

Simvastatin and Moxifloxacin Co-Delivery via ZIF-8/PDA Coating on PEEK Implants: A Strategy for Combating Implant-Associated Infection and Enhancing Osseointegration

Zidong Wu^{1,*}, Kai Ma^{1,2,*}, Zixuan Wu^{1,*}, Xiaoyu Guo¹, Yuning Qu¹, Zheyue Zhu¹, Weidong Zhu^{1,3}, Chengxin Li², Yi Liu^{1,2}, Rui Ma¹, Pei Yang¹

¹Joint and Foot & Ankle Ward of Orthopedic Center, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710004, People's Republic of China; ²State Key Laboratory for Mechanical Behavior of Materials, School of Materials Science and Engineering, Xi'an Jiaotong University, Xi'an, 710049, People's Republic of China; ³Department of Spinal Surgery, No. 215 Hospital of Shaanxi Nuclear Industry, Xianyang, Shaanxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Rui Ma; Pei Yang, Email shandongmarui@126.com; yangpei@xjtu.edu.cn

Background: Polyetheretherketone (PEEK) is a promising orthopedic implant material due to its bone-matched elastic modulus and radiolucency. However, its clinical application is limited by biological inertness and lack of antibacterial activity, which predispose to implant-associated infection (IAI) and poor osseointegration. This study developed a multifunctional coating on sulfonated PEEK (SPEEK) via polydopamine (PDA)-mediated co-delivery of simvastatin-loaded ZIF-8 nanoparticles (SIM@ZIF-8) and moxifloxacin (MOX) to address these limitations.

Methods: A time-programmed release coating was fabricated on SPEEK by embedding SIM@ZIF-8 within a PDA layer and surface-loading MOX onto the surface. The coating's physicochemical properties, drug release profiles, biocompatibility, antibacterial activity, and osteogenic capacity were evaluated in vitro. Antibacterial efficacy and osseointegration were further assessed in vivo using rat subcutaneous infection and rabbit femoral defect models.

Results: Release profiles revealed that MOX exhibited burst release within 24 h, whereas SIM quickly reached therapeutic concentrations during the initial phase and was slowly released over five weeks, while Zn²⁺ from ZIF-8 was released synchronously with SIM. In vitro antimicrobial assays showed that the synergistic release of MOX and SIM exhibited potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, providing sustained inhibition of *Staphylococcus aureus* biofilms. The coating also promoted MC3T3-E1 cell adhesion, spreading, and osteogenic differentiation, as indicated by upregulated osteogenic markers (Runx2, OCN, OPN, COL1A1), enhanced matrix mineralization, and increased BMP-2 expression. In rat models, bacterial colonization on the coated surface and residual bacteria in tissues were significantly reduced. In rabbit femoral defects, sustained co-release of SIM and Zn²⁺ accelerated new bone formation and improved osseointegration, confirmed by micro-CT, double fluorescence labeling, and histological analysis.

Conclusion: This study presents a novel bioactive PEEK implant coating integrating antibacterial and osteogenic functions through a MOX/SIM@ZIF-8/PDA system. The strategy effectively combats IAI and enhances osseointegration, offering potential for clinical orthopedic applications.

Keywords: polyetheretherketone, antibacterial coating, osseointegration, simvastatin, drug delivery

Introduction

Poor osseointegration and implant-associated infection (IAI) present significant challenges for orthopedic implants.^{1,2} IAI frequently necessitates aggressive interventions, including revision surgery or amputation, posing severe risks to patient survival. Furthermore, the avascular implant surface promotes microbial adhesion and biofilm formation.³ These biofilms

shield bacteria from immune clearance and antibiotic penetration, rendering conventional therapies ineffective.^{3,4} Therefore, this study aims to develop an implant surface capable of actively reducing infection risks while establishing a favorable local microenvironment to promote osseointegration.

Polyetheretherketone (PEEK) is considered a viable alternative to metal implants owing to its elastic modulus comparable to that of human cortical bone, thereby effectively preventing the stress-shielding effect commonly associated with metallic implants.⁵ However, PEEK's inherent bioinertness limits its osseointegration capacity, and its lack of antibacterial activity may lead to implant failure during bacterial infection.⁶ Therefore, modifying PEEK to enhance its osteogenic capacity and confer antibacterial activity is necessary. Sulfonation has been widely employed as a simple, yet effective surface modification strategy for PEEK. This treatment method can form a three-dimensional porous network structure and introduce sulfonic acid groups ($-\text{SO}_3\text{H}$), thereby providing a structural foundation for the stable adhesion of the coating.⁷

Simvastatin (SIM), a classical cholesterol-lowering drug, has been reported to demonstrate potential effects in promoting osteogenesis, angiogenesis, and antimicrobial activity, positioning it as a viable candidate for functionalizing PEEK to achieve dual osteogenic and antimicrobial properties.^{8,9} However, studies evaluating the antimicrobial efficacy of SIM have primarily focused on gram-positive pathogens such as *Staphylococcus aureus* (*S. aureus*), and its antibacterial activity against *Escherichia coli* (*E. coli*), a common gram-negative pathogen in IAI, is relatively lacking.^{10,11} Moxifloxacin (MOX), a fluoroquinolone antibiotic, is a broad-spectrum antimicrobial agent with potent activity against both gram-positive cocci and gram-negative bacilli. Incorporating MOX can compensate for the lack of antibacterial efficacy of SIM against gram-negative pathogens, thereby enhancing the broad-spectrum antimicrobial activity of materials.

Metal-organic frameworks (MOFs) are ideal for efficient drug-loading due to their high specific surface areas and tunable three-dimensional porous structures.^{12,13} The zeolite imidazolate framework-8 (ZIF-8) is a subclass of MOFs characterized by high water stability, high porosity, and low toxicity.¹⁴ Using ZIF-8 as a carrier to load SIM and prepare SIM@ZIF-8 nanoparticles enables controlled, sustained drug release. Additionally, ZIF-8 exhibits in vivo degradability for the sustained release of zinc (Zn), which plays a positive role in promoting osteogenesis.

Polydopamine (PDA) is known for its ability to universally adhere to the surfaces of various implant materials, drawing inspiration from mussel foot proteins.¹⁵ The introduction of PDA endows SPEEK with excellent biocompatibility and abundant surface functional groups. Therefore, PDA serves as an ideal bridge connecting the hydrophobic SPEEK surface with functional nanoparticles (ZIF-8) and therapeutic agents (MOX).

This study proposes a three-step fabrication strategy: first, sulfonation of PEEK to improve the stability of subsequent coating adhesion; second, deposition of a mixture of PDA and SIM@ZIF-8 nanoparticles onto SPEEK to form a bioactive sustained-release layer; and finally, surface loading of MOX onto the PDA coating for rapid release. The resulting MOX/SIM@ZIF-8/PDA/SPEEK coating constitutes an innovative system that enables temporally controlled release of multiple functional components. This design achieves rapid bacterial elimination in the early post-implantation phase, long-term suppression of biofilm formation, and enhanced osseointegration (Figure 1 serves as the graphical abstract). Consequently, it simultaneously addresses the dual challenges of IAI and poor osseointegration while mitigating the risk of antibiotic resistance associated with prolonged antibiotic use. The present study systematically investigates the in vitro and in vivo performance of this coating, including its physicochemical properties, drug release behavior, biocompatibility, osteogenic activity, and antimicrobial effect.

Materials and Methods

Sample Preparation

Preparation and Characterization of SIM@ZIF-8 Nanoparticles

First, 20 mg of SIM (Yuanye Bio-Technology, China) and 100 mg of zinc nitrate hexahydrate $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Aladdin, China) were dissolved in 5 mL methanol at 25°C. Subsequently, 300 mg of 2-methylimidazole (Aladdin, China) was dissolved in 5 mL methanol and then added dropwise to the SIM and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ mixture under continuous magnetic stirring for 1 h. The resulting nanoparticles were collected by centrifugation (10,000 x g, 20 min), washed

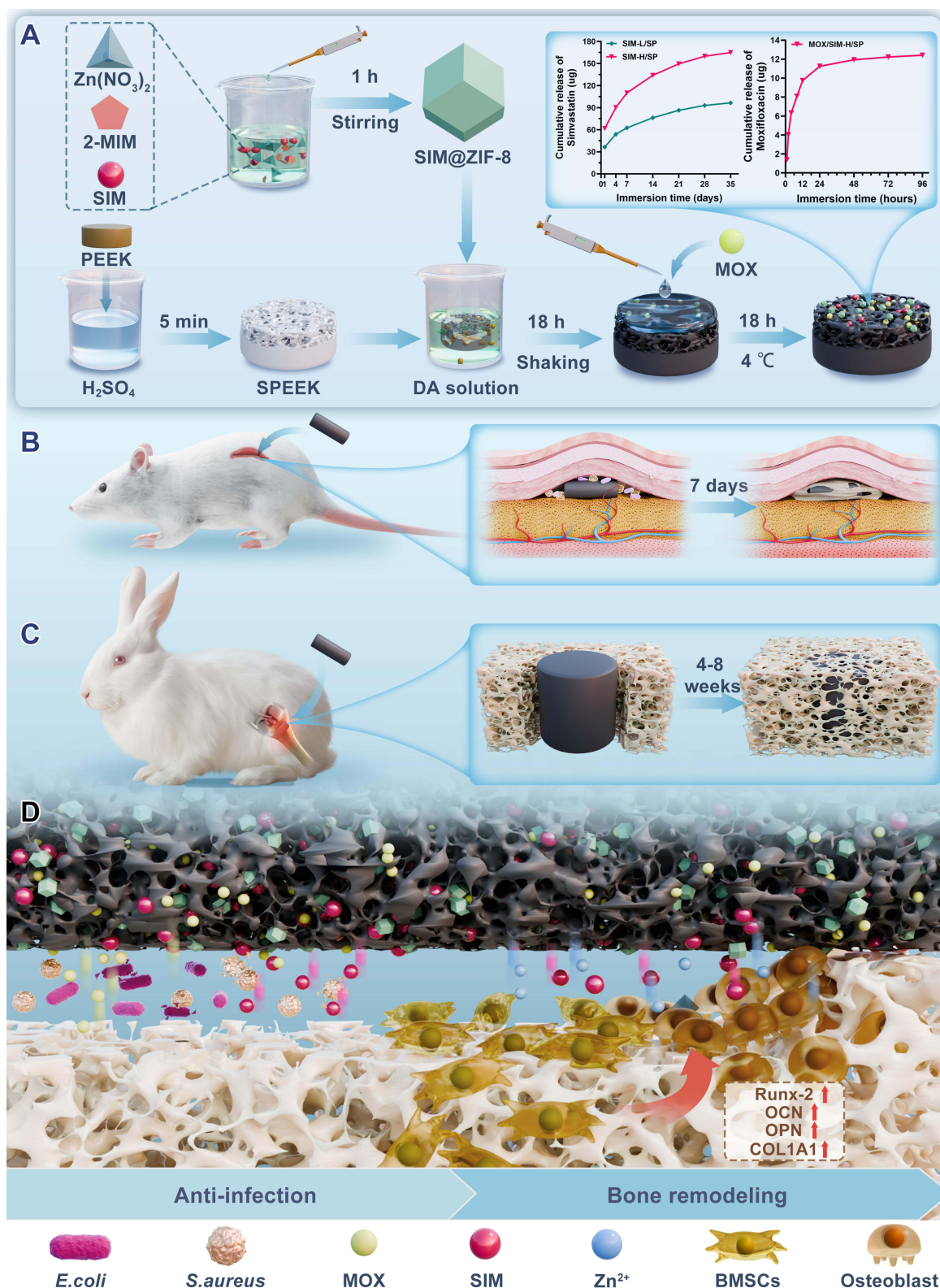


Figure 1 Schematic presentations for material preparation and evaluation in this study. **(A)** Preparation process for MOX/SIM@ZIF-8/PDA/SPEEK, and the graph in the upper right corner displays the cumulative release curves of Simvastatin and Moxifloxacin; **(B)** Rat subcutaneous infection model; **(C)** Rabbit femoral condyle defect model; **(D)** In vivo anti-infection and osseointegration of implants: the implant kills bacteria such as *E. coli* and *S. aureus* to achieve anti-infective effects; it also promotes the differentiation of BMSCs into osteoblasts, upregulates the expression of Runx-2, OCN, OPN, and COL1A1, thereby enhancing osseointegration.

thrice with methanol, and then dried to obtain SIM@ZIF-8 nanoparticles. The preparation method for the unloaded ZIF-8 nanoparticles was the same as that described above, except that SIM was not added.

The morphologies of unloaded ZIF-8 and SIM@ZIF-8 nanoparticles were observed using scanning electron microscopy (SEM, MAIA3 LMH, Tescan, China) and transmission electron microscopy (TEM, Talos L120C TEM, Thermo Fisher, USA). Particle size analyses of both sets of particles were performed using a fully automated surface area and porosity analyzer (ASAP 2460, Micromeritics, USA). To further validate the successful loading of SIM, Fourier-transform infrared spectroscopy (FTIR; Nicolet iS20, Thermo Fisher) was performed on SIM, ZIF-8, and SIM@ZIF-8.

Sulfonation of PEEK

PEEK was purchased from Nanjing Shousu Special Engineering Plastics Co., Ltd. PEEK samples were processed into the following two distinct sizes: disc-shaped ($\Phi 15\text{mm} \times 2\text{ mm}$) for characterization and in vitro experiments, and rod-shaped ($\Phi 5\text{mm} \times 10\text{ mm}$) for in vivo antimicrobial and osteogenic experiments. Prior to the experiment, the PEEK samples were ultrasonically cleaned with acetone for 10 min, followed by ultrasonic cleaning with ultrapure water three times, each for 5 min. The samples were dried at 37°C . PEEK was placed in 95–98% concentrated sulfuric acid and subjected to sulfonation under magnetic stirring for 5 min. The solution was stirred and washed with acetone for 10 min, followed by ultrasonic cleaning with ultrapure water three times for 5 min each. Finally, the sulfonated PEEK was treated at high pressure and 121°C for 30 min, to remove residual sulfur impurities, yielding sulfonated PEEK (SPEEK).¹⁶

Preparation of MOX/SIM@ZIF-8/PDA Coatings

The prepared SIM@ZIF-8 nanoparticles were ultrasonically dispersed in a Tris-HCl buffer (10 mM, pH 8.5). Dopamine hydrochloride (Solarbio, China) was added to achieve a concentration of 2 mg/mL, and the SPEEK sample was immersed in this solution. The mixture was then incubated with shaking at room temperature in the dark for 18 h. Subsequently, the sample was washed with deionized water to remove excess impurities, yielding the SIM@ZIF-8/PDA/SPEEK sample. By controlling the amount of SIM@ZIF-8 nanoparticles added, we prepared the following three sets of samples with different SIM concentrations for subsequent studies: unloaded ZIF-8 alone, SIM@ZIF-8 added at equal masses, and SIM@ZIF-8 added at double masses. Based on clinically safe and effective dosages against the common intraoperative pathogens *S. aureus* and *E. coli*, the optimal MOX concentration was determined to be approximately 2–4 $\mu\text{g/mL}$.^{17,18} Finally, a 5 $\mu\text{g/mL}$ MOX (Solarbio) solution was prepared using Tris-HCl buffer (10 mM, pH 8.5). SIM@ZIF-8/PDA/SPEEK samples were immersed in this solution and incubated overnight at 4°C in the dark. After washing with deionized water, the MOX/SIM@ZIF-8/PDA/SPEEK samples were obtained. Subsequent experiments were conducted using five groups of samples, with the specific groupings and names listed in Table 1. The samples for cell and animal experiments were sterilized with ethylene oxide prior to use.

Characterization of Coating Materials

The surface morphologies and elemental distributions of the different samples were examined using SEM and energy dispersive spectroscopy (EDS). The surface elemental composition of each group was characterized by X-ray photoelectron spectroscopy (XPS; Thermo Scientific K-Alpha, USA), and the chemical states and bonds of the elements were analyzed. Surface roughness and 3D topography images were obtained using atomic force microscopy (AFM; Dimension Icon, Bruker, Germany). Each sample was tested at room temperature with a scanning range set to $5\text{ }\mu\text{m} \times 5\text{ }\mu\text{m}$. Key parameters characterizing the surface roughness, including the mean roughness (R_a) and root mean square roughness (R_q), were software-calculated. Static water contact angle measurements were performed on the samples using a contact-angle measurement instrument (DSA100S, KRÜSS, Germany), to evaluate their hydrophilicity. As the test liquid, 4 μL of ultrapure water was stably deposited onto the horizontally placed sample surface at room temperature. The droplet morphology was captured in real-time, and the water contact angle was measured.

Table 1 Groupings and Abbreviations of Samples

Grouping	Abbreviation	Processing Method
SPEEK	SP	Uncoated sulfonated PEEK as the control group.
ZIF-8/PDA/SPEEK	ZIF/SP	Load unloaded ZIF-8 nanoparticles onto the SPEEK surface via PDA.
SIM@ZIF-8/PDA/SPEEK (Low SIM concentration)	SIM-L/SP	Load SIM@ZIF-8 nanoparticles onto the SPEEK surface via PDA at the same mass as the ZIF-8 in the ZIF/SP group.
SIM@ZIF-8/PDA/SPEEK (High SIM concentration)	SIM-H/SP	Load SIM@ZIF-8 nanoparticles onto the PEEK surface via PDA at twice the mass of SIM@ZIF-8 in the SIM-L/SP group.
MOX/SIM@ZIF-8/PDA/SPEEK	MOX/SIM-H/SP	Immerse the SIM-H/SP group material in MOX solution to load MOX onto the PDA coating.

