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Antimicrobial osteogenic 3D-printed scaffolds driven by photothermal energy for repairing cranial defects in diabetes

Running title: Photothermal 3D-printed scaffolds for cranial defect repair

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Abstract

Diabetes-related bone defects are severely inhibited by high-glucose-induced chronic inflammation, oxidative stress, and angiogenesis impairment, resulting in limited repair efficacy of conventional scaffolds in this complex microenvironment. This study constructed a 3D-printed DexMA/P-HAP/Cu₂O@MXene composite scaffold that integrates photothermal sterilization, osteogenic induction, and immunomodulatory functions. The scaffold utilizes methacrylated dextran (DexMA) as a framework to ensure formability and mechanical properties; apatite modified with dopamine (P-HAP) enhances the bioactivity of the inorganic phase; and the Cu₂O@MXene nanocomposite imparts photothermal response and Cu²⁺ release capabilities, enabling programmable temperature control (50°C for sterilization and 41°C for osteogenic induction) under near-infrared excitation and local microenvironmental regulation. In vitro and in vivo experiments demonstrated that the scaffold effectively scavenged ROS, inhibited inflammatory cytokine expression, promoted M2 macrophage polarization, and synergistically promoted angiogenesis and bone remodeling by activating CD31/VEGF and Runx2 signaling pathways. This study proposed an intelligent scaffold strategy with spatial-temporal response characteristics and multi-dimensional mechanism integration capabilities, providing a new solution for the efficient regenerative repair of complex bone defects in diabetes.

Keywords: Diabetic bone defect; 3D printing scaffold; Osteogenesis; Cu₂O@MXene; Runx2 pathway

1. Introduction

Skull defects are a common and complex form of bone tissue defect in clinical practice [1]. They are often caused by severe trauma, tumor resection, postoperative infection or congenital developmental abnormalities [1, 2]. If not reconstructed in time, it will not only lead to complications such as brain tissue exposure and cerebrospinal fluid leakage, but may also cause intracranial pressure disorders, neurological dysfunction, and even life-threatening conditions [2, 3]. Globally, the demand for skull reconstruction surgery has increased year by year, especially in patients with metabolic diseases such as diabetes [4]. Their bone regeneration capacity is severely impaired, manifested as decreased osteogenesis, blocked angiogenesis and chronic inflammatory activation, which significantly increases the postoperative infection rate and implant failure rate [4, 5]. Existing clinical data show that the failure rate of bone defect repair in diabetic patients is 1.8 to 3.2 times that of non-diabetic patients, and the wound healing time is prolonged by about 40%, indicating that bone regeneration in the context of diabetes faces more severe challenges [6]. Currently, inert materials such as titanium mesh and polyetheretherketone (PEEK) are commonly used in clinical cranial reconstruction [7]. Although these materials provide excellent mechanical support, they lack bioactivity and tissue integration, are prone to foreign body reactions, infection, and even postoperative bone resorption, making functional regeneration difficult [8-10]. Therefore, there is an urgent need to develop a regenerative material system that combines structural support with biofunctional regulation to address the needs of staged repair of complex bone defects, particularly in the context of metabolic disorders.

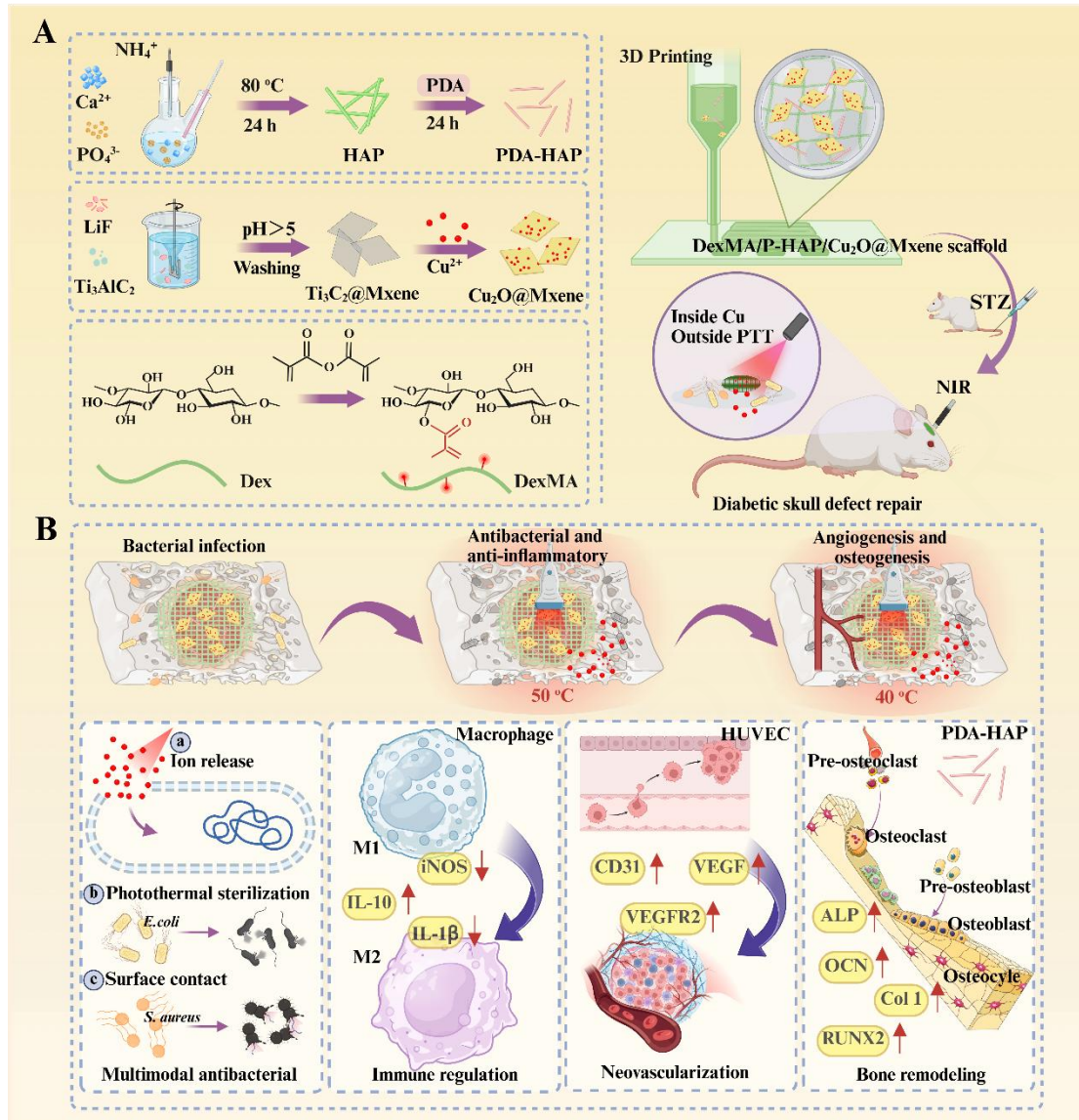
In recent years, additively manufactured hydrogel scaffolds have become an important platform in bone tissue engineering because of their favorable biocompatibility, biomimetic microenvironment, and capacity for patient-specific structural design. Beyond geometric customization, recent advances enable the spatial organization of biomaterials, cells, and bioactive cues, as well as the construction of interconnected or perfusable architectures that support vascularization, matrix mineralization, and osteogenic–angiogenic coupling [11-13]. According to ISO/ASTM 52900, additive manufacturing encompasses several process categories with distinct forming mechanisms and material requirements. For hydrogel fabrication, material extrusion constructs scaffolds through nozzle-based filament deposition and is strongly influenced by ink rheology and filament stability, whereas vat photopolymerization selectively cures liquid photosensitive precursors layer by layer, enabling precise control over scaffold geometry [14, 15]. Therefore, clearly defining the specific additive manufacturing process is essential for interpreting scaffold architecture and performance. Hydrogels can simulate the natural extracellular matrix (ECM)

environment through biomimetic design, promote stem cell adhesion and bone differentiation, and can also be loaded with drugs, growth factors or nanomaterials to enhance their functionality [16]. When combined with additive manufacturing, hydrogel scaffolds can achieve precise matching with the defect site and provide a bone-regeneration platform integrating structural control and biological functionality by regulating pore architecture, mechanical properties, and release behavior [17]. Recent studies further demonstrate that the regenerative performance of additively manufactured scaffolds is closely associated with their surface and interfacial characteristics. Chen et al. developed a neobavaisoflavone-doped GelMA coating for porous Zn–Mg scaffolds, which regulated scaffold degradation and ion release while enhancing osteogenic and angiogenic activities [18]. Liu et al. further demonstrated that surface roughness, composition, and wettability critically affect the osteogenic performance of 3D-printed biodegradable Zn-based scaffolds [19]. These findings indicate that the integration of structural design with surface biofunctionalization is essential for effective bone regeneration. However, how to integrate multifunctional modules such as antibacterial, osteogenesis, angiogenesis and immune regulation on this basis, and achieve "multi-target, multi-stage" precise intervention in diabetic bone defects, is still a key bottleneck facing current research.

