesis and osseointegration, providing a structure-driven strategy to address the infection-regeneration conflict in implant-associated osteomyelitis.

Abstract

Implant-associated osteomyelitis remains a major clinical challenge because antibacterial therapy and bone regeneration impose competing demands within a dysregulated osteoimmune microenvironment. Strategies targeting antibacterial activity or osteogenesis alone are insufficient to address these interlinked challenges. Here, we develop a microenvironment-responsive interface composed of poly(L-lactic acid) (PLLA) and zinc oxide (ZnO) that integrates enhanced piezoelectricity with engineered nanotopography to support bone repair. Surface-exposed ZnO nanowire

arrays markedly enhance the piezoelectric output of the PLLA interface and enable pH-responsive Zn^{2+} release. This synergistic design enables on-demand activation under infection-associated acidic conditions, while the nanowire topography provides potent antibacterial activity through mechanical penetration, thereby addressing the infection-regeneration conflict. The interface thereby enables spatiotemporally coordinated antibacterial, immunomodulatory, and osteogenic responses, reshaping the osteoimmune microenvironment. *In vitro*, the interface exhibits broad-spectrum antibacterial activity, promotes macrophage polarization toward a pro-healing phenotype, and enhances the osteogenic differentiation of mesenchymal stem cells. In a rat model of implant-associated osteomyelitis, the interface significantly suppresses bacterial colonization, attenuates inflammatory responses, and promotes bone regeneration and osseointegration, achieving a 3-fold higher bone volume/tissue volume ratio than uncoated controls while maintaining favorable biosafety. Collectively, this work establishes a microenvironment-adaptive interface that integrates antibacterial and bone-regenerative functions, offering a promising strategy for treating implant-associated osteomyelitis.

Keywords: Piezoelectric; Bone tissue engineering; Antibacterial properties; Biodegradable; Zinc oxide nanowires

1. Introduction

Implant-associated osteomyelitis is characterized by persistent infection, dysregulated inflammation, and impaired bone regeneration within a disrupted osteoimmune microenvironment [1–3]. Bacterial colonization and excessive inflammatory responses suppress osteogenesis and compromise implant integration, while conventional implants lack intrinsic antibacterial and osteoinductive properties [4,5]. The central challenge therefore lies in resolving the conflict between infection control and bone regeneration. However, existing strategies targeting either antibacterial activity or osteogenesis alone fail to address the pathological coupling between infection and impaired regeneration [6–8]. Developing bioactive interfaces capable of coordinated regulation of antibacterial activity, immune response, and osteogenesis remains a critical unmet need.

Piezoelectric materials offer a promising strategy for regulating the osteoimmune microenvironment by converting physiological mechanical stimuli into endogenous electrical signals [9,10]. These electromechanical cues mimic the native electrical microenvironment of bone, promote osteogenic activity, and may also modulate immune responses, thereby providing a potential route to alleviate the inflammation–osteogenesis imbalance in implant-associated osteomyelitis [11–13].

Among candidate materials, poly(L-lactic acid) (PLLA) is biocompatible and biodegradable but exhibits relatively weak piezoelectric output, whereas inorganic piezoelectric materials provide stronger electromechanical performance but are limited by poor flexibility and processability [14–16]. Moreover, PLLA degradation ultimately yields metabolizable lactate-based products that, at appropriate levels, have been reported to contribute to inflammation resolution and tissue repair [17–20]. Zinc oxide (ZnO) is particularly attractive because it exhibits favorable piezoelectric properties and possesses intrinsic antibacterial and osteoimmunomodulatory activities through Zn^{2+} release and interfacial interactions [12,21,22]. However, conventional polymer systems incorporating ZnO nanoparticles often suffer from particle encapsulation and uncontrolled ion release, which restrict interfacial bioactivity and compromise coordinated microenvironmental regulation [23,24]. Although ZnO-based and piezoelectric implant coatings have been explored for antibacterial or osteogenic regulation, how ZnO surface architecture can be used to simultaneously improve electromechanical coupling and ion accessibility remains insufficiently defined.