Drug Release Profile, Encapsulation Efficiency and Loading Capacity

To evaluate drug release behavior in samples, each sample was immersed in 5 mL of phosphate buffered saline (PBS) solution and incubated statically at 37°C. The entire immersion solution was collected at various time points and replaced with an equal volume of fresh PBS. The immersion solutions were retrieved on days 3, 5, 7, 14, 21, 28, and 35, and the concentration of sustained-release Zn²⁺ ions was measured using inductively coupled plasma-optical emission spectrometer (ICP-OES, ICAP 7400, Thermo Fisher). For SIM released from the nanoparticles, the immersion solutions were collected on days 1, 4, 7, 14, 21, 28, and 35 for analysis. The absorbance of SIM standard solutions was measured at 238 nm using a UV-Vis spectrophotometer (UV-3600, Shimadzu, Japan). A calibration curve was plotted, and the concentration of SIM in the samples was determined based on this curve. For immediate-release MOX, the immersion solution was collected for analysis at 1, 2, 4, 8, 12, and 24 h, and on days 2, 3, and 4. The drug concentration was calculated by measuring the fluorescence intensity using a multifunctional microplate reader (SpectraMax iD5e, Molecular Devices, USA) with an excitation wavelength of 294 nm and emission wavelength of 503 nm. A calibration curve was similarly generated, and the MOX concentration in the samples was calculated.

Determination of SIM Encapsulation Efficiency (EE) and Drug Loading Capacity (DLC): After preparation of SIM@ZIF-8, the unloaded SIM in the centrifugation and washing supernatants was measured to determine the amount of SIM encapsulated within the nanoparticles. EE and DLC were then calculated using the following formulas: Determination of MOX Encapsulation Efficiency on PDA: the total amount of MOX on the PDA surface was calculated based on the MOX release profile, and EE was subsequently determined using the established formula.

$$EE\% = \frac{\text{mass of drug in nanoparticles}}{\text{mass of drug initially added}} \times 100\%$$

$$DLC\% = \frac{\text{mass of drug in nanoparticles}}{\text{total mass of nanoparticles}} \times 100\%$$

In vitro Antimicrobial Activity

Bacterial Strain and Culture

The standard strains used in the antimicrobial assays, *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922), were purchased from the China Center for Type Culture Collection. Glycerol stocks were inoculated onto tryptic soy agar (TSA, Solarbio) plates using the streak plate method and incubated at 37°C for 16 h. Single colonies were picked from the plates and cultured in tryptic soy broth (TSB, Solarbio) medium on a 37°C shaking incubator (200 rpm). Subsequent experiments utilized bacteria from the third generation after recovery.

Minimum Inhibitory Concentration Testing of SIM

The minimum inhibitory concentrations (MIC) of SIM against *S. aureus* and *E. coli* were determined. Following the Clinical Laboratory Standards Institute guidelines (CLSI M07) Method 25, bacterial suspensions were prepared using the

direct colony suspension method and were tested using the broth microdilution method. Bacterial suspensions at various drug concentrations were placed in U-bottom 96-well plates and incubated at 37°C for 16 h. The MIC of SIM was determined by visually identifying the lowest drug concentration completely inhibiting bacterial growth.¹⁹

The Spread Plate Method Antimicrobial Test

The spread-plate method was used to evaluate the antibacterial activities of the samples against *S. aureus* and *E. coli* in vitro. The sample was placed in 2 mL of bacterial suspension at a concentration of 1×10^6 CFU/mL and incubated at 37°C for 24 h. The bacterial suspension was collected, diluted, inoculated onto TSA plates, and incubated at 37°C for 24 h. To evaluate the long-term antibacterial performance, the samples were immersed for 4, 7, and 14 days. After retrieval, they were incubated in a *S. aureus* suspension for 24 h, followed by spread plating of the collected bacterial suspensions. Colony counts on the agar plates were determined using the ImageJ software. The SP group, which was devoid of any antimicrobial activity, served as the control; the antimicrobial activity of the samples from each coating group was evaluated.

SEM Analysis

SIM exhibited weak antibacterial activity against *E. coli*; therefore, *S. aureus* was selected to evaluate its anti-biofilm properties. Samples were placed in a 2 mL suspension of *S. aureus* at a concentration of 1×10^8 CFU/mL and incubated statically at 37°C for 48 h. The bacterial suspension was aspirated and the sample surface was carefully washed twice with PBS. Bacteria adhering to the sample surface were fixed using 2.5% glutaraldehyde (Solarbio), followed by ethanol gradient dehydration. The samples were then dried in a vacuum desiccator. After gold coating, the bacterial morphology was observed using SEM (MAIA3 LMH, Tescan, China).

Live/Dead Bacterial Staining

The sample was placed in a 2 mL suspension of *S. aureus* at a concentration of 1×10^8 CFU/mL and incubated at 37°C for 48 h. The sample surface was then washed with PBS to remove non-adherent bacteria. Subsequently, the biofilm on the sample surface was stained using a bacterial live/dead staining kit (DMAO/PI; Beyotime, China) according to the manufacturer's instructions. The staining results were then observed using a confocal laser scanning microscope (CLSM; STELLARIS 5, Leica, Germany).

Crystal Violet Staining

Since coating color affects visual observation, biofilm assessment via crystal violet staining utilizes a sample extract solution for biofilm cultivation. The sample was immersed in TSB medium, incubated at 37°C for 24 h, and the extract solution was then collected. An equal volume of fresh bacterial medium was used for subsequent sample collection. Suspensions of *S. aureus* cultured to the logarithmic growth phase were centrifuged (5000 rpm, 5 min), resuspended in the sample extract, and adjusted to a concentration of 1×10^8 CFU/mL. Cultures were incubated at 37°C in 24-well plates for 24 h. After 24 h, a 50% medium exchange with fresh eluate was performed, and the culture was continued for an additional 24 h before termination. To further evaluate the long-term anti-biofilm performance, the culture duration of bacteria in the sample eluates was extended to 6 and 9 days, with half-volume replacement of fresh eluate every 24 h. Upon completion of the culture, the biofilms were subjected to crystal violet staining. The suspension was aspirated, the adherent bacteria were washed with PBS, and crystal violet (0.1%, Solarbio) was added for staining. Finally, crystal violet was dissolved in 95% ethanol, and the absorbance was measured at 570 nm.

Biocompatibility Evaluation In vitro

Cell Culture

Mouse osteoblast precursor cells MC3T3-E1 were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). MC3T3-E1 cells were cultured in α -Minimum Essential Medium (MEM; Gibco, USA) supplemented with 10% fetal bovine serum (Vazyme, China) and 1% penicillin/streptomycin (Mishushengwu, China) under humidified conditions at 37°C and 5% CO₂.

Cell Proliferation and Viability

The samples were then placed in 24-well plates. Cells were digested with trypsin, passaged when confluency reached 80%, and co-cultured with samples from each group. On days 1, 3, 5, and 7 of culture, the cells were incubated for 2 h with a medium containing 10% CCK-8 (DiNing, China), and the optical density (OD) values were determined at 450 nm using a multifunctional microplate reader. After 24 h of cell culture using the same method, cells on the sample surfaces were stained using a live/dead staining kit (Beyotime, China). Observations were made using CLSM, and cell survival rates were statistically analyzed.

Cell Spreading and Morphology

The cells were co-cultured with the samples, as described previously. After 24 h of co-culturing, the surface cells were fixed using 2.5% glutaraldehyde. Subsequent processing was performed as described in Minimum Inhibitory Concentration Testing of SIM, and the cell morphology was observed via SEM. After 48 h of co-culture, the cells were fixed with 4% paraformaldehyde (Solarbio) and subsequently permeabilized with 0.1% Triton X-100 (Beyotime). Cytoskeletal staining was performed using phalloidin-TRITC (Proteintech Group, China), followed by nuclear staining with 4',6-diamidino-2-phenylindole (DAPI; Beyotime). Finally, the staining results were observed using a CLSM (STELLARIS 5, Leica, Germany).

Osteogenic Differentiation in vitro

Osteogenic Differentiation Cell Culture

MC3T3-E1 cells were seeded onto the sample surface for culture under conditions identical to those described in Cell Culture. After 24 h of cell culture on the sample surface, the complete medium was replaced with an osteogenic induction medium (Oricell, China). Every 2–3 days, half the volume was replaced with fresh osteogenic induction medium. Induction was continued for 7, 14, and 21 d before proceeding with subsequent experiments.

When using sample-soaked extraction liquid for cell culture, the samples were immersed in α -MEM complete medium at 37°C for 24 h prior to cell seeding. After the cells adhered to the newly seeded blank 24-well plates, the extraction liquid was collected and used for cell culture. Simultaneously, the medium was replaced with the osteogenic induction medium to continue soaking the sample. Once the cells reached 70% confluence in a 24-well plate, the medium was replaced with fresh extraction solution for osteogenic induction. Following the same procedure, every 2–3 d, half of the extraction solution was aspirated for osteogenic induction and replenished with an equal volume of fresh osteogenic induction medium to continue immersing the sample.

Alkaline Phosphatase and Alizarin Red Staining

Since sample background color affects staining, alkaline phosphatase (ALP) and Alizarin Red staining were performed using the cell culture extracts. MC3T3-E1 cells undergoing osteogenic induction on days 7 and 14 were stained using an ALP Color Development Kit (Beyotime) according to the manufacturer's instructions. ALP activity was assessed using an ALP Quantification Kit (Beyotime), according to the manufacturer's instructions. On day 21, the cells were stained with alizarin red solution (OriCell) to visualize the degree of cellular matrix mineralization. The mineralized nodules were subsequently dissolved in 10% hexadecylpyridinium chloride (Solarbio), and the OD values were measured at 562 nm using a spectrophotometer.

BMP-2 Immunofluorescence Staining

The MC3T3-E1 cells were seeded in confocal culture dishes (BioSharp). After seven days of osteogenic induction, the cells were fixed with 4% paraformaldehyde. Permeabilization and blocking were performed using 0.1% Triton X-100 and 5% bovine serum albumin (Solarbio). The cells were incubated overnight at 4°C with anti-BMP-2 antibody (Affinity, China). Secondary antibody labeled with FITC (Proteintech Group, China) was added and incubated at 4°C for 2 h. Cell nuclei were stained with DAPI. Images were acquired using CLSM and analyzed semi-quantitatively using the ImageJ software.

Expression of Osteogenic Genes

Osteogenic genes were detected using real-time quantitative polymerase chain reaction (PCR). After seven days of osteogenic induction of MC3T3-E1 cells, total RNA was extracted using an RNA extraction reagent (RNAiso Plus, Takara, Japan). Subsequently, RNA was reverse-transcribed into cDNA using the StarScript II Reverse Transcription Kit (Genstar, China). Real-time PCR was performed using a QuantStudio real-time fluorescent quantitative PCR instrument (Applied Biosystems). Data analysis was performed via the $2^{-\Delta\Delta C_t}$ method using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a reference gene. The osteogenic genes detected included *OCN*, *OPN*, *Runx2*, and *COL1A1*. The corresponding primer sequences are listed in Table 2.

Expression of Osteogenic Protein

Osteogenic protein expression was detected using Western blot analysis. After 14 d of osteogenic induction, MC3T3-E1 cells were lysed with radioimmunoprecipitation assay buffer (DiNing, China) to extract the proteins. Proteins were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membrane was then incubated in a blocking solution (5% skim milk) for 2 h. Subsequently, the membrane was incubated overnight at 4°C with the primary antibody solution. The membrane was then incubated with the corresponding secondary antibodies for 2 h. Chemiluminescent imaging was performed using a chemiluminescent imaging system and semi-quantitative analysis was conducted using the ImageJ software. The primary and secondary antibodies used were as follows: β -actin Polyclonal antibody, Osteopontin Polyclonal antibody, HRP-conjugated Goat Anti-Rabbit IgG (H+L) (Proteintech Group, China), and BMP2 Polyclonal antibody (Affinity, China).

Antibacterial Evaluation in vivo

Surgical Procedure

The Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University approved all animal experimentation protocols (XJTUAE2024-2493), which were conducted in accordance with the standards outlined in the “Guide for the Care and Use of Laboratory Animals” and the “Animal Research: Reporting of In Vivo Experiments”. To investigate the antibacterial efficacy of the samples in deep tissues, a rat model of subcutaneous infection was used. Fifteen male Sprague-Dawley rats (200–250 g; Animal Experiment Center, Xi'an Jiaotong University, China) were randomly divided into five groups of three rats each. The rats were anesthetized with 4% pentobarbital, their dorsal hair was shaved, and a 1 cm incision was made. During protocol optimization, we initially attempted to inject the bacterial suspension prior to implant placement. However, this approach resulted in leakage of the bacterial solution during subsequent procedures, leading to significant variability in the bacterial load contacting different implants. Therefore, we adopted an optimized protocol: A cylindrical sample ($\Phi 5\text{mm} \times 10\text{ mm}$) sterilized with ethylene oxide was implanted subcutaneously, and the incision was sutured. Subsequently, 100 μL of *S. aureus* suspension (concentration: 1×10^5 CFU/mL) was injected onto the surface of the subcutaneous implant using a microinjector. And finally, local massage was performed to ensure uniform distribution of the bacteria around the implant surface. On day 7 post-surgery, the rats were euthanized, and subcutaneous implants along with the surrounding soft tissue were collected.

Table 2 Primer Sequences of Osteogenic Genes

Gene	Forward Primer (5'–3')	Reverse Primer (5'–3')
<i>OCN</i>	CAAGCAGGAGGGCAATAAGGTAGTG	CGGTCTTCAAGCCATACTGGTCTG
<i>OPN</i>	ATGGACGACGATGATGACGATGATG	CTTGTGTACTAGCAGTGACGGTCTC
<i>Runx2</i>	GACTGTGGTTACCGTCATGGC	ACTTGGTTTTTCATAACAGCGGA
<i>COL1A1</i>	TAAGGGTCCCAATGGTGAGA	GGGTCCCTCGACTCCTACAT
<i>GAPDH</i>	ACAACCTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

Detection of Surface Bacteria on in vivo Samples

Under aseptic conditions, the entire sample was excised, along with the surrounding subcutaneous tissue. A small incision was made at one end of the cylindrical sample, the sample was removed, and rolled multiple times on a TSA plate to collect non-adhering surface bacteria. The specimens were then immersed in PBS. In addition, an ultrasonic agitator was used to dislodge adherent bacteria from the specimen surface. The resulting bacterial suspension was diluted and spread evenly on TSA plates. Both the TSA plates were incubated at 37°C for 24 h to observe colony development.

Tissue Staining

The soft tissue surrounding the implant was fixed in 4% paraformaldehyde, dehydrated, and paraffin-embedded for histological sectioning. Hematoxylin and eosin (H&E) staining was used to observe tissue morphology, fibrosis, and inflammatory cell infiltration. Giemsa staining was performed to observe residual bacteria in the tissue.

Osteogenic Activity in vivo

Surgical Procedure

Thirty male New Zealand rabbits each weighing 2.5 kg (Xi'an Jiaotong University Animal Experiment Center, China) were randomly divided into five groups of six rabbits each. Anesthesia was achieved via propofol injection into the marginal ear vein and isoflurane inhalation. Hair around the knee joint was shaved, and the area was disinfected. A longitudinal incision was made lateral to the patellar ligament to expose the femoral condyle. A 5 mm drill bit was used to create a defect in the lateral femoral condyle and establish a defect model. A cylindrical sample ($\Phi 5\text{mm} \times 10\text{ mm}$) sterilized with ethylene oxide was implanted into the defect site. The surgical site was sutured in layers. The rabbits were euthanized at 4 and 8 weeks post-implantation to harvest the femurs.

Micro- Computed Tomography Analysis

At weeks 4 and 8, the rabbit femurs were harvested and fixed in 4% paraformaldehyde. Scanning was performed using a micro-computed tomography (micro-CT) scanner (AX-2000, Always Imaging, China) with the following parameters: voltage 80 kV, current 70 μA , exposure time 0.5s, resolution 17 μm . The three-dimensional image of the distal femur was then reconstructed, and the 500 μm diameter area around the implant was set as the region of interest (ROI), and the tissue morphometric indexes such as bone mineral density (BMD), bone volume fraction (BV/TV), bone surface fraction (BS/BV), trabecular number (Tb.N), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th) were quantitatively analyzed in the ROI.

Double Fluorescent Labeling of New Bone Formation

Fourteen days before being euthanized, the rabbits were administered a solution of alizarin red (Solarbio) prepared in physiological saline via intraperitoneal injection (30 mg/kg). Four days before euthanasia, a solution of calcein (Solarbio) was administered via intraperitoneal injection (20 mg/kg). After the rabbits were euthanized, the collected femurs were subjected to hard-tissue sectioning. Samples were fixed in 4% paraformaldehyde for one week, rinsed with water to remove fixatives, dehydrated using ethanol and n-butanol gradients, washed with xylene, and embedded in methyl methacrylate. Sections were cut parallel to the implant cross-section, ground to 30–50 μm thickness using a microtome, and polished with talcum powder to complete section preparation. The mineral apposition rate (MAR) was calculated by measuring the average distance between two fluorescent labels in the bone tissue using the ImageJ software after observation via CLSM.