The introduction of functional nanomaterials provides an important opportunity for empowering 3D scaffolds. Various metal or metal oxide nanomaterials such as Cu₂O, CeO₂, Fe₃O₄, etc. have attracted widespread attention in the intervention of diabetic and infectious bone defects due to their photothermal effects, ion regulation, antioxidant and immunomodulatory properties [20–22]. For example, Cu-based nanomaterials can regulate macrophage polarization and promote angiogenesis by releasing Cu²⁺ ions, while CeO₂ has a good inhibitory effect on chronic inflammation due to its ROS scavenging ability [23, 24]. In addition, MXene-type two-dimensional materials have also been gradually introduced into the construction of multifunctional bone repair materials due to their high specific surface area, good conductivity and photothermal conversion efficiency [25]. However, current research focuses on the enhancement of a single function, such as antibacterial or osteogenic induction, and lacks a system design that integrates multi-mechanism coupling, spatial/temporal program control and microenvironmental response, making it difficult to achieve synergistic intervention in complex diabetic bone defects.

Based on the key pathological hypothesis that infection interferes with osteogenesis, immune–vascular coupling, and repair in diabetic patients, this study proposed and constructed a DexMA/P-HAP/Cu₂O@MXene composite scaffold fabricated by DLP-based vat photopolymerization, aiming to achieve multidimensional regulation of “infection clearance, immune remodeling, and osteogenic–angiogenic coupling” during bone defect repair (**Scheme 1**). The material composition was selected

to integrate process-specific printability with post-fabrication biological functions. Specifically, the scaffold follows a “photocurable matrix–bioactive interface–therapeutic nanocomposite” design: DexMA enables light-mediated scaffold fabrication, P-HAP provides interfacial reinforcement and osteoconductive cues, and Cu₂O@MXene introduces photothermal and microenvironment-regulatory functions after printing. This system utilizes methacrylated dextran (DexMA) as the photocurable scaffold matrix, providing suitable formability and structural stability. Dopamine-modified hydroxyapatite (P-HAP) improves interfacial compatibility between the inorganic phase and DexMA matrix while providing osteogenic cues through the release of Ca²⁺ and PO₄³⁻. Furthermore, the Cu₂O@MXene nanoplateform enhances photothermal conversion and enables NIR-mediated staged temperature regulation, with approximately 50°C applied for early infection control and 41°C used for subsequent mild photothermal stimulation of regeneration [26]. Copper-ion release may further contribute to inflammatory regulation and angiogenesis. Mechanistically, the scaffold reduced proinflammatory cytokine expression, promoted angiogenesis-related signaling, and enhanced osteogenic differentiation, thereby reconstructing the regenerative microenvironment of infected diabetic bone defects. Overall, this scaffold integrates structural programmability, infection control, and osteogenic–angiogenic regulation, providing a potential engineering strategy for diabetic infected bone-defect repair.



Scheme. 1. Schematic diagram of the construction process and therapeutic mechanism of a 3D-printed DexMA/P-HAP/ $\text{Cu}_2\text{O}@MXene$ composite scaffold for diabetic skull defect repair. (A) Material preparation and scaffold construction process: Hydroxyapatite (HAP) is modified with dopamine to form P-HAP, enhancing its osteogenic inductivity and matrix affinity; Ti_3C_2 MXene is loaded with Cu_2O to form $\text{Cu}_2\text{O}@MXene$, enabling photothermal conversion and copper ion release; Dextran (Dex) is methacrylated to form DexMA, which serves as a 3D-printable scaffold matrix. The resulting DexMA/P-HAP/ $\text{Cu}_2\text{O}@MXene$ composite scaffold was fabricated by 3D printing and applied to a streptozotocin (STZ)-induced diabetic skull defect model for treatment under near-infrared (NIR) irradiation. (B) Multidimensional repair mechanism: Under NIR excitation, the scaffold achieves multimodal synergistic functions, including multimodal antibacterial, immune regulation, promotion of neovascularization, and induction of osteoblastic differentiation, achieving spatial-temporal synergistic bone repair in the diabetic microenvironment.

2. Materials and methods

2.1 Materials

Dextran (Mw=70,000 Da) was purchased from Bai ling wei Co., Ltd. (China). 4-Dimethylaminopyridine (DMAP), tetrahydrofuran (THF), glycidyl methacrylate (GMA), dimethyl sulfoxide (DMSO), aluminum chloride, copper (II) chloride, sodium hydroxide, hydrochloric acid, and ammonia solution were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris), polyvinylpyrrolidone (PVP), acetic acid ($\geq 98\%$), calcium nitrate tetrahydrate, and ferric chloride were purchased from Aladdin Industrial Corporation (Shanghai, China). Ammonium fluoride was supplied by Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Cell Counting Kit-8 (CCK-8) and lipopolysaccharide (LPS, derived from *Escherichia coli* O111:B4) were purchased from Bio-Rad Laboratories (China). Phorbol 12-myristate 13-acetate (PMA, P6741) and ROS-sensitive fluorescent probe DCFH-DA (D6470) were purchased from Solarbio Life Sciences (Beijing, China). Calcein-AM/PI dual staining kit (KGAF001) and CellTracker™ Red CMTPX (200 μ L) were purchased from Jiangsu Kaijie Biotechnology Co., Ltd. (Nanjing, China). Matrigel was purchased from Corning Inc. (USA). Anti-CD206 antibody (60143-1-Ig) and anti-iNOS antibody (18985-1-AP) were purchased from Proteintech Group (Wuhan, China). All cell culture reagents were purchased from Gibco (USA). All chemicals were of analytical grade and used as received without further purification.

2.2 Material preparation and synthesis

2.2.1 Synthesis of Methacrylated Dextran (DexMA)

Dextran (10.0 g) was dissolved in 100 mL DMSO, followed by DMAP (2.0 g) and stirred for 1 h. GMA (3.0 mL) was added, and the reaction proceeded at 25°C in the dark for 48 h. The product was dialyzed (MWCO 3500 Da) against deionized water for 3 days, lyophilized, and stored at -20 °C [27].

2.2.2 Synthesis of hydroxyapatite (HAP) and PDA-modified hydroxyapatite (PDA-HAP)

Hydroxyapatite (HAP) was synthesized by sequentially adding 0.3 M $(\text{NH}_4)_2\text{HPO}_4$ solution to 0.5 M $\text{Ca}(\text{NO}_3)_2$ solution, with a Ca/P molar ratio of 1.67, under vigorous stirring. The pH was adjusted to above 10 using ammonia water, and the reaction was carried out at 37°C for 24 h. The resulting precipitate was collected by centrifugation, washed with deionized water, and dried at 80°C. For PDA-modified hydroxyapatite, a 10% HAP solution was prepared and added to Tris buffer containing 8% hydrochloric acid dopamine, and stirred at room temperature for 24 h. The product was thoroughly

washed with deionized water and dried for later use [28].