Here, we developed a microenvironment-responsive piezoelectric PLLA-ZnO interface with enhanced piezoelectricity and tailored nanotopography by constructing oriented ZnO nanowire arrays on electrospun PLLA fibers and integrating them onto titanium substrates via a polydopamine (PDA) interlayer. This hierarchical design enhances interfacial exposure and piezoelectric performance, enabling pH-responsive, on-demand antibacterial and osteogenic functions under infection-associated acidic conditions. By integrating the enhanced piezoelectricity and tailored nanotopography of the ZnO arrays with ion-mediated biological cues, the system is designed to resolve the infection-regeneration conflict through coordinated antibacterial, immunomodulatory, and osteogenic regulation in implant-associated osteomyelitis (Fig. 1a). Fig. 1b summarizes representative *in vitro* and *in vivo* results demonstrating these multifunctional effects.

Fig. 1. Overview of the PLLA-ZnO microenvironment-responsive piezoelectric interface for the treatment of implant-associated osteomyelitis. (a) Mechanistic overview of the interface for addressing the infection-regeneration conflict. The engineered microenvironment-responsive piezoelectric interface uses the PLLA-ZnO structure to integrate physicochemical and biological cues, enabling the coordinated regulation of osteogenesis, antibacterial activity, and anti-inflammatory responses. (b) *In vitro* and *in vivo* evaluation in an infected rat model. BV/TV, bone volume/tissue volume; NB, new bone.

2. Experimental

2.1. Preparation of piezoelectric interfaces

Poly(L-lactic acid) (PLLA) films were prepared by electrospinning. PLLA-ZnO interfaces were fabricated by plasma treatment, ZnO seed-layer sputtering, and hydrothermal growth of oriented nanowires. PLLA/ZnO control films were prepared by directly blending ZnO nanoparticles into PLLA at matched loadings. A ZnO content of 7.0% was selected following cytocompatibility screening. Titanium implants were coated using a polydopamine interlayer.

2.2. Material characterization

Morphological, structural, mechanical, and surface properties were characterized. Piezoelectric output was measured under cyclic loading at 8 N and 1 Hz. Zn²⁺ release was assessed at pH 5.0 and 7.4, and accelerated degradation was evaluated at 60 °C.

2.3. *In vitro* biological evaluation

Freestanding films were evaluated for cytocompatibility, hemocompatibility, osteogenic differentiation of rat calvarial mesenchymal stem cells (MSCs), and polarization of lipopolysaccharide-stimulated RAW264.7 macrophages. Antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) was assessed by measuring bacterial survival, biofilm formation, oxidative stress, and membrane integrity.

2.4. *In vivo* evaluation

Animal procedures were approved by the Animal Ethics Committee of the Beijing Institute of Nanoenergy and Nanosystems (approval No. 2025007LZ). Rat femoral osteomyelitis was induced by *S. aureus* inoculation followed by implantation. Ti, Ti/PLLA, Ti/PLLA/ZnO, and Ti/PLLA-ZnO groups were assessed for infection and inflammation at 1 week and for bone regeneration and inflammation at 6 weeks. Additional evaluations included myeloperoxidase activity on postoperative day 3 and major-organ histology.

2.5. Statistical analysis

Data are presented as the mean $\pm$ standard deviation. Technical replicates were averaged within each independent *in vitro* experiment. Each animal constituted an independent experimental unit, with tissue measurements averaged per animal. Sample sizes are specified in the figure legends. Comparisons among multiple groups were performed using one-way analysis of variance followed by Tukey's post hoc test, with $P < 0.05$ considered statistically significant.

Complete experimental procedures are provided in the Supplementary material.

3. Results

3.1. Structural design and microenvironment-responsive properties

To validate the structural design and microenvironment-responsive functionality of the PLLA-ZnO piezoelectric interface, its microstructure, physicochemical properties, and ion-release behavior were systematically investigated. For comparison, a control interface (denoted as PLLA/ZnO) was fabricated by directly incorporating ZnO nanoparticles at the same mass fraction into electrospun PLLA fibers, whereas PLLA-ZnO denotes the system with *in situ* grown ZnO nanowire (NW) arrays on the surface of electrospun PLLA fibers.