Tissue Staining

Goldner staining was performed on previously prepared hard tissue sections, and bright-field images were acquired using a slide scanner (Axioscan 7, Zeiss, Germany) to observe the newly formed bone around the implant. ImageJ software was used to analyze a 200- μm -wide region of interest (ROI) surrounding the implants. The bone-implant contact (BIC) ratio was calculated as the percentage of the length of direct contact between new bone and the implant surface relative to the total perimeter.

Statistical Analyses

All data are expressed as mean $\pm$ standard deviation. All in vitro experiments were repeated at least thrice. All statistical analyses were performed using the GraphPad Prism 10.1 statistical software. Statistical differences between groups were assessed using one-way or two-way analysis of variance, followed by Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

Results

Characterization of ZIF-8 and SIM@ZIF-8 Nanoparticles

SEM and TEM observations of ZIF-8 and SIM@ZIF-8 nanoparticles revealed rhombic dodecahedral morphologies with sizes ranging from approximately 100 to 200 nm. Following SIM loading, the nanoparticle size decreased significantly (Figure 2A). Particle size analysis of both groups revealed a BET surface area of 1,856.2703 m²/g for unloaded ZIF-8 particles, with a BJH adsorption average pore diameter of 19.7015 nm; these values decreased to 1,064.1280 m²/g and 12.4165 nm, respectively, in the SIM@ZIF-8 particles. Both sets of particles exhibit Type IV adsorption/desorption isotherms with type H1 hysteresis loops and saturated adsorption plateaus, indicating relatively uniform pore-size distribution (Figure 2B). These findings confirm that SIM molecules were successfully encapsulated within the ZIF-8 frameworks, occupying internal pores and subsequently inducing a contraction of both particle size and pore volume.

FTIR analysis of SIM, ZIF-8, and SIM@ZIF-8 revealed that compared to the ZIF-8 spectrum, the SIM@ZIF-8 spectrum exhibited absorption peaks influenced by SIM loading (Figure 2C). The absorption peak at 3431 cm⁻¹ in ZIF-8 was attributed to the N-H stretching vibration mode. Owing to the presence of adsorbed water on the metal-organic framework surface, this region also contained a significant O-H stretching vibration mode, resulting in a broad peak shape. The strong peaks near 1459 cm⁻¹ and 1420 cm⁻¹ originated from the stretching vibrations of the imidazole ring structure; strong C-N peaks appeared near 1144 cm⁻¹ and 994 cm⁻¹; a characteristic Zn-N stretching absorption peak was observed near 420 cm⁻¹. SIM@ZIF-8 primarily exhibited ZIF-8 characteristics. However, the absorption peaks at 2964 cm⁻¹ and 2969 cm⁻¹ showed significantly increased relative intensities, primarily introduced by C-H stretching vibrations from SIM; a new C=O absorption peak originating from SIM appeared at 1721 cm⁻¹. The relative intensity of the O-H absorption peak at 3432 cm⁻¹ also increased markedly, indicating the introduction of SIM side-chain O-H structures and formation of hydrogen bonds with the ZIF-8 carrier. FTIR spectroscopy further confirmed the successful loading of SIM within ZIF-8 nanoparticles, revealing strong physical interactions between the two components that provide the structural basis for sustained drug release.

Finally, based on the standard concentrations of SIM and the corresponding absorbance values, a standard curve was fitted to calculate the mass of SIM successfully loaded into ZIF-8 nanoparticles. Subsequently, the EE and DLC of SIM were determined, yielding an EE of 87.7% and a DLC of 19.8%, respectively.

Characterization of Coating

The surface morphologies of the coated materials in each group were observed using SEM (Figure 2D). Following sulfonation, the surface of the SPEEK sample exhibited a porous structure. Coating morphology in the other four groups revealed that, following dopamine deposition, the porous SPEEK surface was covered by a PDA coating, with granular material observed on the PDA coating surface. EDS analysis of the SPEEK control group and PDA-coated group loaded with ZIF-8 (Figure 2E) revealed the presence of Zn on the surface of the PDA-coated group, indicating the successful loading of ZIF-8. Visual inspection revealed that the original brown PEEK surface turned white after sulfonation. Following PDA coating, the material surface became black and subsequent MOX loading did not alter the appearance of the material (Figure 2F).

XPS was conducted to examine the surface elemental composition of the coating and the potential interactions among MOX, SIM@ZIF-8, PDA, and SPEEK. Figure 3A displays the XPS full spectra of the four sample groups; the obvious peaks corresponding to C 1s, N 1s, O 1s, and Zn 2p clearly indicate the synchronous decoration of PDA and SIM@ZIF-8 onto the SPEEK substrate, although the successful loading of MOX is not explicitly evident from the full spectra alone. The high-resolution Zn 2p spectrum (Figure S1A) is fitted into two peaks at 1022.3 and 1045.1 eV, attributed to Zn 2p_{3/2}

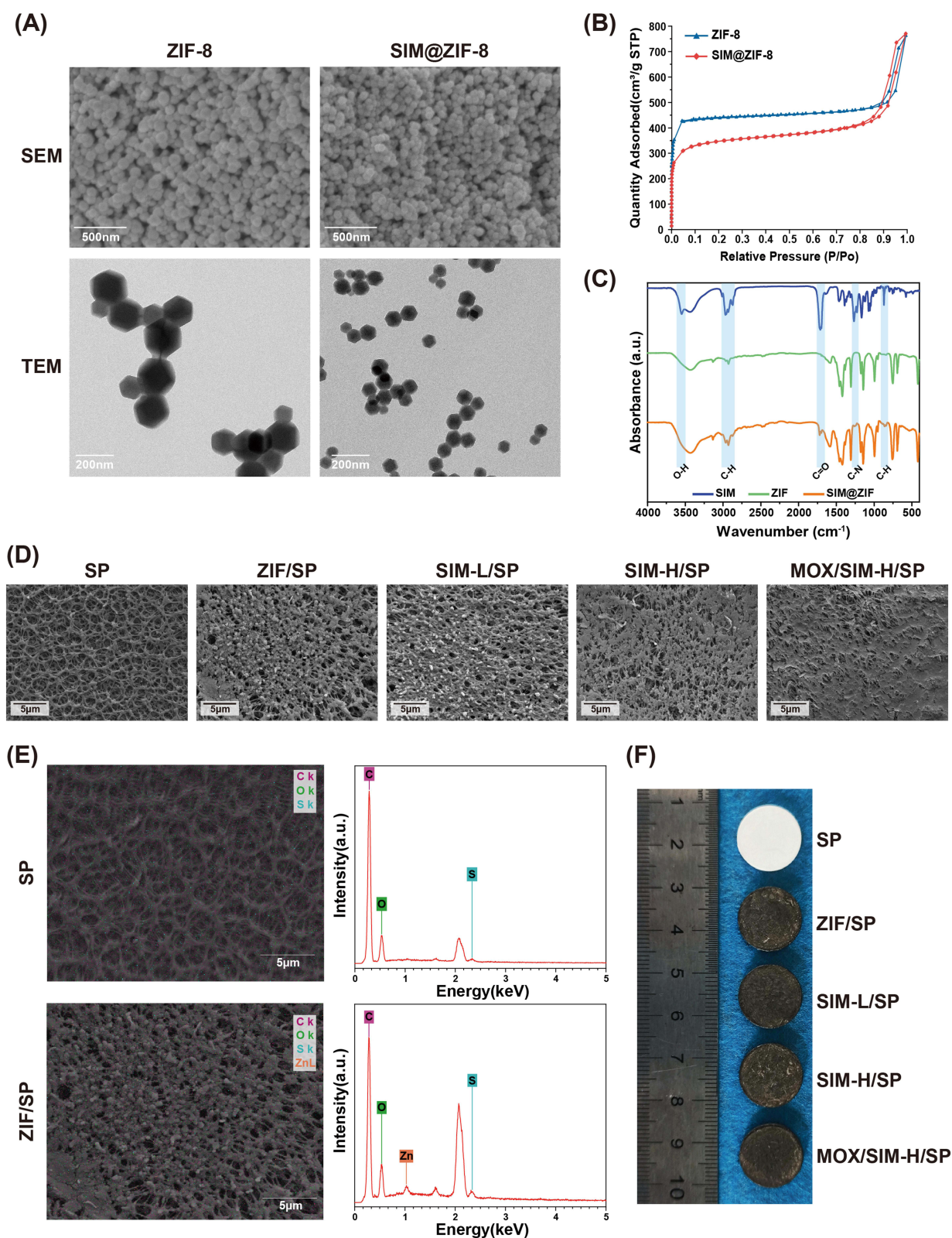


Figure 2 Characterization of SIM@ZIF-8 nanoparticles and MOX/SIM@ZIF-8/PDA/SPEEK coating. **(A)** scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images of ZIF-8 and SIM@ZIF-8 nanoparticles; **(B)** Adsorption/desorption isotherms of ZIF-8 and SIM@ZIF-8 nanoparticles; **(C)** Fourier-transform infrared spectroscopy (FTIR) spectra of SIM, ZIF-8, and SIM@ZIF-8; **(D)** SEM images of each sample group (SP, ZIF/SP, SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP); **(E)** Energy dispersive spectroscopy (EDS) patterns and elemental distribution of SP and ZIF/SP groups; **(F)** Macroscopic morphology photographs of each sample group (SP, ZIF/SP, SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP).

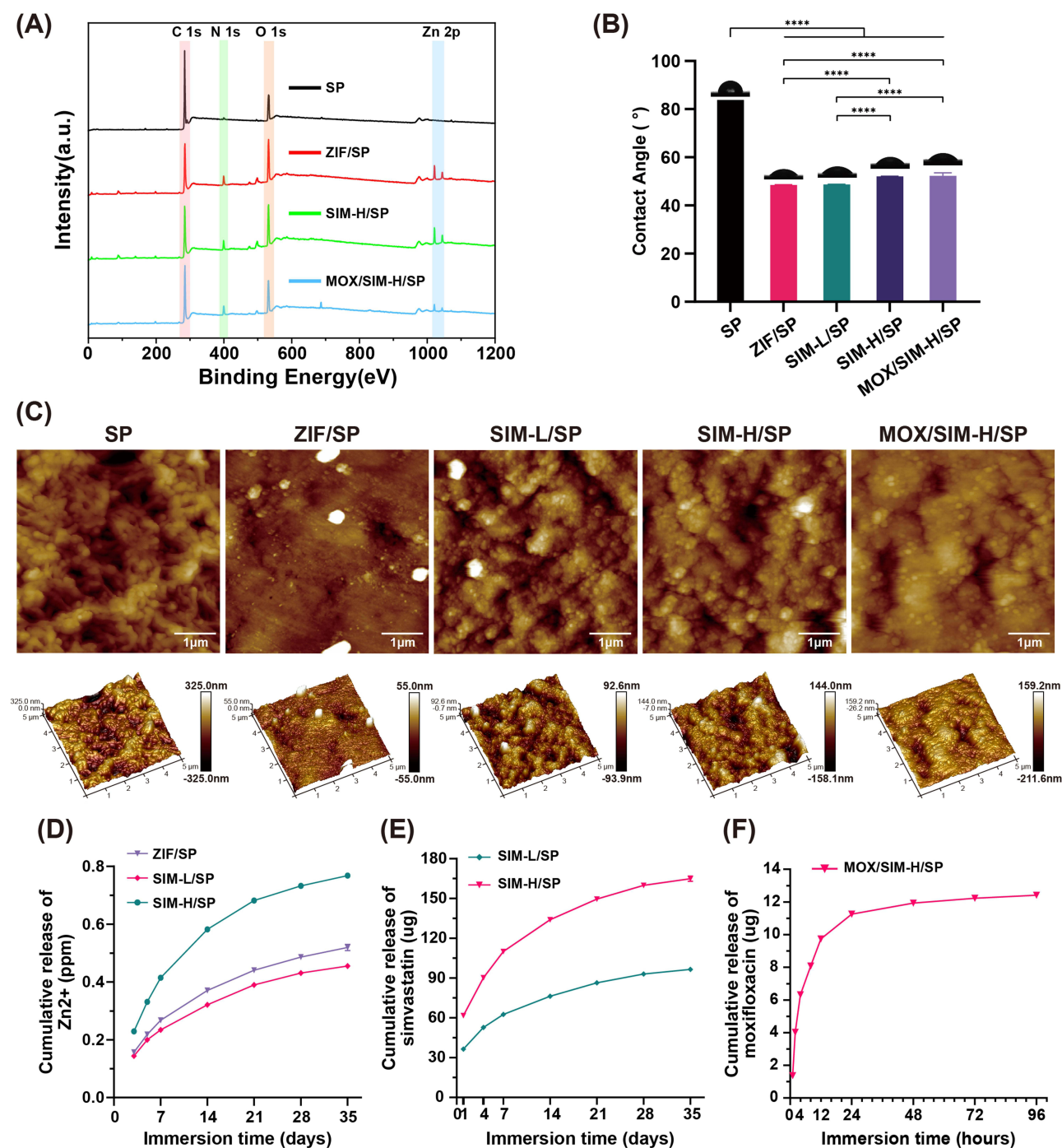


Figure 3 Characterization of MOX/Sim@ZIF-8/PDA/SPEEK Coatings and In Vitro Release Testing. **(A)** XPS spectra of SP, ZIF/SP, SIM-H/SP, and MOX/SIM-H/SP; **(B)** Water contact angles of all sample groups; **(C)** Atomic force microscopy (AFM) images and 3D topography maps of all sample groups; **(D)** Zn²⁺ release curves for ZIF/SP, SIM-L/SP, and SIM-H/SP groups; **(E)** SIM release curves for SIM-L/SP and SIM-H/SP groups; **(F)** MOX release curve for MOX/SIM-H/SP group. ****P < 0.0001.

and Zn 2p_{1/2}, respectively, confirming the presence of ZIF-8 in the PDA layer. The high-resolution N 1s spectrum (Figure S1B) shows a peak at 398.4 eV assigned to C-N bonds. Notably, for the MOX/SIM-H/SP group, the peak around 400.7 eV, associated with four-coordinate nitrogen, exhibits a significant enhancement, while the intensity of the C-N peak decreases; furthermore, a new peak emerges at 404.9 eV, indicating the successful loading of MOX onto the PDA surface. The high-resolution O 1s spectrum (Figure S1C) reveals that, compared to the SP group, the three PDA-coated groups show a remarkable increase in the intensity of the O-C peak at 532.8 eV. Additionally, a peak

appearing at 530.2 eV, typically characteristic of metal oxides, confirms the existence of ZIF-8 within the PDA coating. Finally, the high-resolution C 1s spectrum (Figure S1D) demonstrates that peaks at 284.8 and 288.3 eV are attributed to C-C and C=O bonds, respectively, while the peak at 286.3 eV corresponds to C-N and C-O bonds. The significant elevation of this 286.3 eV peak in the MOX/SIM-H/SP group further reflects the influence of MOX loading on the PDA surface.

The physical properties of a coating surface significantly influence its biological performance. AFM analysis of the surface roughness and 3D topography maps (Figure 3C) revealed that PDA coating substantially reduced the surface roughness of SPEEK. Among the four PDA-coated groups, the SIM-H/SP and MOX/SIM-H/SP groups which were loaded with more SIM@ZIF-8 nanoparticles exhibited greater roughness, as quantitatively determined from the Ra and Rq values (Figure S2A and B).

Surface hydrophilicity is another crucial property influencing material biocompatibility. Water contact angle measurements were conducted on all sample groups to evaluate hydrophilicity of the coating (Figure 3B). The uncoated SP sample exhibited a high average contact angle of 83.78°, which decreased significantly following PDA coating. The ZIF/SP and SIM-L/SP groups, which contained relatively fewer ZIF-8 nanoparticles, exhibited average contact angles of approximately 48°. The SIM-H/SP and MOX/SIM-H/SP groups with higher nanoparticle content exhibited increased average contact angles of approximately 52°. These results clearly demonstrated that the composite coatings effectively enhanced the hydrophilicity of the material surface.

In vitro Release of SIM, MOX, and Zn²⁺ from the Coating

Standard curves for SIM and MOX were first established (Figure S3A and B) to calculate drug loading and release profiles. The standard curve for Zn²⁺ was automatically generated by the ICP-OES system. The release behaviors of Zn²⁺, SIM, and MOX from the samples were evaluated through in vitro immersion degradation. The ZIF/SP, SIM-L/SP, and SIM-H/SP sample groups rapidly released more than one-quarter of the total Zn²⁺ within the first three days, followed by a gradual decrease in the release rate (Figure 3D). They exhibited a slow and stable release for over five weeks, achieving a sustained release of Zn²⁺. Similarly, the SIM-L/SP and SIM-H/SP groups rapidly released approximately one-third of the total SIM on the first day, with the remaining SIM slowly released over the subsequent 4–5 weeks (Figure 3E). The release kinetics of SIM and Zn²⁺ exhibited remarkable synchronicity, with rapid release in the early stage and long-term sustained release in the later stage, which considers the dual needs for rapid initial drug accumulation to provide antibacterial effects and long-term stability during follow-up to promote osteogenic function.