2.2.3 Synthesis of $Ti_3C_2@MXene$

2.0 g LiF was first dissolved in 40 mL of 9 M HCl under stirring for 10 min. Subsequently, 2.0 g of Ti_3AlC_2 was slowly added, and the mixture was stirred at 40 °C for 24 h. The resulting suspension was centrifuged at 8000 rpm for 5 min and repeatedly washed with deionized water until the pH exceeded 5. The obtained $Ti_3AlC_2 @MXene$ precipitate was redispersed in deionized water, followed by ultrasonication and gentle agitation. After centrifugation at 2500 rpm for 2 min, the supernatant was collected and freeze-dried to yield the MXene precursor [29].

2.2.4 Synthesis of $Cu_2O@MXene$

$Cu_2O/MXene$ composite nanosheets were fabricated via an *in situ* reduction strategy. Briefly, 50 mg of PVP and 25 mg of $CuCl_2$ were sequentially added into 100 mL of MXene precursor solution (1 mg/mL) under magnetic stirring. After stirring for 20 min, 10 mL of freshly prepared $NaBH_4$ solution (0.4 mg/mL) was introduced dropwise at a rate of 200 μ L/min. Following further stirring for 30 min, the mixture was centrifuged at 6000 rpm for 10 min. The collected precipitate was washed repeatedly with deionized water and anhydrous ethanol and then collected for subsequent use.

2.3 Fabrication of composite scaffolds by DLP-based vat photopolymerization

The composite scaffolds were fabricated using Digital Light Processing (DLP)-based Vat Photopolymerization (VPP) technology (classified according to ISO/ASTM 52900:2021). A printer (Engineering For Life, EFL-BP-8601P) equipped with a 405 nm light source was used to perform layer-by-layer selective photopolymerization of the DexMA-based liquid precursor. The designed cylindrical scaffolds featured a diameter of 5.0 mm, a thickness of 1.5 mm, a nominal pore size of 500 μ m, a strut width of 300 μ m, and a layer thickness of 100 μ m, utilizing an alternating 0°/90° structural arrangement. The theoretical porosity, calculated based on the CAD model, was approximately 62.5%.

A 25% DexMA solution (containing 0.2% LAP) was prepared, and a certain concentration of $Cu_2O@MXene$ and PDA-HAP were added to it. The solution was poured into a printer to print the hydrogel (light intensity: 9 mW/cm², exposure time: 15 s, number of base layers: 1, base layer exposure time: 18 s). The printed hydrogel scaffold was rinsed 3-6 times with PBS solution to obtain $DexMA@P@CM$ hydrogel scaffolds.

To evaluate printing accuracy, the fabricated scaffolds were equilibrated in PBS for 24 h before measurement. Their external diameter and thickness were measured

using a digital caliper. Hydrated scaffolds were imaged using a stereomicroscope, and the actual pore size and strut width were quantified using ImageJ. Five independently fabricated scaffolds were analyzed, with at least ten pores and ten struts measured per scaffold.

2.4 Chemical composition and crystal structure characterization

To analyze the chemical structure and composition of the materials, XRD, FT-IR, ¹H-NMR, and XPS were performed. XRD (X-ray diffraction, Bruker D8 Advance) was used to analyze the crystal structure and phase composition. FT-IR (Fourier transform infrared spectrometer, Thermo Nicolet iS50) analysis involved grinding 3-5 mg of sample with dry KBr (approximately 5% by mass) and pressing into pellets (vacuum pressure 20 mmHg, 5 min). The scan range was 4000–400 cm⁻¹ to observe changes in functional groups in the materials. ¹H-NMR (nuclear magnetic resonance, Bruker AVANCE III 400 MHz) analysis involved dissolving 8-15 mg of sample in D₂O and transferring it to an NMR tube. Spectra were analyzed at room temperature using MestReNova software. XPS (X-ray photoelectron spectrometer, Thermo ESCALAB 250Xi) was used to analyze the valence state and binding energy of elements in the composite materials.

2.5 Morphology and particle size distribution analysis

The micromorphology of the samples was examined using TEM and SEM. For TEM analysis (JEOL JEM-2100), the samples were dispersed in anhydrous ethanol at a concentration of 1 mg/mL, sonicated to ensure uniform dispersion, and then drop-cast onto copper grids for natural drying before imaging to analyze particle morphology and structure. SEM observations (Zeiss GeminiSEM 500) were performed to evaluate the overall morphology, microporous architecture, and surface features of the 3D-printed scaffolds. Prior to imaging, the samples were freeze-dried and sputter-coated with gold. In addition, the particle size distribution and zeta potential were determined using dynamic light scattering (DLS, Malvern Zetasizer Nano ZS90).

2.6 Mechanical and rheological characterization

The mechanical performance of the hydrogels was assessed by compression testing. After demolding, cylindrical hydrogel specimens prepared from 600 µL precursor solution were compressed to failure using a universal testing machine (Instron 3367). The maximum compressive load was recorded, and the compressive modulus was calculated.

Rheological measurements were carried out on a rheometer (HAAKE MARS 60, Thermo Fisher Scientific) at room temperature. Frequency sweep tests were conducted

over an angular frequency range of 0.1-10 rad/s for hydrogels with concentrations of 15%, 25%, and 35%. The storage modulus (G') and loss modulus (G'') were recorded to evaluate the viscoelastic behavior.

2.7 Analysis of swelling and degradation behavior

In the swelling performance test, the dry hydrogel was weighed (W_0), completely immersed in PBS, and taken out and weighed (W_t) after 1, 2, 4, 6, and 8 h. The swelling ratio was calculated as follows: Swelling ratio (%) = $(W_t - W_0)/W_0 \times 100\%$.

In the degradation performance test, the sample was immersed in the degradation solution, taken out, freeze-dried, and weighed after 1, 3, 5, 7, 14, and 21 days (W_t). The degradation rate was calculated as follows: Degradation ratio (%) = $(W_0 - W_t)/W_0 \times 100\%$.

2.8 Evaluation of Cu^{2+} release behavior

A 400 μL hydrogel scaffold was immersed in 3 mL of PBS (pH 7.4) after gelation and incubated at 37 °C under shaking at 100 rpm. At predetermined time points (1, 2, 4, 6, 12, 24, 48, 72 and 96 h), the supernatant was collected and replaced with an equal volume of fresh PBS. The collected solutions were centrifuged at 4000 rpm for 5 min, and the Cu^{2+} concentration was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer Avio 200) [30].

2.9 Evaluation of the photothermal response performance of the scaffold

The printed scaffolds were irradiated with an 808 nm NIR laser (2.0 W/cm²) for 5 min in a 24-well plate. Temperature changes were recorded every minute using a thermocouple thermometer (Fluke 52 II). Power-dependent heating was evaluated at 0.5, 1.0, 1.5, and 2.0 W/cm², and four on/off irradiation cycles were performed to assess photothermal stability and reversibility.

2.10 Antibacterial performance evaluation

E. coli and *S. aureus* were cultured at 37 °C to the logarithmic phase and adjusted to 1×10^8 CFU/mL. The bacterial suspensions were incubated with different hydrogel scaffold groups for 24 h. Antibacterial activity was first evaluated by plate counting to determine colony-forming units (CFU) and calculate inhibition rates. Live/dead bacterial viability was assessed using SYTO-9/PI fluorescent staining and observed under a fluorescence microscope, where green and red fluorescence represented live and dead bacteria, respectively. Bacterial morphology and ultrastructural changes after treatment were examined by field-emission scanning electron microscopy (FE-SEM, Hitachi SU8010). Samples were fixed with 2.5% glutaraldehyde, washed with PBS, deposited onto silicon wafers, air-dried, and sputter-coated with gold prior to imaging.

In addition, biofilm formation was evaluated by incubating bacteria with the scaffolds for 48 h, followed by methanol fixation and crystal violet staining. The stained biofilms were dissolved, and absorbance at 570 nm was measured to quantify biofilm biomass.

2.11 Cellular function and mechanism verification

To comprehensively evaluate the bioactivity and multidimensional functionality of the composite hydrogel scaffold, a systematic in vitro characterization system was established. Functional validation was performed in different cell types (BMSCs, HUVECs, and RAW264.7 cells). The scaffold's pathway of action was explored in conjunction with relevant molecular mechanisms. The NIR group employed 808 nm laser irradiation (2.0 W/cm², 5 min) to activate the photothermal reaction.