As illustrated in Fig. 2a and Fig. S1 (online), the PLLA-ZnO piezoelectric interface was constructed through a sequential process involving electrospinning of PLLA fibers, deposition of a ZnO seed layer via magnetron sputtering, and subsequent *in situ* hydrothermal growth of ZnO NW arrays. This strategy generated a hierarchical structure with vertically aligned ZnO NWs directly exposed on the fiber surface, in contrast to the nanoparticle-embedded configuration of the PLLA/ZnO control. Such a design is expected to enhance interfacial activity and facilitate effective coupling of physicochemical and biological cues [25].

Fig. 2. Preparation and physicochemical characterization of the PLLA-ZnO piezoelectric interface. (a) Schematic illustration of the two-step fabrication process of the PLLA-ZnO piezoelectric interface. (b) Representative scanning electron microscopy (SEM) image of the PLLA-ZnO interface. (c) Energy-dispersive X-ray spectroscopy (EDS) elemental mapping images of the PLLA-ZnO interface, showing the distribution of Zn, O, and C. (d) Cell Counting Kit-8 (CCK-8) assay of MSC viability after 3 d of co-culture with PLLA-ZnO interfaces containing different ZnO contents. The dashed line indicates the 90% cell-viability biosafety threshold ($n = 3$). (e) Piezoelectric output voltage-time curves of PLLA, PLLA/ZnO, and PLLA-ZnO interfaces with varying ZnO contents under 8 N cyclic mechanical loading. (f) X-ray diffraction (XRD) patterns of PLLA, PLLA/ZnO, and PLLA-ZnO samples. (g) Fourier transform infrared (FT-IR) spectra of PLLA, PLLA/ZnO, and PLLA-ZnO samples. (h) Static water contact angles of PLLA, PLLA/ZnO, and PLLA-ZnO sample surfaces ($n = 3$). (i) Young's modulus of PLLA, PLLA/ZnO, and PLLA-ZnO samples ($n = 3$). (j) Force-displacement curves from 180° peel tests comparing interfacial adhesion in the absence and presence of the PDA interlayer. (k) Force-displacement curves from 0° shear tests comparing interfacial adhesion in the absence and presence of the PDA interlayer. (l) Macroscopic images of the PLLA-ZnO interface during accelerated degradation in PBS at 60 °C ($n = 3$). (m) pH-responsive Zn^{2+} release curves of the PLLA/ZnO and PLLA-ZnO interfaces at 37 °C under pH 7.4 and pH 5.0 conditions ($n = 3$). (n) Optical images of implant samples: bare Ti rod, Ti/PLLA, Ti/PLLA/ZnO, and Ti/PLLA-ZnO.

SEM revealed dense, highly oriented ZnO NW arrays on the surface of electrospun

PLLA fibers, forming a hierarchical architecture with direct interfacial exposure (Fig. 2b and Fig. S2 online). In contrast, the PLLA/ZnO control exhibited a relatively smooth fibrous morphology without surface-exposed ZnO nanowire arrays (Fig. S3 online). Preliminary experiments evaluated the effect of magnetron sputtering time on ZnO NW growth (Fig. S4 online). The sputtering time was therefore set to 15 min, and different ZnO contents were achieved by adjusting the amount of hydrothermal precursors. EDS mapping further confirmed the uniform distribution of Zn, O, and C across the fiber matrix (Fig. 2c). XRD analysis showed an enhanced diffraction peak corresponding to the ZnO (002) plane in the PLLA-ZnO piezoelectric interface (Fig. 2f), indicating preferential ZnO orientation along the c-axis, consistent with the vertically aligned NW morphology. Fourier transform infrared (FT-IR) spectroscopy further characterized the chemical composition and interfacial interactions, confirming successful integration of PLLA and ZnO (Fig. 2g). Compared with conventional nanoparticle-incorporated systems, this surface-exposed nanoarray architecture is expected to facilitate more efficient interfacial interactions and functional activity.