Although SIM and Zn²⁺ were designed for sustained action, the release profiles of MOX were different. In stark contrast to the sustained release of SIM and Zn²⁺, the MOX/SIM-H/SP sample rapidly burst-release 90% of the MOX within 24 h (Figure 3F). Using the release curve of MOX, the DLC on the PDA surface can also be calculated to be 86.17%. This rapid release design compensates for the poor antibacterial efficacy of SIM against Gram-negative bacteria during the early implantation phase, thereby providing broad-spectrum and potent antibacterial properties.

Evaluation of SIM Antibacterial Effect

S. aureus and *E. coli* are common pathogens encountered during surgical procedures. Prior to coating preparation, we determined the MIC of SIM against these two bacteria. Consistent with previous reports, SIM exhibited an MIC of 60 µg/mL against *S. aureus* (Figure S4A and B), while showing no effective antibacterial activity against *E. coli* (Figure S4C and D).^{10,20} Based on the MIC of SIM against *S. aureus*, the amount of SIM@ZIF-8 in the coating was adjusted to ensure rapid drug release at effective antibacterial concentrations during the early phase. This result was confirmed by the sustained release profile of SIM.

Evaluation of in vitro Antibacterial Activity of Coating

We further evaluated the in vitro antibacterial efficacy of the coatings against *S. aureus* and *E. coli* using the uncoated SP group as a control. The spread plate assay (Figure 4A and C) and corresponding inhibition rates for *S. aureus* and *E. coli* (Figure 4B and D) revealed that the ZIF/SP group exhibited no effective antibacterial activity against *S. aureus*. In contrast, the SIM-L/SP group achieved a 64% inhibition rate, while that of the SIM-H/SP group increased to 95%, and

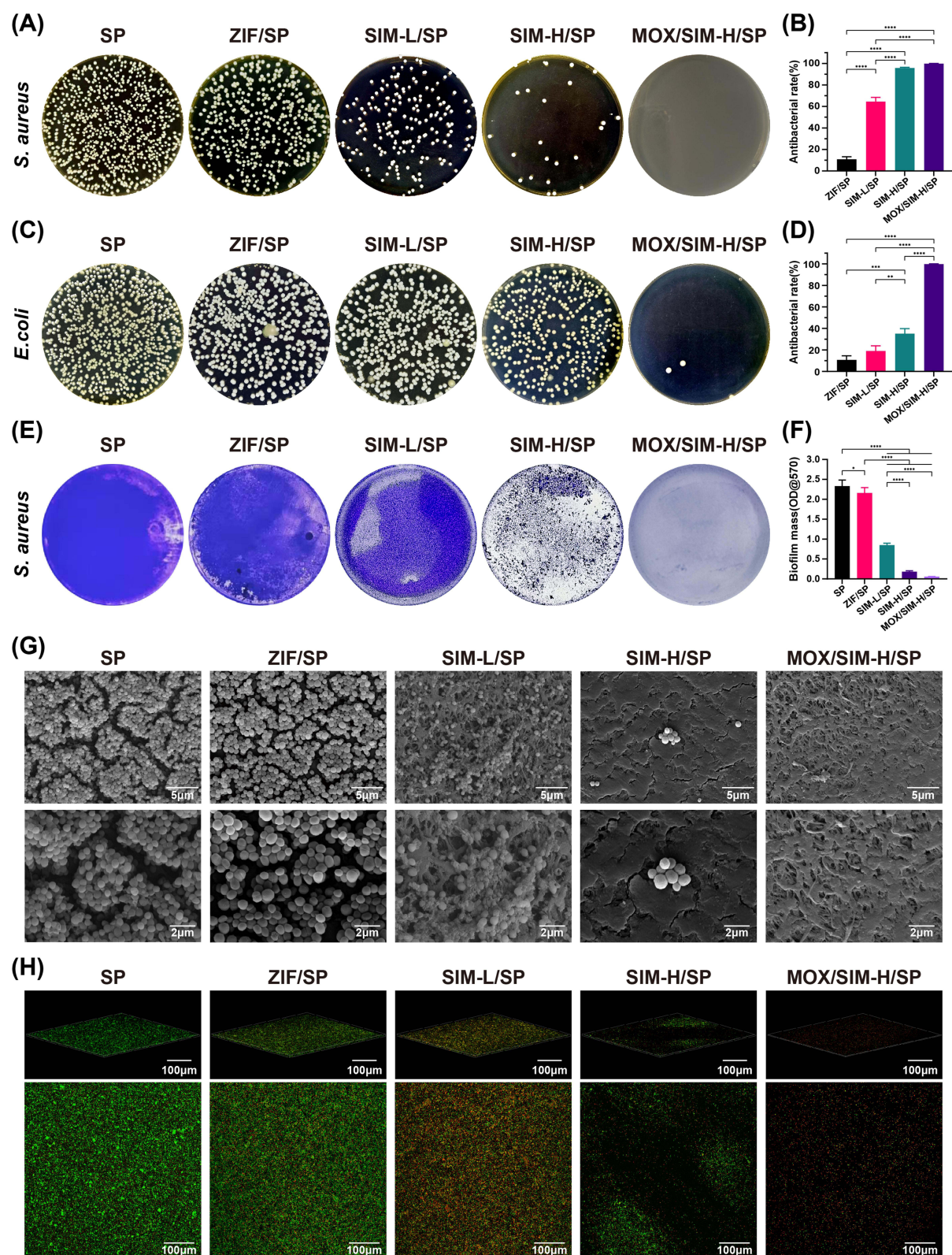


Figure 4 In vitro antibacterial activity of materials. **(A)** Macroscopic photographs of spread plates for *S. aureus* grown on various samples; **(B)** Inhibition rates of various groups against *S. aureus* determined by spread plate assays, with the SP group serving as the control; **(C)** Macroscopic photographs of spread plates for *E. coli* grown on various samples; **(D)** Inhibition rates of various groups against *E. coli* determined by spread plate assays, with the SP group serving as the control; **(E)** Crystal violet staining images of *S. aureus* biofilms cultured in sample eluates; **(F)** Biofilm quantification via crystal violet staining, with data expressed as absorbance at 570 nm after ethanol solubilization. **(G)** SEM images of bacteria and biofilms on the surfaces of each sample group. **(H)** Live/dead staining images of biofilms from each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

the MOX/SIM-H/SP group efficiently killed over 99% of *S. aureus*. The inhibition rate of the ZIF/SP group was < 20%, indicating that the released Zn^{2+} from the coating failed to rapidly reach an effective antimicrobial concentration. The release curve of SIM indicates that the amount of SIM released within 24 hours in the SIM-L/SP group failed to reach the MIC, yet still achieved a 64% antibacterial rate against *S. aureus*, which may be attributed to the synergistic antibacterial effect between Zn^{2+} and SIM co-released together. The SIM-H/SP group demonstrated strong antibacterial activity which was not significantly different from that of the MOX/SIM-H/SP group. For *E. coli*, the ZIF/SP, SIM-L/SP, and SIM-H/SP groups exhibited no effective antibacterial effects, and only the MOX/SIM-H/SP group exhibited antibacterial activity against *E. coli* with the rapid release of MOX. Thus, MOX introduction successfully compensated for the lack of antibacterial activity of SIM against gram-negative bacteria. Next, the long-term antibacterial capability of the coatings was evaluated using only *S. aureus*. As shown in [Figure S5A](#), after immersion for 4, 7, and 14 days, the three groups containing SIM still exhibited certain antibacterial activity. Notably, the two high-concentration SIM groups performed significantly better than the low-concentration group. However, due to the rapid release of all MOX, the MOX/SIM-H/SP group no longer exhibited MOX-dependent bactericidal activity after immersion and performed similarly to the SIM-H/SP group. The antibacterial rates derived from statistical analysis of the spread plate assay ([Figure S5B–D](#)) confirmed these observations. Finally, longitudinal comparison of antibacterial activity across different time points ([Figure S5E](#)) revealed that, over time, the antibacterial efficacy of all groups gradually declined. However, within two weeks, the two high-concentration SIM groups retained acceptable antibacterial performance.

To evaluate the ability of the coatings to inhibit *S. aureus* biofilm formation, crystal violet staining was performed on the biofilms formed by each group of *S. aureus* ([Figure 4E](#)). The SP and ZIF/SP groups formed dense biofilms, whereas the SIM-L/SP group formed very loose biofilms with significantly reduced bacterial counts. SIM-H/SP and MOX/SIM-H/SP groups exhibited pronounced inhibitory effects on biofilm formation. Notably, the MOX/SIM-H/SP group showed virtually no bacterial colonization, demonstrating its superior efficacy. Semi-quantitative analysis of crystal violet staining ([Figure 4F](#)) confirmed this finding, although no statistical difference was observed between the SIM-H/SP and MOX/SIM-H/SP groups. To evaluate the long-term biofilm inhibition capability of the coatings, the biofilm culture period was extended to 6 and 9 days. As shown in [Figure S6A](#), the SP, ZIF/SP, and SIM-L/SP groups exhibited no significant changes; the SIM-H/SP group showed gradual biofilm formation with slowly expanding areas, though still notably less than the first three groups; in contrast, the MOX/SIM-H/SP group only displayed minor bacterial colonization without extensive biofilm formation. Further semi-quantitative analysis of crystal violet staining ([Figure S6B and C](#)) revealed that the SIM-H/SP and MOX/SIM-H/SP groups aligned with visual observations. Notably, while the SIM-L/SP group had formed biofilms, quantitative analysis indicated significantly lower biomass compared to the SP and ZIF/SP groups, likely due to its thinner biofilm structure. This suggests that low-concentration SIM still exerts a mild inhibitory effect on biofilm growth.

SEM observations of biofilm formation on the surfaces of each sample group ([Figure 4G](#)) revealed substantial *S. aureus* adhesion on the SP and ZIF/SP surfaces. No significant biofilm formation was observed on the surfaces of the SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP groups, and scattered bacterial adhesion progressively decreased across the groups. Finally, CLSM was used to observe *S. aureus* in each sample group after live/dead staining, where green fluorescence indicated viable bacteria and red fluorescence indicated dead bacteria. The results revealed that the biofilm thickness in the SP and ZIF/SP groups was greater than that in the SIM-L/SP group, which exhibited some antibacterial activity. Furthermore, biofilms in the SP and ZIF/SP groups exhibited almost no bacterial death, whereas the SIM-L/SP group contained abundant dead bacteria. In contrast, the surfaces of the SIM-H/SP and MOX/SIM-H/SP groups did not form complete biofilms. Dead bacteria could not be immobilized on the sample surfaces along with the biofilms and only a small number of live bacteria colonized ([Figure 4H](#)).

These consistent observations from crystal violet staining, SEM, and CLSM indicate that *S. aureus* biofilm formation was significantly inhibited as the SIM content of the coating increased. The biofilm structure of the SIM-L/SP group loosened, whereas the surfaces of the SIM-H/SP and MOX/SIM-H/SP groups were nearly incapable of forming complete biofilm structures. The combined results demonstrated that the MOX/SIM@ZIF-8/PDA/SPEEK coating effectively eradicated *S. aureus* and inhibited biofilm formation. Additionally, MOX exhibited activity against *E. coli*, significantly

reducing viable bacteria around the implant and providing a favorable microenvironment for subsequent osseointegration.

Biocompatibility Evaluation in vitro

Mouse osteoblast precursor cells MC3T3-E1 were seeded onto the sample surfaces. After 24 h of culturing, live/dead staining was performed (Figure 5A). No significant red fluorescence, representing dead cells, was observed in any of the sample groups. The density of green fluorescence indicated that the abundance of live cells was similar among the groups. Quantitative analysis of live/dead staining further confirmed the absence of significant cytotoxicity across all sample groups (Figure 5B). Cell viability was detected using the CCK-8 assay (Figure 5C), and the cells in each group proliferated well. There were no significant differences in the number of cells in each group until the seventh day. Thus, the coating had good cytocompatibility and cells grew efficiently on the implant surface, providing favorable conditions for subsequent osteogenic differentiation.

Additionally, SEM images of cells cultured for 24 h revealed that cells adhering to the SP group exhibited a spindle-shaped morphology. Compared to the SP group, cells in the ZIF/SP group remained predominantly spindle-shaped overall but displayed improved spreading. In contrast, cells on the surfaces of the SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP groups were spread out in an irregular shape (Figure 5D). CLSM images of the cytoskeleton were aligned with the SEM images (Figure 5E). Cells in the SP and ZIF/SP groups exhibited poor overall spreading morphology, although the ZIF/SP group with the PDA coating showed slightly better spreading than that in the SP group. Following the introduction of SIM, cells on the surfaces of all the three groups exhibited more extensive spreading and a more developed cytoskeleton. Furthermore, the SIM-H/SP and MOX/SIM-H/SP groups demonstrated better spreading morphologies than that of the SIM-L/SP group.

Evaluation of Osteogenic Differentiation in vitro

The osteogenic differentiation and mineralization abilities of MC3T3-E1 cells were evaluated using ALP and alizarin red staining. ALP staining was performed on days 7 and 14 following osteogenic induction in MC3T3-E1 cells. On day 7, the ALP staining intensity gradually increased in the SP, ZIF/SP, SIM-L/SP, and SIM-H/SP groups, with no significant difference between the SIM-H/SP and MOX/SIM-H/SP groups (Figure 6A). The trend for quantitative detection of ALP activity was similar (Figure 6C), with gradual increases in ALP activity in the SP, ZIF/SP, SIM-L/SP, and SIM-H/SP groups; however, there was no significant difference between the SP and ZIF/SP groups ($P > 0.05$). On day 14, the ALP staining intensity in the ZIF/SP and SIM-L/SP groups was not significantly higher than that in the uncoated SP group; similarly, the staining intensity in the SIM-H/SP and MOX/SIM-H/SP groups was not significantly higher than that in the SIM-L/SP group (Figure 6A). Quantitative detection of the ALP activity suggested similar overall trends for the gradual enhancement of activity; however, unlike the macroscopic observation, the ALP activity of the SIM-L/SP group was stronger than that of the ZIF/SP group (Figure 6C).

After 21 d of osteogenic induction in MC3T3-E1 cells, alizarin red staining and semi-quantitative analysis were performed. The overall trend was similar to that of ALP detection, and the results revealed that the degree of matrix mineralization of SP, ZIF/SP, SIM-L/SP, and SIM-H/SP gradually increased, with significant differences among the groups. The SIM-H/SP and MOX/SIM-H/SP groups demonstrated the highest degree of mineralization, which was not significantly different from each other (Figure 6B and D).

In summary, the high-dose SIM groups (SIM-H/SP and MOX/SIM-H/SP) were significantly better than the other groups in terms of ALP activity (early osteogenic differentiation marker) and mineralized nodule formation ability (late osteogenic differentiation marker). In addition, the ALP activity and mineralized nodule formation ability of the SIM-L/SP group were similar to those of the ZIF/SP group, compared to those of the high-dose SIM group. This suggests that SIM released by the low-dose SIM group was insufficient to improve the osteogenic capacity of MC3T3-E1 cells. The ZIF/SP group showed better osteogenic differentiation ability than that of the SP group on days 14 and 21, suggesting that ZIF-8 successfully released Zn^{2+} to promote osteogenesis.

To verify the ability of the material to promote osteogenic differentiation at the molecular level, BMP-2 expression in MC3T3-E1 cells after 7 d of osteogenic induction was analyzed via immunofluorescence. As shown in Figure 7A, Green

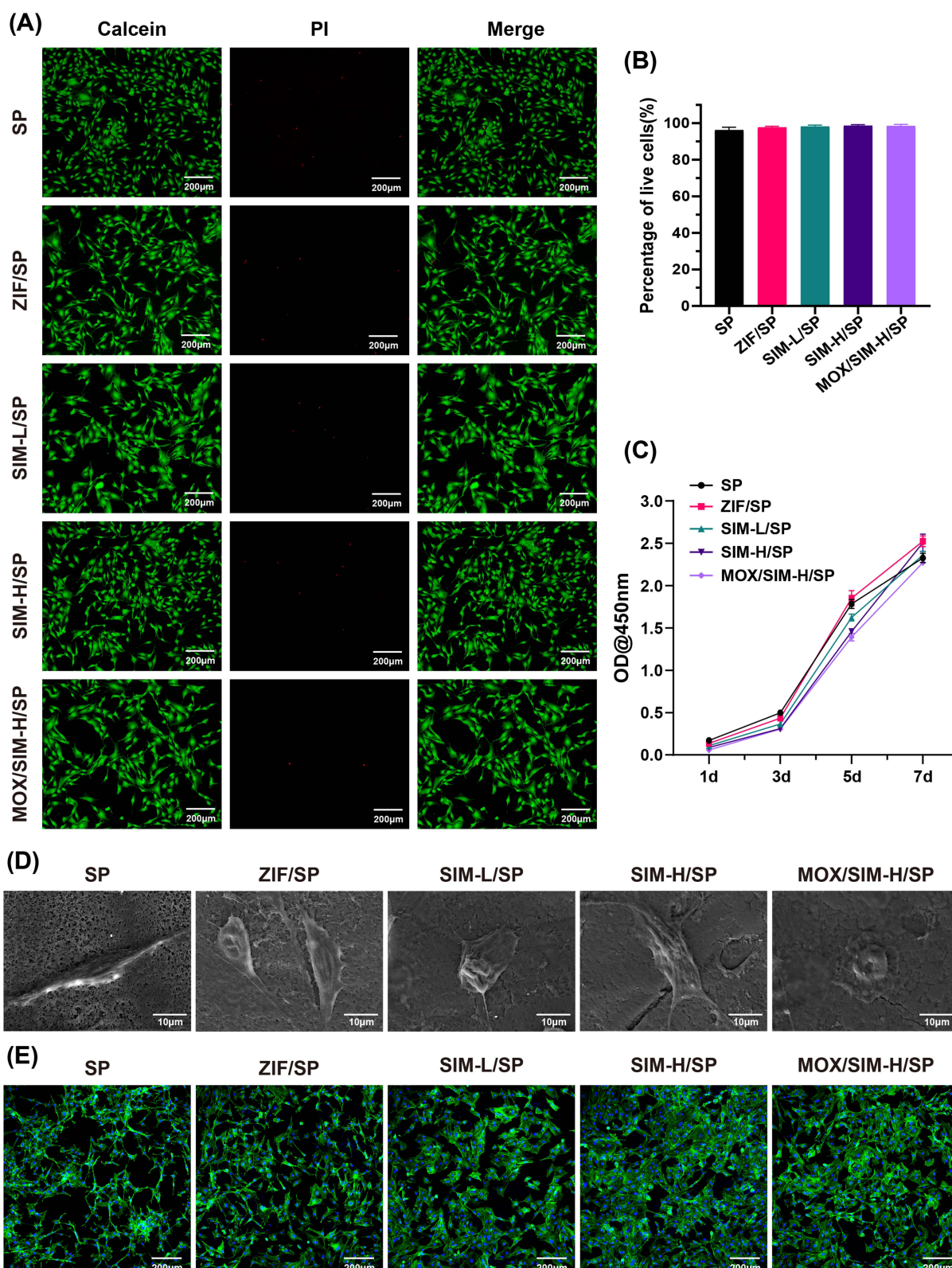


Figure 5 Biocompatibility of the materials. **(A)** Live/dead staining images of MC3T3-E1 cells co-cultured on the surface of each group of samples; **(B)** Quantitative analysis of live/dead staining of samples from each group; **(C)** Proliferation curves of MC3T3-E1 cells in each group, the data show the OD value at 450 nm obtained using the CCK-8 method; **(D)** SEM images of MC3T3-E1 cells on the surface of each group of samples; **(E)** Cytoskeletal staining CLSM images of MC3T3-E1 cells in each group.