2.11.1 Biocompatibility assessment and cell morphology analysis

The biocompatibility of the materials toward BMSCs and HUVECs was assessed using a CCK-8 assay (KGAF001, KeyGen). Cells were seeded in 96-well plates and incubated with material extracts (material to medium ratio = 1:10, 24 h extraction). Cell viability was measured on days 1, 3, and 5 by recording absorbance at 450 nm. Live/dead staining was further performed using a Calcein-AM/PI kit (Beyotime). Cells were cultured in 48-well plates, incubated with extracts for 24 h, stained in the dark for 30 min, and imaged using an inverted fluorescence microscope (Nikon Eclipse Ti-U). For cytoskeletal analysis, F-actin and nuclei were labeled with FITC-Phalloidin and DAPI, respectively, to evaluate cell adhesion, spreading, and morphology under fluorescence microscopy at designated time points.

2.11.2 Cell migration assay

The effect of the materials on HUVEC migration was evaluated using scratch wound and Transwell assays. For the wound healing assay, a linear scratch was created on a confluent HUVEC monolayer using a sterile pipette tip. After washing with PBS, culture medium containing the material extracts was added. Images were captured at 0, 12, 24, and 36 h, and the wound closure area was quantified using ImageJ. For the Transwell assay, HUVECs (1×10^5 cells/well) suspended in serum-free medium were seeded into the upper chamber of Transwell inserts (8.0 μ m pore size, Corning). The lower chamber was filled with medium containing different extracts and 10% FBS. After 24 h incubation, the migrated cells were fixed, stained, and counted under a microscope to assess migration ability.

2.11.3 Assessment of angiogenic potential

HUVEC suspensions (1×10^4 cells/well) were seeded in Matrigel-coated 96-well plates and incubated with the material extract for 6 h. The total length, number of nodes,

and number of branches of the formed capillary-like structures were photographed and counted under a microscope. To further explore the molecular mechanisms, immunofluorescence staining was performed to detect the expression of CD31, VEGF, and VEGFR2, and mean fluorescence intensity was analyzed using ImageJ. Furthermore, after protein extraction, Western blotting was performed to detect VEGF and CD31, key factors associated with angiogenesis, to clarify the angiogenic mechanism [31].

2.11.4 Analysis of osteogenic induction ability

BMSCs were seeded at a cell density of 2×10^4 cells/mL in a 24-well plate and subsequently cultured in a new hydrogel extract. Alkaline phosphatase (ALP) activity was stained (Beyotime ALP Kit) on day 7, and activity levels were measured using a multi-function microplate reader (405 nm). Alizarin red staining (ARS) was performed on day 14 to observe the formation of calcified nodules. Calcification nodules were then quantitatively analyzed by measuring absorbance at 562 nm after dissolution with anhydrous ethanol. Furthermore, immunofluorescence was used to assess the expression of early (RUNX2), mid (COL-I), and late (OCN) osteogenic markers. Western blotting was also used to assess RUNX2, ALP, OCN, and β -catenin proteins to further elucidate whether the material promotes osteogenic differentiation by activating osteogenic signaling pathways such as Wnt/ β -catenin.

2.11.5 Verification of macrophage polarization and immune modulation

Using RAW264.7 cells as a model, the material's regulatory effects on macrophage immune phenotype were investigated. Under inflammatory conditions containing LPS (5 μ g/mL), cells were exposed to the extracts from each group. After 24 hours of culture, qPCR was performed to assess the expression levels of M1 phenotype factors (IL-1 β) and M2 phenotype factors (IL-10). Immunofluorescence staining was performed to detect CD86 and CD206 protein expression at excitation wavelengths of 555 nm and 488 nm, respectively, to visualize M1/M2 polarization trends. Flow cytometry (BD FACS Aria III) was used to quantify the ratio of CD80+ (M1) to CD206+ (M2) cells, assessing the distribution of immune polarization induced by the material and reflecting its potential to modulate the inflammatory microenvironment.

2.11.6 Real-time quantitative PCR (RT-qPCR)

RAW264.7 cells (5×10^5 cells/well) were seeded in 12-well plates and stimulated with LPS (5 μ g/mL) overnight, followed by treatment with hydrogel extracts for 24 h. Total RNA was extracted using TRIzol and quantified with a NanoDrop 2000. cDNA was synthesized and analyzed by qPCR (ABI 7500, SYBR Premix Ex Taq, Takara). GAPDH was used as an internal control, and IL-1 β and IL-10 expression levels were

calculated using the $2^{-\Delta\Delta C_t}$ method.

2.11.7 Western Blot (WB)

HUVEC and RAW264.7 cells were seeded in 6-well plates and treated with the hydrogel extracts of each group for 24 h after attachment. Cell lysates were lysed with RIPA, centrifuged, and protein quantified before loading onto SDS-PAGE electrophoresis and transferring to PVDF membranes. After blocking, the corresponding primary antibodies and HRP-conjugated secondary antibodies were applied, and the cells were developed using ECL developer. Protein bands were acquired using the ChemiDoc MP Imaging System (Bio-Rad), and grayscale values were quantified using ImageJ.

2.11.8 Molecular mechanism and pathway verification

Total RNA was extracted from BMSCs (TRIzol), quantified by NanoDrop, and quality-checked by Agilent 2100 ($RIN \geq 7.0$). Strand-specific libraries were constructed and sequenced (PE150) on an Illumina NovaSeq 6000 (≥ 6 GB/sample). Reads were processed by fastp, aligned to GRCm38/mm10 using HISAT2, and DEGs were identified with DESeq2 ($|\log_2FC| \geq 1$, $P < 0.05$). GO and KEGG analyses were performed using clusterProfiler.

2.12 Construction of diabetic infected skull defect model and evaluation of treatment effects

2.12.1 Animal Grouping

36 male Sprague-Dawley (SD) rats (200-220 g) were randomly divided into six groups ($n=6$ per group): Control, DexMA, DexMA@P, DexMA@P+NIR, DexMA@P@CM, and DexMA@P@CM+NIR. All animals were housed under specific pathogen-free conditions with free access to food and water. This study adhered to the Principles of Laboratory Animal Care (National Institutes of Health Publication No. 85-23, revised 1996) and was approved by the National Institutional Animal Care Committee. The animal ethics approval number is 202501007.

2.12.2 Construction of diabetic infected skull defect model

A diabetic-infected calvarial defect model was established to evaluate scaffold efficacy. Diabetes was induced by intraperitoneal injection of STZ (50 mg/kg, 0.1 M citrate buffer, pH 4.5) for 3 consecutive days, and rats with fasting blood glucose > 16.7 mmol/L for two consecutive days were regarded as diabetic. A mixed bacterial suspension (*S. aureus* and *E. coli*, 1:1, 1×10^8 CFU/mL) was used to infect the scaffolds by immersion for 2 h. Bilateral critical-size calvarial defects (5 mm) were created, and the infected scaffolds were implanted. Postoperative photothermal treatment was

applied using an 808 nm laser (50 ± 1 °C, 5 min/day on days 1–3; 41 ± 1 °C, 3 times/week thereafter). Animals were sacrificed at 6 and 12 weeks for evaluation [32, 33].

2.12.3 In vivo antibacterial performance evaluation

On the third postoperative day, the implanted stents and surrounding tissues were harvested for antibacterial performance evaluation. Tissue homogenization was performed, serially diluted, and plated onto LB solid medium. Colony-forming units (CFU) were counted after incubation at 37°C for 24 h.

2.12.4 Photothermal image evaluation

To verify the *in vivo* photothermal conversion capacity and spatial localization effect of the 3D-printed stents under near-infrared (NIR) laser irradiation, infrared thermal images of the top of the rat head were acquired immediately after surgery and at different time points. In the experiment, an 808 nm NIR laser (power density: 2 W/cm²) was applied to the surgical area for 5 min. Simultaneously, an infrared thermal imager (FLIR E60, FLIR Systems, USA) was used to record the surface temperature changes and distribution of the stents in different groups in real time.