To balance biological activity and cytocompatibility, PLLA-ZnO piezoelectric interfaces with different ZnO contents were evaluated using CCK-8 assays (Fig. 2d and Fig. S5 online) and live/dead fluorescence staining (Fig. S6 online). A ZnO content of 7% was selected as the optimal condition because it maintained cell viability above 90% while providing enhanced functional performance.

The ion-release behavior of the piezoelectric interfaces was evaluated using inductively coupled plasma mass spectrometry (ICP-MS) under physiological (pH 7.4) and acidic (pH 5.0) conditions (Fig. 2m). Both PLLA/ZnO and PLLA-ZnO piezoelectric interfaces exhibited sustained, pH-responsive Zn^{2+} release profiles, while the PLLA-ZnO piezoelectric interface showed a significantly greater cumulative release at an equivalent ZnO content. Notably, Zn^{2+} release was markedly accelerated under acidic conditions, which are characteristic of infection-associated microenvironments [26]. Considering the heterogeneous acidity reported in *S. aureus* biofilms, pH 5.0 was selected as a representative acidic condition for evaluating Zn^{2+} release [27–29]. This pH-responsive behavior enables infection-triggered ion release, allowing rapid antibacterial action during early inflammatory stages while maintaining a sustained release profile under physiological conditions to support osteogenesis. The greater Zn^{2+} release from PLLA-ZnO at comparable ZnO loading indicates that surface exposure increases ion accessibility, particularly under acidic conditions.

The electromechanical performance of the piezoelectric interfaces was further evaluated to assess their ability to provide physicochemical cues. Under an external force of 8 N, the PLLA-ZnO piezoelectric interface exhibited a significantly higher piezoelectric output voltage than the PLLA/ZnO interface at the same ZnO content (Fig. 2e and Fig. S7 online), demonstrating that the oriented ZnO NW arrays effectively amplify the piezoelectric response. Long-term cyclic loading tests demonstrated stable

electromechanical performance (Fig. S8 online), and the interface maintained stable piezoelectric output after cell and bacterial co-culture (Fig. S9 online). In addition, ZnO incorporation increased the Young's modulus of the composite piezoelectric interface (Fig. 2i), suggesting improved mechanical stability under physiological loading. ZnO nanowire growth also increased surface hydrophilicity (Fig. 2h), which may facilitate protein adsorption and cell adhesion at the interface.

Mechanical testing, including 180° peel and 0° shear tests, confirmed that the PDA interlayer significantly increased resistance to detachment, demonstrating robust interfacial adhesion between the piezoelectric coating and the titanium substrate (Fig. 2j, k). Accelerated degradation testing showed progressive structural breakdown of the PLLA-ZnO piezoelectric interface over time (Fig. 2l). To further evaluate its applicability for implant surface modification, the piezoelectric interfaces were fabricated on titanium substrates, forming uniform, stable coatings without visible delamination (Fig. 2n). These results indicate good interfacial adhesion and adaptability to complex geometries.

3.2. Microenvironment regulation: osteogenesis and immunomodulation

Previous studies have shown that cell-derived traction forces can mechanically deform piezoelectric scaffolds and generate localized piezoelectric potentials, thereby enabling endogenous electrical stimulation in the absence of external mechanical loading [30–33]. *In vivo*, physiological loading associated with limb movement and weight bearing may further activate the piezoelectric response. Meanwhile, ZnO releases Zn^{2+} in a controlled manner under physiological conditions [34,35]. Together, these physicochemical and biological cues are expected to synergistically regulate the osteoimmune microenvironment, promoting osteogenesis while modulating immune responses (Fig. 3a).