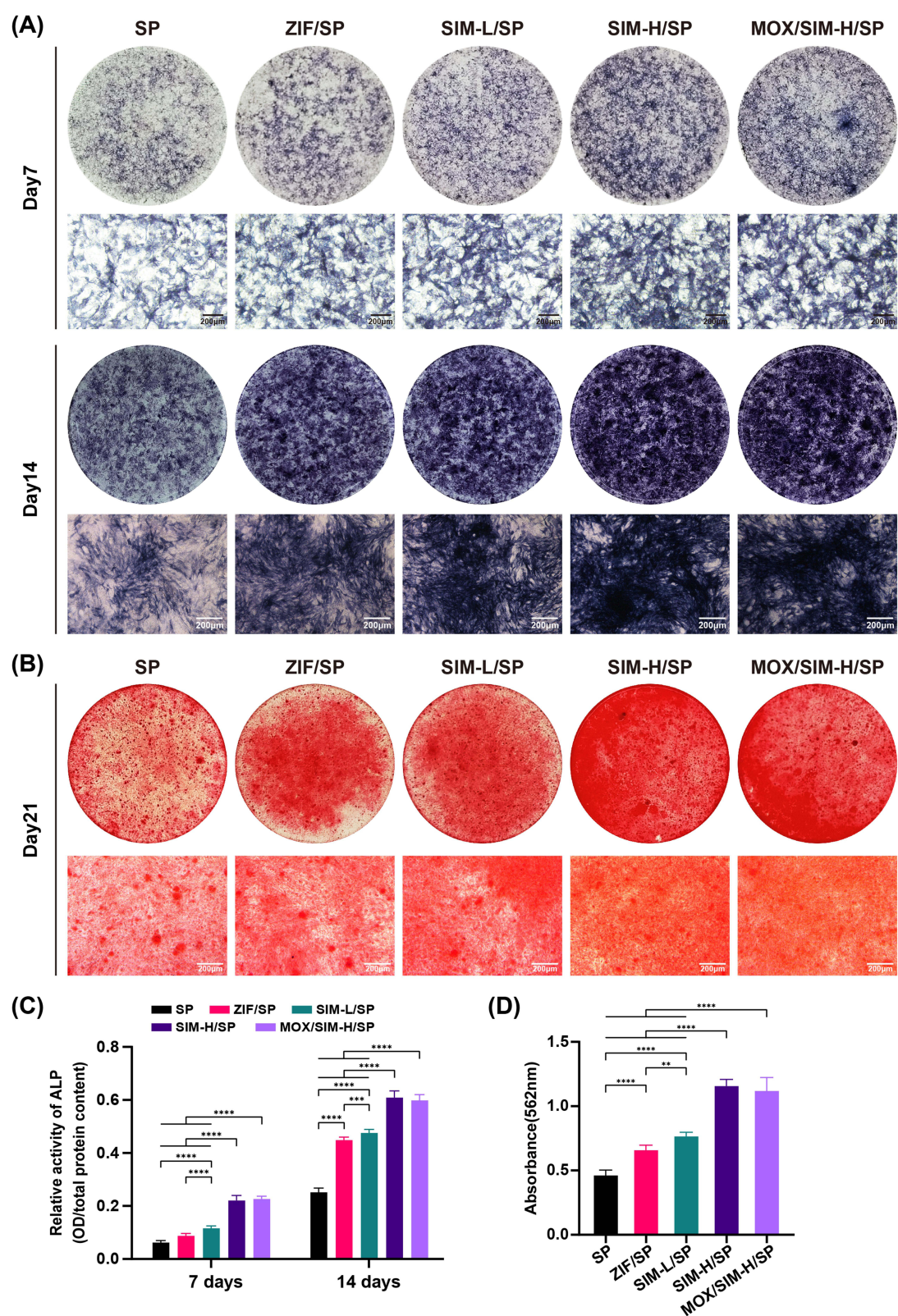


Figure 6 Evaluation of the osteogenic differentiation of MC3T3-E1 cells cultured in sample extracts. **(A)** Gross images and light microscopy images of alkaline phosphatase (ALP) staining on the seventh and 14th days of MC3T3-E1 osteogenesis induction in each group; **(B)** Gross images and light microscope images of alizarin red staining on day 21 of osteogenesis induction of MC3T3-E1 cells in each group; **(C)** Quantitative detection of ALP activity on the seventh and 14th days of osteogenic induction of MC3T3-E1 cells in each group; **(D)** Semi-quantitative analysis of alizarin red staining in each group, the data showed the OD value at 562 nm after dissolving the alizarin red staining solution. **P<0.01, ***P<0.001, ****P<0.0001.

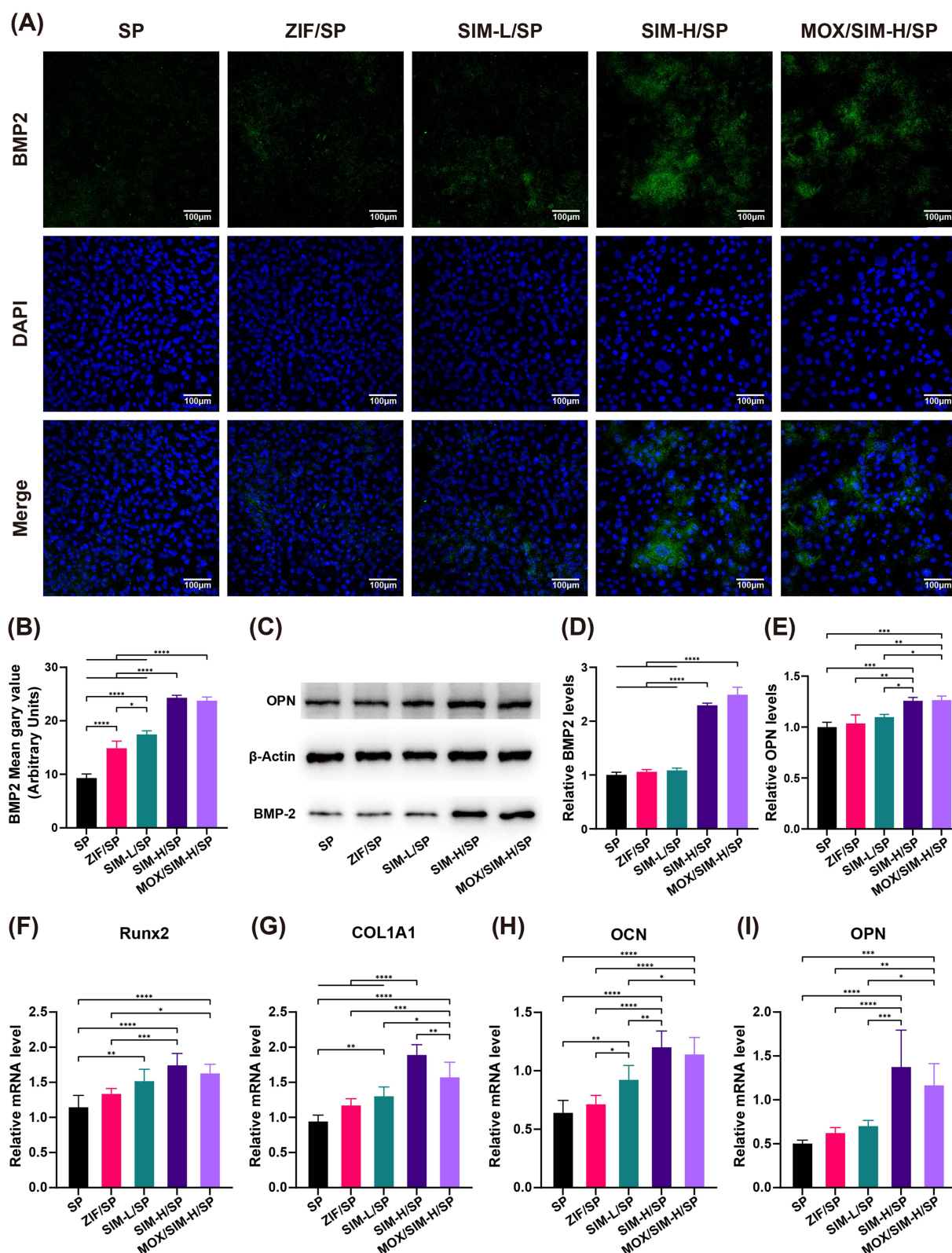


Figure 7 Expression of osteogenic proteins and genes in MC3T3-E1 cells. **(A)** Immunofluorescence staining images of BMP-2 in MC3T3-E1 cells on the surface of each group; **(B)** Semi-quantitative analysis of BMP-2 fluorescence intensity; **(C–E)** Expression of osteogenic proteins in MC3T3-E1 cells in each group; **(F–I)** Expression of osteogenic genes in MC3T3-E1 cells in each group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

fluorescence representing BMP-2 in the SP, ZIF/SP, and SIM-L/SP groups gradually increased, the fluorescence intensity of the high-dose SIM group (SIM-H/SP and MOX/SIM-H/SP) greatly increased, and there was no significant difference between the MOX/SIM-H/SP and SIM-H/SP groups (Figure 7B). This was also confirmed via semi-quantitative analysis of the CLSM images (Figure 7B), where the expression of BMP-2 gradually increased with an increase in SIM content; the SIM-H/SP and MOX/SIM-H/SP groups exhibited the strongest fluorescence intensity with no significant difference between them.

Western blotting revealed osteogenic proteins (OPN and BMP-2) 14 d after the osteogenic induction of MC3T3-E1 cells in each group (Figure 7C–E). The expression levels of osteogenic proteins in the SIM-H/SP and MOX/SIM-H/SP groups were significantly upregulated relative to those in the SP, ZIF/SP, and SIM-L/SP groups. Unlike other osteogenic evaluations, there was no significant enhancement trend among the SP, ZIF/SP, and SIM-L/SP groups.

After 7 d of osteogenic induction of MC3T3-E1 cells in each group, real-time quantitative PCR analysis significantly increased expression levels of the osteogenic genes (*Runx2*, *COL1A1*, *OPN*, and *OCN*) in both the SIM-H/SP and MOX/SIM-H/SP groups (Figure 7F–I). The expressions of some genes (*Runx2*, *COL1A1*, and *OCN*) in the SIM-L/SP group with a small amount of SIM was higher than those in the SP group, whereas the expression of only *OCN* was significant compared to that of the ZIF/SP group ($P < 0.05$). Compared with the uncoated SP group, the ZIF/SP group demonstrated a non-significant up-regulated trend ($P > 0.05$).

In summary, the MOX/SIM@ZIF-8/PDA/SPEEK material can effectively promote the osteogenic differentiation and mineralization of MC3T3-E1 cells. The SIM-H/SP and MOX/SIM-H/SP groups, which contained higher SIM concentrations, exhibited the strongest osteogenic promotion effects, with no statistically significant difference between them.

Evaluation of Antibacterial Capacity in vivo

Each group of samples and the surrounding tissue were removed together, the tissue was then carefully separated, and the sample was rolled directly on an agar plate to collect non-adherent colonies. After 24 h of agar plate culture, *S. aureus* was abundantly present on the surface of SP and ZIF/SP group samples. The number of viable bacteria on the surface of the SIM-L/SP and SIM-H/SP groups after SIM introduction decreased, and very few bacteria survived on the surface of the MOX/SIM-H/SP group following MOX introduction (Figure 8A). Adherent *S. aureus* cells were collected by ultrasonic shaking. After 24 h of incubation on agar plates, abundant *S. aureus* adhered to the surface of the SP and ZIF/SP groups, and the number of surface-adhered bacteria in the SIM-L/SP group was significantly reduced. In contrast to the spread-plate results for non-adherent bacteria, the surface-adherent bacteria in the SIM-H/SP group were greatly reduced, and the surface of the MOX/SIM-H/SP group was almost non-adherent (Figure 8B). Figure 8C shows the counting statistics of the *S. aureus* spread plates adhered to the sample surface. Although there was a slight decreasing trend among the SP, ZIF/SP, and SIM-L/SP groups, the gap did not reach an order of magnitude, indicating that it did not effectively inhibit bacterial colonization and biofilm formation. However, SIM-H/SP and MOX/SIM-H/SP exhibited clear inhibitory effects.

Slice staining of the subcutaneous tissue near the implant was performed to evaluate the antibacterial activity of the samples. The overall structure of the subcutaneous tissue and the infiltration of inflammatory cells were observed using H&E staining (Figure 8D); a large number of residual traces of neutrophil infiltration were observed in the SP and ZIF/SP groups under a high-power field of view. Neutrophil infiltration was reduced in the SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP groups, among which the MOX/SIM-H/SP group exhibited the weakest inflammatory response. Neutrophil infiltration is a marker of acute inflammatory response, and the degree of infiltration directly reflects the severity of infection.^{21,22} Furthermore, we determined whether the antibacterial effect successfully guided the tissue from a pathological inflammatory state to a tissue microenvironment more suitable for damage repair. The average thickness of the fiber layer wrapping the implant in H&E staining was counted; the results revealed that the fiber layer was significantly thickened in the SP and ZIF/SP groups; it gradually became thinner in the SIM-L/SP, SIM-H/SP, and MOX/SIM-H/SP groups; and was the thinnest in the MOX/SIM-H/SP group, which was similar to the trend of inflammatory cell infiltration (Figure 8E). The large number of neutrophil infiltrations and thick fibrous envelopes in the SP and ZIF/SP groups indicate the body's dual rejection and immune response to foreign bodies and infections. This also simulates the common "fiber wrapping" dilemma of biologically inert PEEK implants in clinical practice: the dense fibrous connective