2.12.5 Micro-CT imaging and bone quantification analysis

Skull specimens were collected at 6 and 12 weeks postoperatively and imaged using a micro-computed tomography system (SkyScan 1176, Bruker). Scanning parameters were set at 65 kV, 380 µA, and a resolution of 18 µm/voxel. Three-dimensional reconstruction of the defect area was performed using NRecon and CTAn software. Indicators such as bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp) were analyzed to assess the amount and structural quality of newly formed bone.

2.12.6 Histological staining and quantitative analysis

Fixed skull tissue was decalcified, paraffin-embedded, and sectioned (5 µm thickness). Hematoxylin and Erythrocyte Embedding (H&E) and Masson's trichrome staining were performed to assess tissue structure and collagen deposition, respectively. Additionally, Goldner staining was performed to evaluate endochondral ossification.

2.12.7 Immunofluorescence detection of osteogenesis and angiogenesis protein expression

Immunofluorescence staining was used to detect proteins associated with new bone formation and angiogenesis, including OSX, Col-I, and CD31. Sections were deparaffinized, antigen retrieval, and blocked with BSA. The sections were then

incubated with primary antibodies (overnight at 4°C) and fluorescently labeled secondary antibodies (1 h at room temperature). Cell nuclei were counterstained with DAPI. Images were acquired using a confocal microscope, and fluorescence intensity and area of positively expressed regions were quantified using ImageJ.

2.12.8 Immunohistochemical analysis of inflammatory cytokine expression

To evaluate the regulation of the local inflammatory microenvironment, immunohistochemical staining for TNF- α , IL-1 β , and IL-6 was performed. After deparaffinization and antigen retrieval, sections were incubated with primary antibodies overnight, followed by HRP-conjugated secondary antibodies and DAB visualization, and counterstained with hematoxylin. Images were captured under a light microscope, and the positive staining area was quantified using ImageJ.

2.13 Statistical analysis

All data are presented as mean $\pm$ SD. Statistical analyses were performed using GraphPad Prism 9.0. Differences between two groups were analyzed by unpaired Student's *t*-test, and multiple comparisons were assessed using one-way ANOVA with Tukey's post hoc test. A *p* value < 0.05 was considered statistically significant. All experiments were repeated at least three times. Significance levels were defined as **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

3. Results and discussions

3.1 Structure confirmation and dispersion stability evaluation of composite nanomaterials.

In this study, we successfully synthesized and systematically characterized the key nanocomponents of the DexMA/P-HAP/Cu₂O@MXene composite scaffold. TEM images (**Fig. 1(A)**) reveal that HAP exhibits regular nanorod morphology with uniform particle size and smooth surfaces [34]. Following PDA modification, a uniform coating layer appears on the particle surfaces, confirming successful PDA attachment to HAP surfaces. This modification enhances particle surface activity and subsequent functionalization potential. XRD results (**Fig. 1(B)**) reveal that the diffraction peaks of the synthesized HAP highly match those of the standard card (JCPDS 73-0293), exhibiting characteristic peaks at (100), (002), (211), and (300), indicating the phase purity of the sample [35]. The major diffraction peaks remained unchanged after PDA modification, suggesting no alteration to the HAP crystal structure. The FT-IR spectrum (**Fig. 1(C)**) further confirmed the modification effect. HAP exhibited typical characteristic absorption peaks, with absorption peaks at 564, 603, 962, 1032, and 1094 cm⁻¹ corresponding to the bending and stretching vibrations of phosphate groups,

respectively. The absorption peak at 871 cm^{-1} corresponds to HPO_4^{2-} , while those at 3570 cm^{-1} and 633 cm^{-1} represent hydroxyl stretching vibrations. The peak at 1641 cm^{-1} likely originates from hydroxyl groups corresponding to water absorbed from the atmosphere during the experiment [34]. Following PDA modification, the O-H stretching vibration peak around 3400 cm^{-1} indicates abundant surface hydroxyl groups. This peak broadens and intensifies due to the overlapping stretching vibrations of both -OH and -N-H groups present in dopamine, confirming successful PDA encapsulation on the HAP surface. In PDA-HAP, besides retaining the typical phosphate and hydroxyl absorption peaks of HAP, characteristic peaks such as C-N and aromatic C=C appear. The enhanced and broadened O-H stretching peak indicates the successful introduction of the dopamine coating layer.

SEM images (**Fig. 1(D)**) reveal uniform loading of Cu_2O nanoparticles on the MXene sheet surfaces. EDS mapping results (**Fig. 1(E)**) indicate uniform distribution of Cu, Ti, and O elements, confirming the strong bonding between Cu_2O and MXene. XRD patterns (**Fig. 1(F)**) show characteristic peaks of the MAX phase in Ti_3AlC_2 , with increased interlayer spacing in MXene after Al layer removal [36]. Following Cu_2O loading, diffraction peaks at $2\theta = 36.4^\circ$, 42.3° , and 61.3° appeared, corresponding to the (111), (200), and (220) crystal planes of Cu_2O , further confirming its successful incorporation. XPS analysis (**Fig. 1(G)**) similarly detected Ti 2p and C 1s signals from MXene. In $\text{Cu}_2\text{O}@MXene$, a new Cu 2p peak (approximately 932.5 eV) appeared, while the O 1s peak morphology changed, indicating that the presence of Cu_2O altered the surface chemical state of the material. Meanwhile, the Tyndall effect demonstrated the dispersion of the materials. Results showed (**Fig. 1(H)**) that no significant light scattering was observed in pure water, while the PDA-HAP aqueous solution exhibited faint scattered light paths. In contrast, the $\text{Cu}_2\text{O}@MXene$ dispersion displayed distinct light paths, indicating the formation of a stable colloid in the aqueous solution. Zeta potential measurements (**Fig. 1(I)**) revealed MXene at -37.1 mV and $\text{Cu}_2\text{O}@MXene$ at -16.8 mV, both within stable ranges, confirming excellent dispersion. The decrease in absolute potential indicates that Cu_2O introduction alters the overall surface charge distribution. This change enhances colloidal stability and may modulate interactions with cells. The particle size distribution results (**Fig. 1(J)**) further verify the effect of structural modification on particle size. The average particle size of HAP is $218.3 \pm 7.91\text{ nm}$, with a narrow distribution. The average particle size of PDA-HAP is $236.3 \pm 19.11\text{ nm}$, slightly larger than HAP, consistent with the increase of the PDA coating layer. The average particle size of MXene is $256.8 \pm 4.66\text{ nm}$, with a relatively wide distribution, reflecting its heterogeneous lamellar structure. The average particle size of $\text{Cu}_2\text{O}@MXene$ further increases to $399.4 \pm 31.18\text{ nm}$, indicating that the loading of Cu_2O significantly increases the overall particle size. Therefore, these materials not

only maintain good crystal structure and dispersibility, but also have controllable surface modification and suitable particle size characteristics, laying a solid material foundation for their application in 3D printed photothermal composite scaffolds.