Fig. 3. *In vitro* biocompatibility, pro-osteogenic activity, and immunomodulatory properties of the PLLA-ZnO piezoelectric interface. (a) Schematic illustrating the proposed enhancement of osteogenic differentiation through the combined effects of piezoelectric stimulation and sustained Zn^{2+} release from the interface. (b) Hemolysis assay of different material groups, with H_2O as the positive control and PBS as the negative control ($n = 3$). (c) Live/dead fluorescence staining of MSCs cultured on different material surfaces for 120 h. (d) Alizarin Red S (ARS) staining of MSCs cultured on different material surfaces for 7 and 14 d. (e) Quantitative analysis of relative mineralization levels based on ARS staining at 7 and 14 d ($n = 3$). (f) MSC proliferation on different material surfaces after 1, 3, and 5 d, assessed by CCK-8 assay ($n = 3$). (g–h) Immunofluorescence staining of key osteogenic proteins in MSCs, including bone morphogenetic protein 2 (BMP2) and runt-related transcription factor 2 (RUNX2). (i) Immunofluorescence staining of the macrophage

polarization-associated markers Arg-1 and iNOS in macrophages cultured with different material groups. (j) Quantitative analysis of RUNX2 and BMP2 fluorescence intensity and the percentage of Arg-1-positive macrophages ($n = 6$). (k) Relative mRNA expression levels of alkaline phosphatase (ALP), osteopontin (OPN), and RUNX2 in MSCs cultured on different material surfaces for 7 d, determined by quantitative real-time polymerase chain reaction (qRT-PCR) ($n = 3$).

The biocompatibility of the piezoelectric interfaces was first assessed by hemolysis assays, live/dead staining, and CCK-8 proliferation tests (Fig. 3b, c, f), which indicated negligible cytotoxicity and favorable hemocompatibility and cytocompatibility across all tested groups.

Osteogenic performance was evaluated using multiple complementary assays. ARS staining and semiquantitative analysis revealed that extracellular calcium deposition was highest on the PLLA-ZnO piezoelectric interface, reflecting enhanced late-stage osteogenic differentiation (Fig. 3d, e). Additional ARS staining showed that treatment with the Zn^{2+} -containing extract enhanced mineralization compared with PLLA, while the PLLA-ZnO group exhibited a further increase (Fig. S10 online). Immunofluorescence staining of the osteogenic markers BMP2 and RUNX2 further supported this pro-osteogenic effect (Fig. 3g, h, j and Figs. S11–S14 online). Quantification of ALP activity at early time points (3 and 7 d) and qRT-PCR analysis of key osteogenic genes (ALP, OPN, and RUNX2) after 7 d further supported enhanced osteogenic differentiation on the PLLA-ZnO piezoelectric interface (Fig. 3k and Fig. S15 online).

The PLLA-ZnO piezoelectric interface also exhibited immunomodulatory effects within the peri-implant regenerative microenvironment. Immunofluorescence analysis showed decreased iNOS expression together with increased Arg-1 expression in the PLLA-ZnO group, indicating a shift toward a pro-healing macrophage phenotype (Fig. 3i, j). Together, these findings indicate that the PLLA-ZnO piezoelectric interface creates a favorable microenvironment for bone tissue repair and regeneration.

3.3. Antibacterial performance and microenvironmental regulation

Effective antibacterial activity is essential for restoring the osteoimmune microenvironment, as bacterial colonization is a major driver of persistent inflammation and impaired osteogenesis [36,37]. The antibacterial performance of the piezoelectric interfaces was evaluated using *S. aureus* and *E. coli* as representative Gram-positive and Gram-negative bacteria.