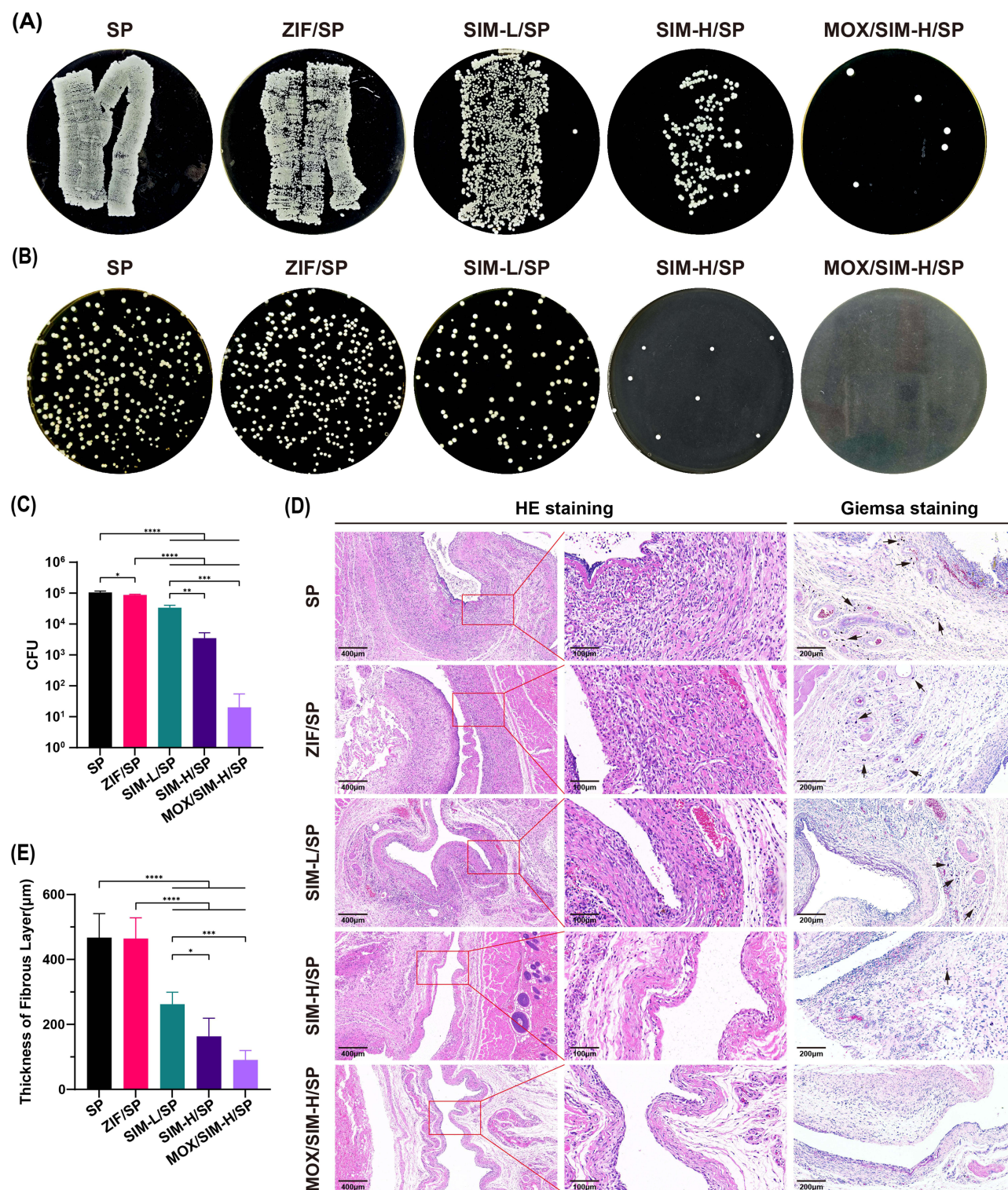


Figure 8 In vivo antibacterial capacity of the material. **(A)** the spread plates of non-adherent bacteria on the surface of each group of samples in the subcutaneous tissue of rats (n=3); **(B)** the spread plates of bacteria adhering to the sample surfaces of each group in rat subcutaneous tissue (n=3); **(C)** Count statistics for the spread plates of adherent bacteria; **(D)** Hematoxylin and eosin (H&E) and Giemsa staining of the subcutaneous tissue around each group of samples, with black arrows indicating residual bacteria in the tissue (n=3); **(E)** Statistics on the average thickness of the fibrous layer of the subcutaneous tissue around the samples in each group. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

tissue not only hinders osseointegration, but also provides a shelter for bacteria from immune clearance.^{23,24} In contrast, the MOX/SIM-H/SP group exhibited the thinnest fiber layer and the lightest inflammatory cell infiltration, providing a good microenvironment for osseointegration. Giemsa staining results were consistent with those of H&E staining, with more bacterial colonies visible in the SP and ZIF/SP groups. The bacterial colonies in the SIM-L/SP and SIM-H/SP groups gradually decreased, while the MOX/SIM-H/SP group showed almost no bacterial invasion following MOX introduction (Figure 8D).

The findings of the comprehensive *in vitro* and *in vivo* evaluations showed that the MOX/SIM@ZIF-8/PDA/SPEEK coating achieved early rapid sterilization and long-term efficient antibacterial activity through the synergistic release of MOX and SIM, while significantly lowering the bacterial load. This mitigated excessive inflammatory responses triggered by infection and prevented the formation of dense fibrous capsules on the implant surface.

Evaluation of Osteogenic Ability *in vivo*

The micro-CT results of the rabbit femurs are shown in Figure 9A and B. The coronal and sagittal CT images were used to determine whether the material implantation position was suitable, and 500 μm -thick bone tissue around the implant was selected for three-dimensional image reconstruction. The progression from purple to green indicated increased mineral content in the bone tissue and better mineralization, suggesting more mature bone tissue. Vertically, at 4 weeks post-surgery, the material surface had a significant amount of newly formed bone and defects with unformed bone tissue, whereas at 8 weeks post-surgery, the bone defect area decreased and the bone tissue had matured further. Laterally, at the same time points, the SP and ZIF/SP groups exhibited more defects without bone tissue, and the bone tissue was predominantly less-mineralized newly formed bone. Compared with the SP and ZIF/SP groups, the SIM-L/SP group showed increased amounts of newly formed bone and higher mineralization. The SIM-H/SP and MOX/SIM-H/SP groups showed the highest amounts of newly formed bone and the best mineralization.

Statistical analyses were performed using multiple bone analysis parameters obtained using micro-CT. At week 4 postoperatively (Figures 9C–F, S7A and B), the SIM-H/SP and MOX/SIM-H/SP groups showed significantly better performances in multiple indicators than those of the other three groups. The significant increase in BV/TV and BMD indicated higher amounts of newly formed bone and better mineralization in these two groups, while increased Tb.N and decreased Tb.Sp suggested a denser and more connected trabecular bone structure, which is crucial for restoring bone mechanical properties. The SIM-L/SP group also performed well in terms of BV/TV, BMD, and Tb.N, demonstrating that SIM exhibited osteoinductive ability even at lower concentrations. In contrast, compared to the SP group, the ZIF/SP without SIM group showed significant differences only in the Tb.Sp indicator.

At the eighth week after surgery (Figures 9G–J, S7C and D), the SIM-H/SP and MOX/SIM-H/SP groups showed the best results, with more newly formed bone, thicker and denser trabeculae, and better BV/TV, Tb. Sp, and Tb. Th than those of the other three groups. However, they failed to widen the gap with the SIM-L/SP group in the Tb.N and BS/BV indicators representing the number and structural maturity of the trabecular bone and were only better than the SP and ZIF/SP groups without SIM. The SIM-L/SP group outperformed the SP and ZIF/SP groups without SIM in terms of multiple indicators (BV/TV, BS/BV, Tb.Sp, and Tb.N). Finally, unlike in week 4, the ZIF/SP group outperformed the uncoated SP group in several indicators (BV/TV, Tb.Sp, and Tb. N), owing to the sustained release of Zn^{2+} by ZIF-8. A possible reason for the different results is the relatively short 4-week period, which makes it impossible to widen the significant gap between the SP group and ZIF/SP group, which has a relatively weaker bone-forming effect.

Following micro-CT detection, the rabbit femurs and implants were sectioned into hard tissues. First, newly formed bone labeled with the dual fluorescence of alizarin red and calcein green was observed using CLSM (Figure 10A), and the average distance between the fluorescent labels in the two groups was statistically analyzed, along with the calculation of MAR. At week 4, the SIM-H/SP and MOX/SIM-H/SP groups with high SIM concentrations exhibited significantly higher MAR than those of the other three groups (Figure 10C). Similarly, the SIM-L/SP group with SIM showed a better MAR than that of the SP group, whereas there was no significant difference between the ZIF/SP and SP groups. This demonstrates that coatings with SIM, particularly in the high-concentration group, greatly accelerate the rate of bone mineralization and deposition during the active phase of bone healing, providing strong evidence that coatings promote osteoblast activity and bone matrix maturation. However, when the time was extended to the eighth week, the

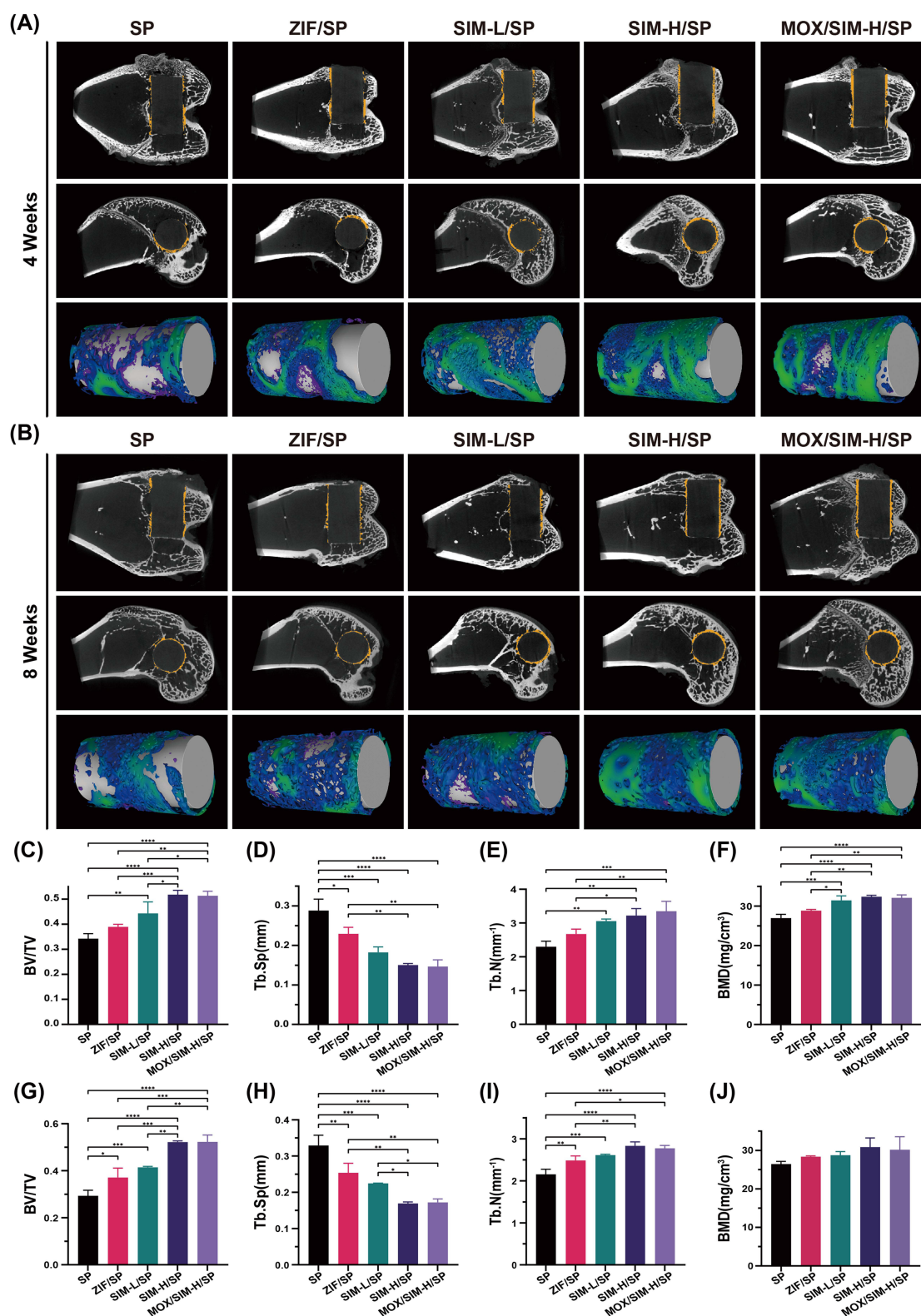


Figure 9 Micro-computed tomography (CT) analysis of rabbit femur and implants. **(A)** Micro-CT images of rabbit femur at week 4 (coronal, sagittal, and 3D reconstruction images from top to bottom, $n=4$); **(B)** Micro-CT images of the rabbit femur at week 8 ($n=4$); **(C–F)** Quantitative micro-CT parameters (BV/TV, Tb.Sp, Tb.N, BMD) of the rabbit femur at week 4 post-operation; **(G–J)** Quantitative micro-CT parameters (BV/TV, Tb.Sp, Tb.N, BMD) of the rabbit femur Micro-CT at week 8 post-operation. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$.

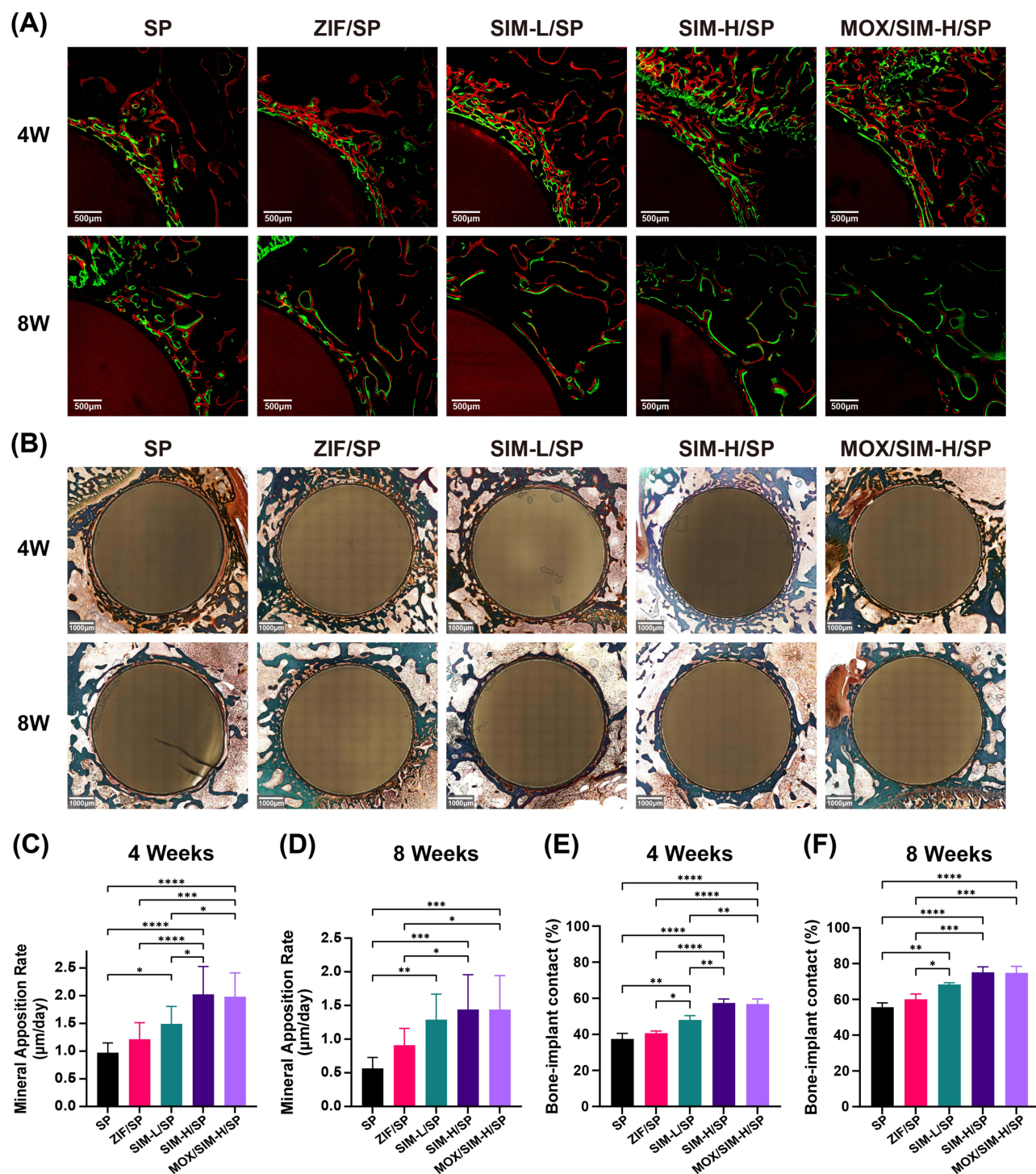


Figure 10 Double fluorescence labeling and Goldner staining of newly formed bone in rabbit femurs. **(A)** Double fluorescence labeling of newly formed bone in rabbit femurs of each group (n=4). **(B)** Goldner staining of newly formed bone in rabbit femurs of each group (n=4). **(C and D)** Bone formation deposition rate obtained from the statistical analysis of double fluorescence labeling; **(E) (F)** Bone-implant contact ratio obtained from statistical analysis of Goldner staining. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

differences among the SIM-H/SP, MOX/SIM-H/SP, and SIM-L/SP groups decreased not significantly (Figure 10D). This suggests that in the later stages of defect repair, the other groups also entered the mineralization stage. However, the high mineralization rate in the early stage ensured that the SIM-H/SP and MOX/SIM-H/SP groups completed osseointegration earlier.

Finally, the hard tissue sections were stained with Goldner's stain (Figure 10B). The light-red tissue in the newly formed bone around the material was an osteoid with a low degree of mineralization, and the blue-green tissue was a bone with a high degree of mineralization. BIC statistical analysis of the 200-micrometer region of interest around the implant (Figure 10E and F), combined with microscopic observation, revealed: In longitudinal comparison, the number of newly formed bone in each group increased over time. Horizontal comparison at the same time point revealed that there were more highly mature blue-green neonatal bones in the SIM-H/SP and MOX/SIM-H/SP groups, and some light red newly formed bones which were undergoing mineralization. Bone maturation in the SP group, with no promotion of osteogenic function, was slower, mainly at the light red neobone stage. The newly formed bone in the ZIF/SP and SIM-L/SP groups was between that in the SP and SIM-H/SP groups. A comprehensive analysis across all cohorts revealed that the MOX/SIM-H/SP coating not only stimulated robust neo-bone formation but also significantly accelerated the mineralization process, leading to enhanced structural maturity. This is consistent with the MAR results obtained via double fluorescence labeling, which more intuitively proves that MOX/SIM@ZIF-8/PDA/SPEEK has good osteogenic ability.