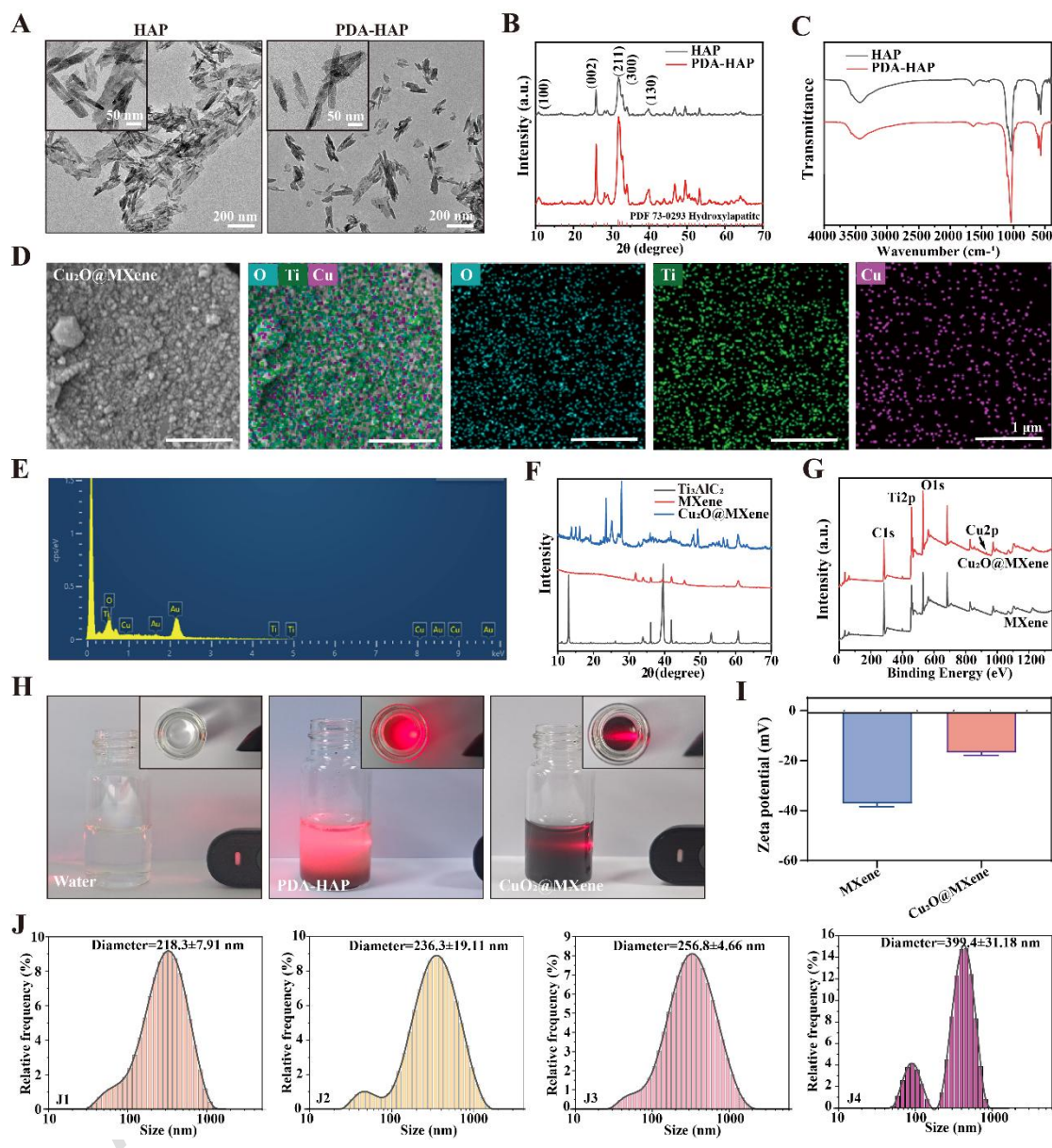


Fig. 1. Chemical structure characterization of nanoparticles. (A) TEM images of HAP and PDA-HAP. (B) XRD patterns of HAP and PDA-HAP. (C) FT-IR spectra of HAP and PDA-HAP. (D) SEM image and EDS mapping of $\text{Cu}_2\text{O}@MXene$. (E) EDS mapping of $\text{Cu}_2\text{O}@MXene$. (F) XRD patterns of Ti_3AlC_2 , MXene, and $\text{Cu}_2\text{O}@MXene$. (G) XPS spectra of MXene and $\text{Cu}_2\text{O}@MXene$. (H) Tyndall effect of water, PDA-HAP aqueous solution, and $\text{Cu}_2\text{O}@MXene$ aqueous solution. (I) Zeta potential of MXene and $\text{Cu}_2\text{O}@MXene$. (J) Particle size distribution histograms of nanoparticles (J1-J4 represent HAP, PDA-HAP, MXene, and $\text{Cu}_2\text{O}@MXene$, respectively).

3.2 Structural controllability and functional performance evaluation of 3D printed scaffolds.

To verify the comprehensive performance of the constructed DexMA@P@CM 3D-printable scaffold in terms of mechanical strength, structural stability, and intelligent responsiveness, this study conducted a systematic physicochemical and functional evaluation. First, proton nuclear magnetic resonance (^1H -NMR) and Fourier transform infrared spectroscopy (FT-IR) confirmed the successful grafting of Dex with methacrylic acid (MA) groups. The ^1H NMR spectrum (**Fig. 2(A)**) reveals characteristic double bond hydrogen peaks at 5.67 ppm and 6.12 ppm in DexMA, distinct from the Dex signal, indicating successful double bond introduction [37, 38]. The FT-IR spectrum (**Fig. 2(B)**) further reveals the presence of a stretching vibration peak at 1702 cm^{-1} , corresponding to the C=C double bond of the methacrylic acid group, and a reduction in the hydroxyl absorption peak at 3440 cm^{-1} [27]. This further validates the effectiveness of the methacrylation reaction, enabling photocrosslinking, and provides a foundation for subsequent 3D printing and mechanical property manipulation. The scaffold was designed with a nominal pore size of $500\text{ }\mu\text{m}$, a theoretical porosity of approximately 62.5%, and a $0^\circ/90^\circ$ alternating architecture to generate interconnected channels. Representative macroscopic top, side, and cross-sectional images (**Fig. A1**, see Appendix) showed that both DexMA and DexMA@P@CM scaffolds maintained the intended cylindrical geometry. The cross-sectional images further demonstrated the preservation of the layered and interconnected internal architecture after the incorporation of P-HAP and $\text{Cu}_2\text{O@MXene}$. SEM images (**Fig. 2(C)**) showed that DexMA formed a regular grid-like structure with uniformly arranged pores. Following the incorporation of P-HAP (P) and $\text{Cu}_2\text{O@MXene}$ (CM), the DexMA@P@CM scaffold retained its overall structural integrity, indicating that the functional components did not substantially disrupt scaffold formation. Quantitative dimensional analysis showed that the hydrated scaffolds had an external diameter of $5.08 \pm 0.06\text{ mm}$, a thickness of $1.56 \pm 0.04\text{ mm}$, a pore size of $462 \pm 28\text{ }\mu\text{m}$, and a strut width of $326 \pm 21\text{ }\mu\text{m}$, compared with the corresponding CAD values of 5.00 mm , 1.50 mm , $500\text{ }\mu\text{m}$, and $300\text{ }\mu\text{m}$, respectively. The corresponding absolute dimensional deviations were 1.6%, 4.0%, 7.6%, and 8.7%. The slight reduction in pore size and increase in strut width were likely caused by light scattering and lateral overcuring during vat photopolymerization, whereas the minor deviations in overall dimensions may also be associated with post-printing swelling. Overall, the fabricated scaffolds reproduced the intended architecture with acceptable, although not perfect, dimensional fidelity. This interconnected macroporous architecture is expected to facilitate mass transport, vascular infiltration, and tissue ingrowth [39].