Colony formation assays and quantitative survival analysis demonstrated that the PLLA-ZnO piezoelectric interface exhibited enhanced antibacterial efficacy compared with the PLLA and PLLA/ZnO controls (Fig. 4b, c). This effect is attributed to the

unique nanowire array architecture, which enables direct interfacial interactions with bacteria and supports multiple antibacterial mechanisms. Mechanistically, the exposed ZnO NWs combine nanotopographical disruption, oxidative stress, and ion-mediated interference, leading to bacterial membrane damage and metabolic inhibition (Fig. 4a). These mechanisms were supported by increased intracellular protein leakage (Fig. 4g), elevated intracellular reactive oxygen species (ROS) levels (Fig. 4d and Fig. S16 online), and inhibited bacterial biofilm formation (Fig. 4e, f), indicating compromised membrane integrity and suppressed bacterial proliferation. SEM observations further confirmed severe morphological damage, including collapsed *S. aureus* cells and damaged *E. coli* structures on the PLLA-ZnO surface (Fig. 4h), while live/dead bacterial staining verified the robust bactericidal effect of the interface (Fig. 4i and Figs. S17 and S18 online).

Fig. 4. *In vitro* antibacterial performance of the PLLA-ZnO piezoelectric interface. (a) Schematic illustration of the triple antibacterial mechanism of the PLLA-ZnO piezoelectric interface. (b) Qualitative colony formation assay of *S. aureus* and *E. coli* on agar plates after 6 h of co-culture with different material groups. (c) Bacterial survival rate of *S. aureus* and *E. coli* corresponding to panel (b) ($n = 3$). (d) DCFH-DA fluorescence staining images for intracellular ROS detection in *S. aureus* and *E. coli* after 6 h of co-culture with different materials. (e) Crystal violet staining images of biofilm formation by *S. aureus* and *E. coli* after 24 h on different material surfaces. (f) Quantitative analysis of biofilm biomass based on absorbance at 565 nm ($A_{565\text{ nm}}$), corresponding to panel (e) ($n = 4$). (g) Quantification of intracellular protein leakage from bacteria after 6 h of co-culture with different material groups ($n = 4$). (h) SEM images showing morphological changes in *S. aureus* and *E. coli* after 6 h of co-culture with different material surfaces. Red arrows indicate bacteria with damaged and abnormal morphology. (i) Live/dead fluorescence staining of *S. aureus* and *E. coli* on different material surfaces after 12 h of incubation using DMAO/PI dual staining. Green (DMAO): total bacteria; red (PI): dead bacteria; yellow (merged): dead bacteria.

3.4. *In vivo* evaluation in an infection model

To evaluate the *in vivo* performance of the PLLA-ZnO piezoelectric interface under infection conditions, a rat femoral implant-associated osteomyelitis model was established by local injection of *S. aureus* followed immediately by implantation of a coated Ti rod (Fig. 5a and Figs. S19 and S20 online). Four groups were investigated: Ti, Ti/PLLA, Ti/PLLA/ZnO, and Ti/PLLA-ZnO. Biological responses were analyzed at both the acute inflammatory stage (week 1) and the chronic repair stage (week 6).

Fig. 5. *In vivo* antibacterial, anti-inflammatory, and pro-osteogenic efficacy of the PLLA-ZnO piezoelectric interface at the acute inflammatory stage in an implant-associated osteomyelitis model. (a) Schematic illustration of the establishment of the rat femoral implant-associated osteomyelitis

model and the experimental workflow. (b) Schematic of the implant-surface bacterial colony formation assay (left) and representative colony-growth images on LB agar plates after the retrieved implants were rolled (right). (c) Schematic of the bacterial turbidity assay (left) and representative LB-medium turbidity images after retrieved implants were incubated for 2 h (right). (d) Quantitative analysis of relative bacterial survival rate corresponding to panel (c). (e) Peripheral blood neutrophil percentages at the acute inflammatory stage after implantation. (f) Hematoxylin and eosin (H&E) staining of peri-implant tissues at the acute inflammatory stage (green arrows indicate infiltrating neutrophils) and semiquantitative analysis of the neutrophil infiltration area. (g) Giemsa staining of peri-implant tissues at the acute inflammatory stage (blue arrows indicate residual bacteria) and semiquantitative analysis of the residual bacterial area. (h–j) Immunohistochemical (IHC) staining of peri-implant tissues at the acute inflammatory stage, including the pro-inflammatory factor TNF- α (h), anti-inflammatory factor IL-10 (i), and early osteogenic marker osteocalcin (OCN) (j), with corresponding quantitative analyses of staining intensity (IOD/Area). All quantitative analyses were performed with $n = 3$ animals per group.