In summary, *in vivo* studies strongly confirmed that MOX/SIM@ZIF-8/PDA/SPEEK significantly promoted high-quality osseointegration in rabbit femoral condyle defect models.

Discussion

This study aims to develop a dual-functional surface based on PEEK that can both actively reduce the risk of infection and establish a local osteogenic microenvironment. To impart antibacterial properties to PEEK, common strategies involve loading antibiotics or antimicrobial metal ions such as silver or zinc ions.^{20,25,26} Although traditional antibiotics are effective, they are ill-suited for long-term sustained release due to the risk of inducing antibiotic-resistant bacteria. Metal ions, while safer, require costly and complex preparation processes involving specialized equipment such as magnetron sputtering, low-temperature plasma systems, or laser processing.^{25,27,28} These limitations hinder the large-scale clinical translation of these commonly used antibacterial strategies in orthopedic implants. Previous studies have demonstrated that the PDA/SPEEK system features a simple fabrication process, low cost, and high versatility for subsequent modifications. Li et al constructed an ultrasonically responsive coating on SPEEK using PDA,²⁹ Yang et al successfully fabricated graphene oxide coatings on microporous SPEEK via PDA modification.³⁰ Both methods allowed the successful coating of PDA on SPEEK surfaces through a simple "one-pot" co-deposition method. Therefore, this study selected the PDA/SPEEK system and introduced SIM@ZIF-8 and MOX to construct a dual-functional coating with antibacterial and osteogenic effects. SIM possesses dual functions of promoting osteogenesis and combating infection, and its safety has been proven through years of clinical application, making it an ideal drug for long-term sustained release in coatings as an alternative to antibiotics.³¹ ZIF-8 is widely used as a sustained-release carrier. For instance, Li et al leveraged ZIF-8's drug-loading capacity to achieve controlled copper ion release, where the rapidly released copper oxide provides antibacterial effects, and the sustainably released Zn^{2+} promotes osteogenic differentiation.³² Yu et al loaded luteolin onto ZIF-8 nanoparticles and incorporated them into hydrogel scaffolds. The synergistic release of luteolin and Zn^{2+} simultaneously promotes osteogenesis and modulates the immune microenvironment.³³ However, SIM@ZIF-8 still lacks antibacterial activity against Gram-negative bacteria,^{10,11} necessitating the introduction of MOX, which exhibits rapid burst release, to provide short-term broad-spectrum antibacterial activity.

The successful preparation of the coating was attributed to the complex physical embedding and chemical chelation among PDA, ZIF-8 nanoparticles, and the SPEEK substrate. As a biopolymer with strong adhesion and abundant functional groups, PDA provides an ideal secondary loading platform. Its surface catechol and amino groups offer multiple binding sites for anchoring ZIF-8 and MOX.³⁴ Furthermore, the ZIF-8 and PDA nanoparticles on SPEEK substrates are interconnected through π - π - π stacking interactions between benzene rings in SPEEK and PDA and imidazole units in ZIF-8.³⁵ The homogeneous embedding of ZIF-8 within the PDA coating not only enhances structural stability but also effectively inhibits ZIF-8 hydrolysis in tissues, thereby prolonging the release duration of SIM and Zn ions.^{36,37} Conversely, MOX, loaded only on the surface of the PDA coating without a sustained-release barrier, exhibited a rapid burst release, ultimately achieving a temporally coordinated and synergistic release of multiple components.

The release profile results demonstrated that MOX exhibited an early burst release to achieve broad-spectrum and efficient bactericidal effects, aligning with the critical clinical window for postoperative acute infection and ensuring that the majority of bacteria introduced during surgery are rapidly eliminated before adhering and forming biofilms. The non-antibiotic drug (SIM) rapidly accumulated effective concentrations followed by prolonged sustained release to provide long-term biofilm inhibition and bone integration promotion. The bioactive metal ion (Zn^{2+}) was synchronously sustained-released with SIM, further enhancing bone integration efficiency. This temporally coordinated, synergistic working strategy of the coating's multi-drug release not only achieved functional diversification but also promoted component synergy, enabling simultaneous antibacterial and osteogenic effects, reducing the risk of bacterial resistance, prolonging biofilm suppression, and more efficiently accelerating bone integration.

To determine the optimal load concentration of SIM, we conducted a preliminary MIC test for SIM. The MIC results indicated that SIM indeed exhibited significant limitations in its antimicrobial spectrum. Its potential mechanism involves statins interfering with isoprenoid lipid synthesis in bacterial cell membranes, thereby disrupting membrane integrity and forming functional microdomains, ultimately affecting bacterial growth and biofilm formation.^{11,38,39} However, the unique structure of the outer membrane of Gram-negative bacteria acts as a permeation barrier, restricting the entry of many hydrophobic drugs.⁴⁰ This limitation of SIM prompted us to introduce MOX, a broad-spectrum antibiotic. However, unlike existing strategies that aim to prolong the release duration of MOX to achieve prolonged antibacterial effects,⁴¹ this study intends for MOX to be fully released within a short timeframe, enabling rapid bactericidal action. Once the initial bacterial load is rapidly eliminated, SIM becomes sufficient to maintain long-term antibacterial efficacy. The strong tissue penetration capability of MOX enables it to reach effective concentrations in bone tissues and biofilms, and studies have reported its application in treating orthopedic infections.^{42,43} Additionally, various antimicrobial compounds, including quinolones, can enhance their antimicrobial activity by interacting with Zn^{2+} to form complexes.⁴⁴ Therefore, although the independent antibacterial contribution of Zn^{2+} in the coating remains unclear, Zn^{2+} may potentially exert synergistic antibacterial effects with SIM and MOX during antimicrobial processes, thereby enhancing the overall antibacterial activity.⁴⁵

Once IAI occurs, it is difficult to treat effectively. This arises from bacteria adhering to, proliferating on, and ultimately forming biofilm structures on implant surfaces.⁴⁶ As a protective fortress for bacteria, biofilms significantly enhance resistance to antibiotics and the host immune system, leading to persistent infections. Thus, an ideal antimicrobial coating must possess dual capabilities: eliminating planktonic bacteria and inhibiting biofilm formation. In this study, the MOX/SIM@ZIF-8/PDA/SPEEK coating demonstrated excellent biofilm inhibition capability. The superior performance of this coating likely stems from the synergistic action of multiple mechanisms: first, SIM, MOX, and Zn^{2+} work together to efficiently eliminate planktonic bacteria, reducing the bacterial load available for biofilm formation at the source; second, SIM disrupts bacterial membrane lipid metabolism, potentially directly affecting the initial stages of biofilm formation-bacterial adhesion and aggregation; finally, previous studies have shown that SIM exhibits anti-inflammatory properties, suppressing inflammatory responses by downregulating NOD-like receptor protein 3 (NLRP3) inflammasome activation.^{47,48} The synergistic interaction between anti-inflammatory and bactericidal effects may inhibit the formation of dense fibrous layers and create a favorable environment for the host immune system's antimicrobial response.⁴⁹ Experimental results from the subcutaneous infection model in rats corroborate the aforementioned findings. However, future studies should further validate the *in vivo* antibacterial efficacy of this coating using infected bone defect models that more closely mimic clinical pathological conditions.

The PDA coating significantly enhanced the hydrophilicity of the material surface, thereby promoting initial cell adhesion, which is critical for the successful application of implantable materials.^{50,51} In this study, SEM and cytoskeletal staining revealed that, compared to the hydrophobic SP group, cells on the coated surface exhibited superior spreading morphology and a more developed actin cytoskeleton, which are positive morphological indicators of vigorous cell activity and osteogenic differentiation potential.^{52,53} Moreover, samples treated with SIM further enhanced cell spreading on the material surface, demonstrating the potential of SIM to improve cell adhesion. These morphological observations clearly indicate that the MOX/SIM@ZIF-8/PDA/SPEEK coating effectively mitigated the biologically inert surface of SPEEK materials, laying a solid foundation for subsequent osteogenic differentiation.

The osteogenic effects of SIM have been extensively studied, and the results of this study align with previous findings.^{54,55} The primary molecular mechanism involves the activation of the BMP-2/SMAD signaling pathway, which rapidly initiates and promotes the differentiation of mesenchymal stem cells into osteoblasts, ultimately enhancing the maturation and mineralization of the extracellular matrix.^{56,57} However, the optimal SIM concentration determined by MIC could not serve as a reference for osteogenic evaluation; thus, coatings with two different concentrations (SIM-L/SP and SIM-H/SP) were prepared to test whether the osteogenic capacity of SIM reached its maximum. Both in vitro and in vivo results demonstrated that the SIM-H/SP and MOX/SIM-H/SP groups showed the strongest osteogenic capabilities. Moreover, micro-CT analysis revealed that the high-dose SIM groups (SIM-H/SP and MOX/SIM-H/SP) developed more mature and superior trabecular bone structures. Studies have shown that SIM can inhibit excessive osteoclastic activity by upregulating osteoprotegerin, thereby maintaining a balanced bone formation-resorption ratio during remodeling, which favors the development of thicker and more stable trabecular bone structures.^{55,58} SIM also promotes osteogenesis by providing blood supply essential for bone regeneration by enhancing vascular endothelial cell migration and angiogenesis.^{54,59} Additionally, no significant differences were observed between the SIM-H/SP and MOX/SIM-H/SP groups in any osteogenic evaluations, confirming that MOX solely provides basic antibacterial functionality without interfering with or enhancing osteogenic differentiation.

Zn is an essential trace element that plays multiple roles in bone metabolism. It not only directly stimulates osteoblast proliferation and differentiation but also suppresses osteoclast activity, thereby bidirectionally regulating bone formation and resorption processes to promote osseointegration.^{60,61} The osteogenic potential of Zn^{2+} released from ZIF-8 has been validated in previous studies.⁶² However, in this study, the ZIF/SP group released a relatively low concentration of Zn^{2+} , which failed to provide stable and significant osteogenic effects in vitro. Notably, in the rabbit femoral defect model, although Zn^{2+} showed no obvious osteogenic activity in the early stages, the ZIF/SP group outperformed the SP group in several key indicators by week 8, demonstrating its delayed but measurable effects. Additionally, as an essential cofactor for various cellular enzymes, Zn^{2+} provides a stable microenvironmental support for the long-term functional activity of osteoblasts.⁶³ Therefore, the superior osseointegration performance of the MOX/SIM@ZIF-8/PDA/SPEEK coating may arise from the synergistic interaction between SIM and Zn^{2+} , which collectively forms the molecular basis for high-quality osseointegration.

Despite the positive findings of this study, we remain acutely aware of its limitations, which also point the way for future research. First, the mechanical wear resistance and long-term stability of the coating require further investigation, as these properties are critical for clinical translation. Second, the independent and synergistic mechanisms of the coating components must be further elucidated. For example, the synergistic mechanism by which SIM and Zn^{2+} promote osteogenesis at the signaling pathway level remains unclear. Similarly, the synergistic mechanism of Zn^{2+} , SIM, and MOX in antibacterial activity has yet to be elucidated. Third, while MC3T3-E1 cells were used in this study, employing primary bone marrow stromal cells (BMSCs), which better mimic physiological conditions, would provide more compelling evidence. Fourth, the long-term biosafety of the implanted devices within animal models has not been evaluated, making it impossible to verify whether the sustained-release SIM and Zn^{2+} pose any accumulation risks over extended periods. Fifth, the antibacterial evaluation utilized a rat subcutaneous infection model, which represents a superficial tissue infection and fails to fully replicate deep bone infections. Future studies should adopt more representative osteomyelitis models. Additionally, the osteogenic evaluation relied on a healthy animal model, which does not account for clinical comorbidities (eg, osteoporosis and diabetes) that negatively impact bone healing and infection risks.^{64–66} Finally, the coating design solely depended on the burst release of MOX to kill *E. coli* introduced during surgery, yet its long-term antibacterial efficacy against *E. coli* during osseointegration remains limited. Furthermore, studies have shown that SIM inhibits methicillin-resistant *S. aureus* (MRSA),²⁰ but whether the designed coatings in this study retain anti-drug-resistant bacterial activity requires further validation.

Conclusion

In this study, a MOX/SIM@ZIF-8/PDA/SPEEK multifunctional active coating system was successfully constructed on the surface of SPEEK. The system is based on SIM, which exhibits osteogenesis promoting, antibacterial, and anti-inflammatory functions, combined with the broad-spectrum quinolone antibiotic MOX, with the help of a ZIF-8 vector

which can release bioactive Zn^{2+} . This synergistic drug delivery system with time-programmed release characteristics has been constructed to simultaneously address two major clinical problems, IAI and poor osseointegration, enabling the implant to actively reduce infection risks and create a favorable local microenvironment for osseointegration. In the early post-implantation phase, SIM and MOX quickly reach effective antibacterial concentrations, which can promptly eliminate pathogens introduced during surgery and synergistically exert a broad-spectrum antibacterial effect to establish an aseptic environment for tissue repair. During the subsequent bone-healing stage, the sustained release of SIM and Zn^{2+} allows the implant to maintain prolonged antibacterial efficacy without relying on MOX, thereby mitigating the risk of bacterial resistance caused by long-term antibiotic exposure. SIM and Zn^{2+} effectively promoted osteogenic differentiation and osseointegration. A series of material characterizations, along with in vitro and in vivo experiments, confirmed the excellent performance of the composite coating in delivering early broad-spectrum antibacterial activity, long-term inhibition of *S. aureus* biofilms, and enhanced osseointegration. More importantly, this study demonstrates a new strategy for modular and multifunctional implant surface modification based on metal-organic frameworks and PDA coatings. This strategy overcomes the limitations of traditional single-antibiotic or single-growth-factor coatings, such as mono-functionality, short action durations, and a high propensity for inducing drug resistance. This study provides a solid experimental basis and feasible technical route for the development of a new generation of orthopedic implant materials with both antibacterial and biological activities. This not only expands the clinical application potential of PEEK materials, but is also expected to extend to the surface functionalization of other medical implants, with broad clinical translation prospects.

Abbreviations

AFM, atomic force microscopy; ALP, alkaline phosphatase; BMD, Bone mineral density; BMPs, bone morphogenetic proteins; BS/BV, bonesurface fraction; BV/TV, bone volume fraction; CLSI, Clinical Laboratory Standards Institute guidelines; CLSM, confocal laser scanning microscope; EDS, energy dispersive spectroscopy; FTIR, Fourier-transform infrared spectroscopy; H&E, Hematoxylin and eosin; IAI, implant-associated infection; MAR, mineral apposition rate; MEM, Minimum Essential Medium; MIC, minimum inhibitory concentration; micro-CT, micro-computed tomography; MOFs, metal-organic frameworks; MOX, moxifloxacin; OD, optical density; PBS, phosphate buffered saline; PDA, polydopamine; PEEK, Polyetheretherketone; PVDF, polyvinylidene fluoride; Ra, mean roughness; ROI, region of interest; Rq, root mean square roughness; SEM, scanning electron microscopy; SIM, simvastatin; SPEEK, sulfonated PEEK; Tb.N, trabecular number; Tb.Sp, trabecular resolution; Tb.Th, trabecular thickness; TEM, transmission electron microscopy; TSA, tryptic soy agar; TSB, tryptic soy broth; ZIF-8, zeolite imidazolate framework-8.

Ethics Statement

The Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University approved all animal experimentation protocols (XJTUAE2024-2493), which were conducted in accordance with the standards outlined in the "Guide for the Care and Use of Laboratory Animals" and the "Animal Research: Reporting of In Vivo Experiments".

Author Contributions

Zidong Wu: Writing-original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Kai Ma: Validation, Methodology, Investigation, Conceptualization. Zixuan Wu: Methodology, Investigation, Formal analysis. Xiaoyu Guo: Investigation, Formal analysis. Yuning Qu: Methodology, Formal analysis. Zheyue Zhu: Methodology, Formal analysis. Weidong Zhu: Investigation. Chengxin Li: Supervision, Resources. Yi Liu: Supervision, Resources, Project administration. Rui Ma: Writing-review & editing, Methodology, Supervision, Resources, Conceptualization. Pei Yang: Supervision, Resources, Project administration, Funding acquisition. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The research was funded by the National Natural Science Foundation of China (No.82272503 and No.82472429), the Fundamental Research Funds for the Central Universities (No. 11913224000029), Team and Talent Support-Interdisciplinary Team Project of Xi'an Jiaotong University (xtr062025011), IIT Clinical Research Fund of the Second Affiliated Hospital of Xi'an Jiaotong University (No. 2024 M060), Key Research and Development Projects of Shaanxi Province (Social Development, No. 2025SF-YBXM-452) and 2025 Shaanxi Provincial High-Level Health Talent (Team) Cultivation Program (Bioactive PEEK Orthopedic Implant R&D and Translational Innovation Team of the Second Affiliated Hospital of Xi'an Jiaotong University).