In terms of mechanical control, rheological and compression testing (**Fig. 2(D), (E)**) further revealed the balance between material concentration and structural density. 35% DexMA exhibited the highest modulus and stress-bearing capacity, but excessive concentrations resulted in decreased flexibility. 15% DexMA exhibited poor morphology due to excessive swelling (**Fig. 2(F)**). Based on the tested formulations, 25% DexMA was selected because it provided the most appropriate balance among formability, mechanical strength, and swelling stability. Furthermore, the DexMA@P@CM scaffold exhibits dual properties: biodegradable and therapeutic. Degradation experiments (**Fig. 2(G)**) demonstrated accelerated degradation in the presence of lysozyme, meeting the basic requirements of a biodegradable implant. To impart therapeutic properties to the material, Cu₂O@MXene was incorporated into the scaffold, providing antibacterial and photothermal responsiveness. The sustained release curve of Cu²⁺ (**Fig. 2(H)**) showed that the material stably released nearly 90% of copper ions within 72 h, demonstrating excellent ion release stability and potential advantages in infection control and vascularization promotion [40]. Regarding photothermal performance, thermal imaging (**Fig. 2(I)**) revealed that the DexMA@P@CM rapidly heated up under near-infrared (NIR) laser irradiation (808 nm, 2.0 W/cm²), reaching a core temperature of 50.3°C within 5 min, significantly outperforming both the DexMA and DexMA@P groups. This demonstrates that the introduction of the functional Cu₂O@MXene composite significantly enhanced photothermal conversion [41]. Further temperature rise curves (**Fig. 2(J)**) also show that the temperature of DexMA@P@CM rises rapidly in a short period of time and tends to be stable, making it suitable for mild photothermal therapy. The power gradient test (**Fig. 2(K)**) confirmed that the temperature rise amplitude is proportional to the laser power and is controllable; the cyclic switching experiment (**Fig. 2(L)**) shows that DexMA@P@CM has excellent photothermal response reversibility and cyclic stability, and can still maintain good performance in multiple heating/cooling cycles, ensuring the continuity and safety of the thermal effect in practical applications. These results also clarify the rationale for the scaffold composition. DexMA primarily ensures photocurable fabrication and structural stability, whereas P-HAP and Cu₂O@MXene mainly provide osteogenic and photothermal functions after printing. Consistent with previous studies showing that biomaterial composition influences both printing performance and the mechanical and thermal properties of fabricated scaffolds [42, 43], the present formulation maintained a regular printed architecture while integrating multiple therapeutic functions. However, direct benchmarking against other scaffold systems was not performed because of differences in materials, fabrication processes, and testing conditions. In summary, the DexMA@P@CM scaffold not only has good structural printability and mechanical strength, but also has controllable degradation, adjustable photothermal properties and functional ion release characteristics, showing

a wide range of application potential in the repair of diabetic skull defects.

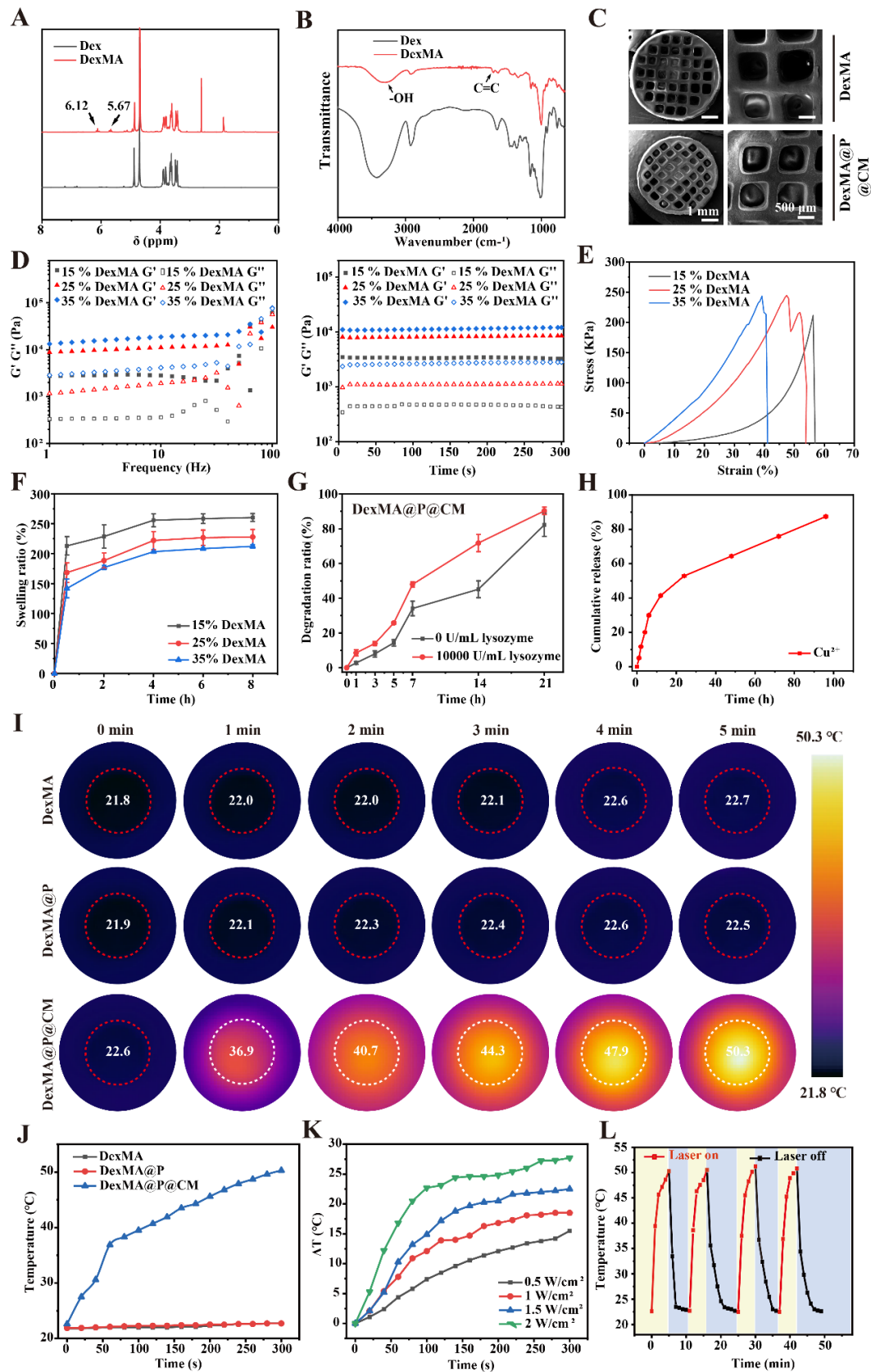


Fig. 2. Preparation and performance characterization of 3D-printed scaffolds. (A) ^1H -NMR spectra of Dex and DexMA. (B) FT-IR spectra of Dex and DexMA. (C) SEM images of DexMA and DexMA@P@CM scaffolds. (D) Rheological frequency and time-scan curves of 15%, 25%, and 35% DexMA hydrogels. (E) Compressive stress-strain curves of 15%, 25%, and 35% DexMA hydrogels. (F) Swelling performance curves of 15%, 25%, and 35% DexMA hydrogels. (G) Degradation rate curves of DexMA@P@CM scaffolds under different external environments. (H) Cumulative release curve of Cu^{2+} from DexMA@P@CM scaffolds at designated time points. (I) Infrared thermography of DexMA, DexMA@P, and DexMA@P@CM scaffolds in deionized water. NIR conditions: 808 nm, 2.0 W/cm², height: 15 cm. (J) Photothermal heating curve of 3D-printed scaffolds immersed in deionized water under NIR irradiation. (K) Photothermal heating curve of DexMA@P@CM scaffolds at different NIR powers. (L) Cyclic heating curve of DexMA@P@CM scaffolds undergoing four on/off cycles in deionized water.

3.3 Evaluation of photothermal synergistic antibacterial properties of 3D printed scaffolds.

To systematically evaluate the antibacterial properties of the constructed 3D-printed scaffolds, this study conducted co-culture antibacterial experiments, biofilm inhibition assessments, live-dead staining, and scanning electron microscopy observations against two typical pathogens-*Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) (**Fig. 3**). In **Fig. 3(A)**, after co-culture of each scaffold with *E. coli* and *S. aureus* for 24 h, the bacterial broth was plated on LB agar plates and observed for colony growth. The results showed that the DexMA, DexMA@P, and DexMA@P+NIR groups exhibited dense colony growth, similar to that of the control group, indicating no significant antibacterial activity. In contrast, the DexMA@P@CM group exhibited significantly fewer colonies, while the DexMA@P@CM+NIR group exhibited almost no colony formation, demonstrating the scaffold's potent antibacterial properties under light irradiation. Quantitative analysis results (**Fig. 3(B), (C)**) showed that the DexMA@P@CM group exhibited approximately 90.56% and 90.12% inhibition rates against *E. coli* and *S. aureus*, respectively, while the DexMA@P@CM+NIR group exhibited inhibition rates exceeding 99%, both with statistically significant differences (** $p < 0.001$), confirming the highly effective antibacterial activity achieved through the synergistic photothermal mechanism. **Fig. 3(D)** shows the effects of different scaffold groups on biofilm formation by the two bacterial species. Crystal violet staining revealed that a significant amount of biofilm remained in the pores of the DexMA and DexMA@P groups, while the DexMA@P@CM+NIR group exhibited the weakest staining, with virtually no biofilm remaining. Absorbance measurements (**Fig. 3(E)**) further demonstrated that the DexMA@P@CM+NIR group exhibited significantly lower OD₅₇₀ absorbance than the