At week 1, the PLLA-ZnO piezoelectric interface exhibited markedly enhanced antibacterial efficacy *in vivo*. Colony formation assays showed substantial bacterial clearance from the implant surface, with survival rates below 10% and significantly lower than those of all control groups (Fig. 5b–d). This bacterial clearance was accompanied by reduced systemic inflammation, as evidenced by a lower peripheral blood neutrophil percentage (Fig. 5e). Consistently, myeloperoxidase (MPO) activity in peri-implant tissues on postoperative day 3 was significantly lower in the PLLA-ZnO group than in the other groups (Fig. S21 online), indicating attenuated local neutrophil-associated inflammatory activity. Histological analysis further confirmed reduced neutrophil infiltration and minimal bacterial colonization in peri-implant tissues (Fig. 5f, g), indicating effective suppression of infection at both systemic and local levels.

Importantly, the PLLA-ZnO piezoelectric interface also demonstrated early immunomodulatory effects. Immunohistochemical staining revealed downregulation of the pro-inflammatory factor TNF- α and upregulation of the anti-inflammatory cytokine IL-10 (Fig. 5h, i), indicating a shift toward a pro-healing immune microenvironment. Meanwhile, enhanced expression of the osteogenic marker OCN was observed (Fig. 5j), suggesting the initiation of early osteogenic activity under infection conditions. Collectively, these results demonstrate that the PLLA-ZnO piezoelectric interface coordinates antibacterial activity and immune modulation, establishing a favorable microenvironment for subsequent bone regeneration.

At week 6, long-term bone regeneration and microenvironmental remodeling were further evaluated. Micro-CT analysis showed substantial new bone formation surrounding the PLLA-ZnO implants, with dense and continuous bone structures in both cancellous bone and marrow cavity regions (Fig. 6a, b). Quantitative analysis confirmed significantly higher BV/TV values than those in all control groups (Fig. 6d, f). Histological staining further revealed mature bone tissue tightly integrated with the

implant surface, indicating superior osseointegration (Fig. 6c).

Fig. 6. Long-term pro-osteogenic and anti-inflammatory efficacy of the PLLA-ZnO piezoelectric interface at the chronic repair stage. (a) Schematic illustration of the regions of interest (ROIs) used to evaluate bone regeneration, including the cancellous bone and marrow cavity regions around the implant in the rat femur. (b) 3D micro-CT reconstruction images of new bone formation in the cancellous bone and marrow cavity ROIs around the implants. (c) Masson's trichrome staining of cross-sections perpendicular to the long axis of the femur at the same implant depth, showing new bone (NB) formation around titanium rods modified with different interfaces. (d) Quantitative analysis of the bone volume/tissue volume (BV/TV) ratio of new bone in the cancellous bone ROI. (e) Peripheral blood lymphocyte percentages at the chronic repair stage. (f) Quantitative analysis of the BV/TV ratio of new bone in the marrow cavity ROI. (g–i) IHC staining of peri-implant tissues at the chronic repair stage, including the pro-inflammatory factor TNF- α (g), anti-inflammatory factor IL-10 (h), and osteogenic marker OCN (i), with corresponding quantitative analyses of staining intensity (IOD/Area). All quantitative analyses were performed with $n = 4$ animals per group.

In parallel, the PLLA-ZnO group maintained effective control over chronic inflammation. Peripheral blood lymphocyte percentages were significantly reduced (Fig. 6e), while local tissues exhibited sustained downregulation of TNF- α and upregulation of IL-10 (Fig. 6g, h), indicating sustained immunomodulatory effects. Enhanced OCN expression further demonstrated sustained osteogenic activity during the late stage (Fig. 6i). These findings indicate that the PLLA-ZnO piezoelectric interface not only suppresses early infection but also sustains regulation of the immune microenvironment to support bone regeneration.