Disclosure

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

References

- Ghimire A, Song J. Anti-periprosthetic infection strategies: from implant surface topographical engineering to smart drug-releasing coatings. *ACS Appl Mater Interfaces*. 2021;13(18):20921–20937. doi:10.1021/acsami.1c01389
- Deng J, Van Duyn C, Cohen DJ, Schwartz Z, Boyan BD. Strategies for improving impaired osseointegration in compromised animal models. *J Dent Res*. 2024;103(5):467–476. doi:10.1177/00220345241231777
- Huang S, Zhong Y, Fu Y, Zheng X, Feng Z, Mo A. Graphene and its derivatives: “one stone, three birds” strategy for orthopedic implant-associated infections. *Biomater Sci*. 2023;11(2):380–399. doi:10.1039/d2bm01507b
- Chen X, Zhou J, Qian Y, Zhao L. Antibacterial coatings on orthopedic implants. *Mater Today Bio*. 2023;19:100586. doi:10.1016/j.mtbio.2023.100586
- He M, Huang Y, Xu H, et al. Modification of polyetheretherketone implants: from enhancing bone integration to enabling multi-modal therapeutics. *Acta Biomater*. 2021;129:18–32. doi:10.1016/j.actbio.2021.05.009
- Zheng Z, Liu P, Zhang X, et al. Strategies to improve bioactive and antibacterial properties of polyetheretherketone (PEEK) for use as orthopedic implants. *Mater Today Bio*. 2022;16:100402. doi:10.1016/j.mtbio.2022.100402
- Xie M, Xiao GY, Song ZG, Lu YP. The formation process and mechanism of the 3D porous network on the sulfonated PEEK surface. *ACS Appl Mater Interfaces*. 2024;16(11):13585–13596. doi:10.1021/acsami.4c00055
- Al-Baadani MA, Xu L, Cai K, et al. Preparation of co-electrospinning membrane loaded with simvastatin and substance P to accelerate bone regeneration by promoting cell homing, angiogenesis and osteogenesis. *Mater Today Bio*. 2023;21:100692. doi:10.1016/j.mtbio.2023.100692
- Zhao Y, He P, Yao J, et al. pH/NIR-responsive and self-healing coatings with bacteria killing, osteogenesis, and angiogenesis performances on magnesium alloy. *Biomaterials*. 2023;301:122237. doi:10.1016/j.biomaterials.2023.122237
- Sun T, Huang J, Zhang W, et al. Simvastatin-hydroxyapatite coatings prevent biofilm formation and improve bone formation in implant-associated infections. *Bioact Mater*. 2023;21:44–56. doi:10.1016/j.bioactmat.2022.07.028
- Goldie SP, Lau LP, Jones HAS, Harries PG, Walls AF, Salib RJ. Antimicrobial activity of simvastatin against chronic rhinosinusitis-related *Staphylococcus aureus*: an in vitro study. *Rhinology*. 2025;63(5):600–607. doi:10.4193/Rhin25.023
- Liu C, Tian C, Guo J, et al. Research progress of metal-organic frameworks as drug delivery systems. *ACS Appl Mater Interfaces*. 2024;16(33):43156–43170. doi:10.1021/acsami.4c09536
- Raza A, Wu W. Metal-organic frameworks in oral drug delivery. *Asian J Pharm Sci*. 2024;19(5):100951. doi:10.1016/j.ajps.2024.100951
- Ren N, Liang N, Dong M, et al. Stem cell membrane-encapsulated zeolitic imidazolate framework-8: a targeted nano-platform for osteogenic differentiation. *Small*. 2022;18(26):e2202485. doi:10.1002/sml.202202485
- Li H, Jia Y, Bai S, Peng H, Li J. Metal-chelated polydopamine nanomaterials: nanoarchitectonics and applications in biomedicine, catalysis, and energy storage. *Adv Colloid Interface Sci*. 2024;334:103316. doi:10.1016/j.cis.2024.103316
- Ma R, Wang J, Li C, et al. Effects of different sulfonation times and post-treatment methods on the characterization and cytocompatibility of sulfonated PEEK. *J Biomater Appl*. 2020;35(3):342–352. doi:10.1177/0885328220935008
- Krasemann C, Meyer J, Tilotson G. Evaluation of the clinical microbiology profile of moxifloxacin. *Clin Infect Dis*. 2001;32 (Suppl 1):S51–63. doi:10.1086/319377
- Odenholt I, Cars O. Pharmacodynamics of moxifloxacin and levofloxacin against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*: simulation of human plasma concentrations after intravenous dosage in an in vitro kinetic model. *J Antimicrob Chemother*. 2006;58(5):960–965. doi:10.1093/jac/dkl356
- CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th. CLSI; 2024.
- Figueiredo EP, Ribeiro JM, Nishio EK, et al. New approach for simvastatin as an antibacterial: synergistic effect with bio-synthesized silver nanoparticles against multidrug-resistant bacteria. *Int J Nanomed*. 2019;14:7975–7985. doi:10.2147/ijn.S211756
- Li Y, Li C, Tu Y, et al. In vivo assessing colitis severity by topical administration of fluorescent probe against neutrophils. *Talanta*. 2021;233:122519. doi:10.1016/j.talanta.2021.122519
- Cho Y, Szabo G. two faces of neutrophils in liver disease development and progression. *Hepatology*. 2021;74(1):503–512. doi:10.1002/hep.31680
- Zhang Z, Zhang X, Zheng Z, et al. Latest advances: improving the anti-inflammatory and immunomodulatory properties of PEEK materials. *Mater Today Bio*. 2023;22:100748. doi:10.1016/j.mtbio.2023.100748
- Chen Z, Chen Y, Wang Y, et al. Polyetheretherketone implants with hierarchical porous structure for boosted osseointegration. *Biomater Res*. 2023;27(1):61. doi:10.1186/s40824-023-00407-5

25. Malheiros SS, Borges MHR, Rangel EC, et al. Zinc-doped antibacterial coating as a single approach to unlock multifunctional and highly resistant titanium implant surfaces. *ACS Appl Mater Interfaces*. 2025;17(12):18022–18045. doi:10.1021/acsami.4c21875
26. Jin X, Xie D, Zhang Z, et al. In vitro and in vivo studies on biodegradable Zn porous scaffolds with a drug-loaded coating for the treatment of infected bone defect. *Mater Today Bio*. 2024;24:100885. doi:10.1016/j.mtbio.2023.100885
27. Ravanbakhsh S, Paternoster C, Barucca G, et al. Improving the radiopacity of Fe-Mn biodegradable metals by magnetron-sputtered W-Fe-Mn-C coatings: application for thinner stents. *Bioact Mater*. 2022;12:64–70. doi:10.1016/j.bioactmat.2021.10.022
28. Yin S, Sun N, Jiang F, et al. The translation from in vitro bioactive ion concentration screening to in vivo application for preventing peri-implantitis. *ACS Appl Mater Interfaces*. 2021;13(4):5782–5794. doi:10.1021/acsami.0c19698
29. Li Y, Fan Y, Zhao S, Cheng B. Ultrasound-triggered piezoelectric polyetheretherketone with boosted osteogenesis via regulating Akt/GSK3 β /catenin pathway. *J Nanobiotechnology*. 2024;22(1):539. doi:10.1186/s12951-024-02814-9
30. Yang C, Zhu K, Cheng M, et al. Graphene oxide-decorated microporous sulfonated polyetheretherketone for guiding osteoporotic bone regeneration. *J Control Release*. 2024;374:15–27. doi:10.1016/j.jconrel.2024.07.054
31. Karthikeyan S, Somasundaram P, Karimi I, et al. Statin drugs and lipid modulation: mechanistic basis considering lipid rafts, kinase signaling, myopathy, and cancer. *Pharmacol Res*. 2025;220:107912. doi:10.1016/j.phrs.2025.107912
32. Li W, Zhong J, Wang X, et al. Intelligent responsive zeolitic imidazolate framework-8@copper oxide nanocomposite 3d-printed scaffolds for efficient repair of infected bone defects. *ACS Nano*. 2025;19(39):35154–35180. doi:10.1021/acsnano.5c13201
33. Yu SY, Wu T, Xu KH, et al. 3D bioprinted biomimetic MOF-functionalized hydrogel scaffolds for bone regeneration: synergistic osteogenesis and osteoimmunomodulation. *Mater Today Bio*. 2025;32:101740. doi:10.1016/j.mtbio.2025.101740
34. Yan M, Jiang F, Fei H, Ma F, Yan J, Wu Y. Polydopamine-based multilevel molecularly imprinted nanocomposite membranes comprising metal organic frameworks for selective recognition and separation. *J Colloid Interface Sci*. 2022;606(Pt 1):696–708. doi:10.1016/j.jcis.2021.08.076
35. Deng Y, Gao XY, Shi XL, et al. Graphene oxide and adiponectin-functionalized sulfonated Poly(etheretherketone) with effective osteogenicity and remotely repeatable photodisinfection. *Chem. Mater*. 2020;32(5):2180–2193. doi:10.1021/acs.chemmater.0c00290
36. Wang X, Ren M, Wang N, et al. Zeolitic imidazolate framework-8@polydopamine decorated carboxylated chitosan hydrogel with photocatalytic and photothermal antibacterial activity for infected wound healing. *J Colloid Interface Sci*. 2024;675:1040–1051. doi:10.1016/j.jcis.2024.07.087
37. Yin X, Ran S, Cheng H, et al. Polydopamine-modified ZIF-8 nanoparticles as a drug carrier for combined chemo-photothermal osteosarcoma therapy. *Colloids Surf B Biointerfaces*. 2022;216:112507. doi:10.1016/j.colsurfb.2022.112507
38. Hu X, Li H, Zhao X, et al. Multi-omics study reveals that statin therapy is associated with restoration of gut microbiota homeostasis and improvement in outcomes in patients with acute coronary syndrome. *Theranostics*. 2021;11(12):5778–5793. doi:10.7150/thno.55946
39. Polita AR, Bagdonaitė RT, Shivabalan AP, Valinčius G. Influence of simvastatin and pravastatin on the biophysical properties of model lipid bilayers and plasma membranes of live cells. *ACS Biomater Sci Eng*. 2024;10(9):5714–5722. doi:10.1021/acsbomaterials.4c00911
40. Qiao Y, Xu Y, Liu X, et al. Microwave assisted antibacterial action of garcinia nanoparticles on gram-negative bacteria. *Nat Commun*. 2022;13(1):2461. doi:10.1038/s41467-022-30125-w
41. Pouroutzidou GK, Papadopoulou L, Lazaridou M, et al. Composite PLGA-nanobioceramic coating on moxifloxacin-loaded akermanite 3D porous scaffolds for bone tissue regeneration. *Pharmaceutics*. 2023;15(3):819. doi:10.3390/pharmaceutics15030819
42. Greimel F, Scheuerer C, Gessner A, et al. Efficacy of antibiotic treatment of implant-associated *Staphylococcus aureus* infections with moxifloxacin, flucloxacillin, rifampin, and combination therapy: an animal study. *Drug Des Devel Ther*. 2017;11:1729–1736. doi:10.2147/ddt.S138888
43. Aguilera-Correa JJ, Garcia-Casas A, Mediero A, et al. A new antibiotic-loaded sol-gel can prevent bacterial prosthetic joint infection: from in vitro studies to an in vivo model. *Front Microbiol*. 2019;10:2935. doi:10.3389/fmicb.2019.02935
44. Liu J, Li H, Li H, et al. Rational design of dipicolylamine-containing carbazole amphiphiles combined with Zn(2+) as potent broad-spectrum antibacterial agents with a membrane-disruptive mechanism. *J Med Chem*. 2021;64(14):10429–10444. doi:10.1021/acs.jmedchem.1c00858
45. Dey S, Patel A, Haloi N, et al. Quinoline thiourea-based zinc ionophores with antibacterial activity. *J Med Chem*. 2023;66(16):11078–11093. doi:10.1021/acs.jmedchem.3c00368
46. Yao K, Cheung SW, Tang Y, et al. Photoelectron therapy preventing the formation of bacterial biofilm on titanium implants. *Small*. 2025;21(6):e2409824. doi:10.1002/sml.202409824
47. Mohaghegh N, Ahari A, Buttles C, et al. Simvastatin-loaded polymeric nanoparticles: targeting inflammatory macrophages for local adipose tissue browning in obesity treatment. *ACS Nano*. 2024;18(40):27764–27781. doi:10.1021/acsnano.4c10742
48. Wang X, Huo R, Liang Z, et al. Simvastatin inhibits NLRP3 inflammasome activation and ameliorates lung injury in hyperoxia-induced bronchopulmonary dysplasia via the KLF2-mediated mechanism. *Oxid Med Cell Longev*. 2022;2022:8336070. doi:10.1155/2022/8336070
49. Li H, Wang Y, Liu J, et al. Endothelial Klf2-Foxp1-TGF β signal mediates the inhibitory effects of simvastatin on maladaptive cardiac remodeling. *Theranostics*. 2021;11(4):1609–1625. doi:10.7150/thno.48153
50. Zhang B, Leng J, Ouyang Z, et al. Superhydrophilic and topography-regulatable surface grafting on PEEK to improve cellular affinity. *Biomater Adv*. 2023;146:213310. doi:10.1016/j.bioadv.2023.213310
51. Shi M, Mo W, Qi H, et al. Oxygen ion implantation improving cell adhesion on titanium surfaces through increased attraction of fibronectin PHSRN domain. *Adv Healthc Mater*. 2022;11(10):e2101983. doi:10.1002/adhm.202101983
52. Han T, Xue P, Ju S, et al. RNA-seq reveals correlations between cytoskeleton-related genes and the osteogenic activity of mesenchymal stem cells on strontium loaded titania nanotube arrays. *Mater Sci Eng C Mater Biol Appl*. 2021;122:111939. doi:10.1016/j.msec.2021.111939
53. Wang X, Yang Y, Wang Y, et al. Focal adhesion and actin orientation regulated by cellular geometry determine stem cell differentiation via mechanotransduction. *Acta Biomater*. 2024;182:81–92. doi:10.1016/j.actbio.2024.05.017
54. Tao S, Yu F, Song Y, et al. Water/pH dual responsive in situ calcium supplement collaborates simvastatin for osteoblast promotion mediated osteoporosis therapy via oral medication. *J Control Release*. 2021;329:121–135. doi:10.1016/j.jconrel.2020.11.059
55. Wu T, Sun J, Tan L, et al. Enhanced osteogenesis and therapy of osteoporosis using simvastatin loaded hybrid system. *Bioact Mater*. 2020;5(2):348–357. doi:10.1016/j.bioactmat.2020.03.004
56. Sun T, Meng C, Ding Q, et al. In situ bone regeneration with sequential delivery of aptamer and BMP2 from an ECM-based scaffold fabricated by cryogenic free-form extrusion. *Bioact Mater*. 2021;6(11):4163–4175. doi:10.1016/j.bioactmat.2021.04.013
57. Jiang F, Qi X, Wu X, et al. Regulating macrophage-MSC interaction to optimize BMP-2-induced osteogenesis in the local microenvironment. *Bioact Mater*. 2023;25:307–318. doi:10.1016/j.bioactmat.2023.02.001

58. Parolini C. Pathophysiology of bone remodelling cycle: role of immune system and lipids. *Biochem Pharmacol.* **2025**;235:116844. doi:10.1016/j.bcp.2025.116844
59. Rezk AI, Yeon Kim J, Su Kim B, Hee Park C, Sang Kim C. De novo dual functional 3D scaffold using computational simulation with controlled drug release. *J Colloid Interface Sci.* **2022**;625:12–23. doi:10.1016/j.jcis.2022.05.020
60. Wang S, Gu R, Wang F, et al. 3D-Printed PCL/Zn scaffolds for bone regeneration with a dose-dependent effect on osteogenesis and osteoclastogenesis. *Mater Today Bio.* **2022**;13:100202. doi:10.1016/j.mtbio.2021.100202
61. Bartmański M, Pawłowski Ł, Knabe A, et al. The effect of marginal Zn(2+) excess released from titanium coating on differentiation of human osteoblastic cells. *ACS Appl Mater Interfaces.* **2024**;16(36):48412–48427. doi:10.1021/acsami.4c13529
62. Tang H, Yu Y, Zhan X, et al. Zeolite imidazolate framework-8 in bone regeneration: a systematic review. *J Control Release.* **2024**;365:558–582. doi:10.1016/j.jconrel.2023.11.049
63. Kiouri DP, Chasapis CT, Mavromoustakos T, Spiliopoulou CA, Stefanidou ME. Zinc and its binding proteins: essential roles and therapeutic potential. *Arch Toxicol.* **2025**;99(1):23–41. doi:10.1007/s00204-024-03891-3
64. Li Z, Yue M, Zhou Y. Advances in material-based strategies for diabetic bone regeneration. *Stem Cells Transl Med.* **2024**;13(3):243–254. doi:10.1093/stcltm/szad092
65. Zhang X, Xu D, Zhang R, Wang H, Yang G. Interaction between diabetes and osteoporosis: imbalance between inflammation and bone remodeling. *Osteoporos Int.* **2025**;36(12):2401–2409. doi:10.1007/s00198-025-07636-5
66. Hejazi F, Zare F, Asgari M. Enhanced differentiation of mesenchymal stem cells in coral-incorporated PCL/elastin scaffold enables 3D defect healing in osteoporosis rat model. *Int J Biol Macromol.* **2024**;277(Pt 4):134483. doi:10.1016/j.ijbiomac.2024.134483

International Journal of Nanomedicine

Publish your work in this journal

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®/Clinical Medicine, Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/international-journal-of-nanomedicine-journal>

Dovepress
Taylor & Francis Group