Collectively, the PLLA-ZnO piezoelectric interface achieves spatiotemporally coordinated regulation of antibacterial activity, immune responses, and osteogenesis, effectively resolving the infection–regeneration conflict and enabling successful bone healing under infectious conditions. No obvious histopathological abnormalities were observed in major organs (Fig. S22 online), supporting its favorable *in vivo* biosafety.

4. Discussion and conclusion

Implant-associated osteomyelitis arises from the coupled disruption of antibacterial defense, immune regulation, and bone regeneration within the osteoimmune microenvironment [1–3]. Conventional piezoelectric implant interfaces, which typically address a single function, are insufficient to resolve this intrinsic conflict [38–40]. In this study, we developed a microenvironment-responsive PLLA-ZnO interface that integrates enhanced piezoelectricity and nanotopography with electromechanical

stimulation and ion-mediated signaling, thereby addressing the long-standing infection-regeneration conflict in implant-associated osteomyelitis.

The key advantage of this system lies in the synergistic integration of physicochemical and biological cues. The exposed and oriented ZnO nanowire arrays provide nanotopographical cues, generate electrical signals upon deformation, and facilitate Zn^{2+} release. Cellular responses to these nanowires are also influenced by their morphology and interfacial properties. In the present system, the ZnO nanowires exhibit diameters on the order of tens of nanometers and submicron-scale lengths, forming a uniformly distributed architecture with a moderate aspect ratio. This morphology, together with improved surface hydrophilicity and mechanical stiffness, may favor cell adhesion and mechanotransduction without inducing evident cytotoxic stress, as supported by the maintained MSC viability at the selected ZnO content. These material-derived cues may act synergistically to regulate MSC osteogenic differentiation and enhance matrix mineralization. However, the specific molecular pathway underlying piezoelectricity-mediated osteogenic differentiation was not investigated in the present study. In parallel, the nanotopographical features and ion-mediated effects jointly confer potent antibacterial activity. Together, these coupled effects enable the PLLA-ZnO interface to suppress bacterial colonization, modulate immune responses, and promote bone regeneration.

Importantly, the *in vivo* results further demonstrate the capacity of this system for spatiotemporal regulation. During the early stage after implantation, rapid bacterial clearance and suppression of acute inflammation create a favorable microenvironment for tissue repair. At the later stage, sustained immunomodulation and enhanced osteogenesis drive robust bone regeneration and osseointegration, achieving a 3-fold higher bone volume/tissue volume (BV/TV) ratio than uncoated controls while limiting chronic inflammation. This sequential regulation across different healing stages highlights the ability of the piezoelectric interface to dynamically adapt to the evolving pathological microenvironment.

Overall, this study presents a microenvironment-responsive design strategy that resolves the long-standing conflict between infection control and bone regeneration. By integrating electromechanical stimulation with ion-mediated bioactivity, the PLLA-ZnO interface provides a multifunctional, dynamically responsive platform for implant surface modification. This work not only offers new insights into osteoimmune microenvironment regulation but also establishes a promising paradigm for developing next-generation orthopedic implants for implant-associated osteomyelitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Changxu Chen conceived the study, designed and performed the experiments, analyzed the data, and wrote the original draft. Jianfeng Sun and Zijian Wang contributed to methodology development, experimental investigation, data analysis, and validation. Jiaxuan Li, Xiaozhou Wen, Xuqiao Zhao, Yilin Qi, Shuncheng Yao, and Zhipeng Qu contributed to experimental investigation and data collection. Jinxi Liu contributed to methodology development and data analysis. Yong Chen and Engui Wang contributed to experimental support and resources. Han Ouyang contributed to methodology development, supervision, and manuscript revision. Swee Hin Teoh contributed to supervision and manuscript review and editing. Xi Cui contributed to study conception, supervision, project administration, and manuscript review and editing. Zhou Li conceived and supervised the study, acquired funding, administered the project, and reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

Appendix A. Supplementary material

References

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