other groups, demonstrating its effective inhibitory effect on bacterial biofilm formation. Live/Dead fluorescence staining was used to further analyze bacterial activity (**Fig. 3(F)**), with green representing live bacteria and red representing dead bacteria. The results showed that a large number of green signals were observed in the DexMA and DexMA@P groups, while the DexMA@P@CM+NIR group was dominated by red fluorescence, indicating that a large number of bacteria were killed, demonstrating its strong killing ability against both Gram-negative and Gram-positive bacteria. Scanning electron microscopy results (**Fig. 3(G)**) further supported the antibacterial effect from a morphological perspective. The bacterial cells in the control and DexMA groups were intact and densely arranged; in the DexMA@P@CM+NIR group, *Escherichia coli* showed obvious cell membrane rupture and collapse, and *S. aureus* showed agglomerate dissolution and cell membrane shrinkage, suggesting that the structural integrity of the bacteria was destroyed under photothermal induction, further confirming that its bactericidal mechanism is closely related to membrane damage [44].

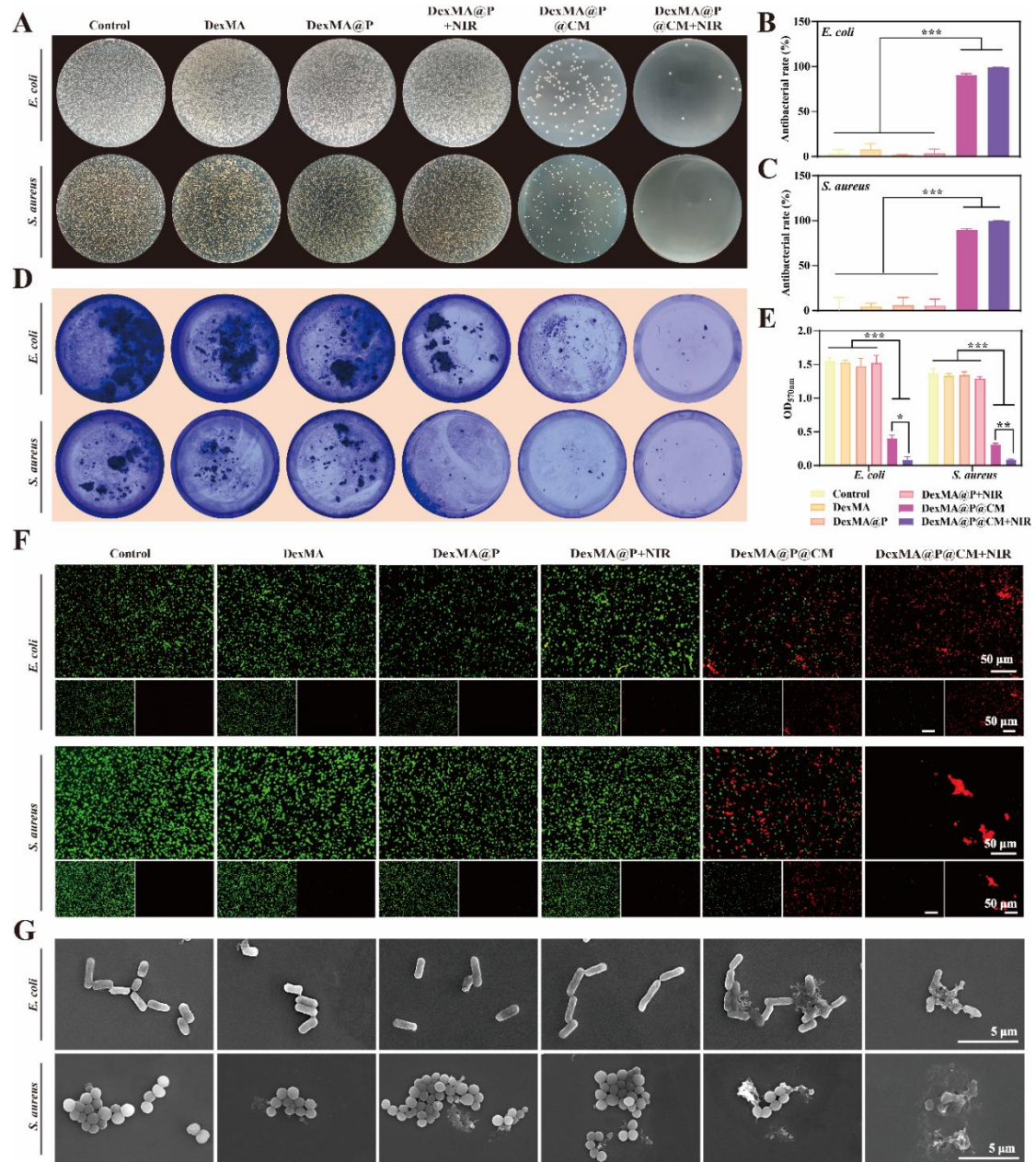


Fig. 3. Evaluation of the antimicrobial properties of 3D-printed scaffolds. (A) Agar plate images after co-culturing scaffolds with *Escherichia coli* and *Staphylococcus aureus*. (B) Quantitative analysis of *Escherichia coli* inhibition rate. (C) Quantitative analysis of *Staphylococcus aureus* inhibition rate. (D) Crystal violet staining images of biofilms. (E) Absorbance measurements (OD₅₇₀) after biofilm dissolution. (F) Fluorescent staining images of live/dead bacteria after co-culturing scaffolds with *Escherichia coli* and *Staphylococcus aureus*. (G) SEM images of bacteria after co-culturing scaffolds with *Escherichia coli* and *Staphylococcus aureus*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4 Photothermal synergistic enhancement of cell activity and immune homeostasis remodeling.

An ideal bone repair scaffold must not only possess excellent cell adhesion and proliferation properties but also be able to modulate the local immune microenvironment and promote the transition from inflammation to repair [45]. Therefore, we systematically evaluated its cytocompatibility and immunomodulatory effects. Live-dead fluorescence staining (**Fig. 4(A)**) showed that after 24, 72, and 120 h of culture, all groups supported the survival of BMSCs. However, the DexMA@P@CM+NIR group showed a significant increase in cell density and more uniform cell morphology, suggesting that the composite scaffold promoted cell proliferation under the synergistic effect of photothermal therapy [46]. Quantitative cell viability analysis (**Fig. 4(B), (C)**) further confirmed this trend: at 72 and 120 h, the viability of HUVECs and BMSCs in the DexMA@P@CM and DexMA@P@CM+NIR groups was significantly higher than that in the control group ($***p < 0.001$), demonstrating the material's excellent cytocompatibility and its ability to enhance cell proliferation through the photothermal effect. Scaffold fluorescence staining results (**Fig. 4(D)**) showed that in the DexMA@P@CM+NIR group, the BMSC scaffold structure was intact and expanded, with significantly enhanced cell adhesion. This characteristic has positive implications for subsequent osteogenic differentiation and matrix deposition.

Cell migration experiments further revealed the scaffold's ability to promote repair. Wound healing experiments (**Fig. 4(E), (G)**) showed that the DexMA@P@CM+NIR group had the fastest wound closure, achieving near-complete healing after 36 h. Transwell assays (**Fig. 4(F), (H)**) also demonstrated that this group exhibited the highest number of cells migrating to the lower chamber, significantly higher than the other groups ($***p < 0.001$). These results demonstrate that DexMA@P@CM significantly enhances the migration of HUVECs and BMSCs under photothermal treatment, providing favorable conditions for angiogenesis and bone tissue regeneration.

Regarding immune regulation, qPCR results (**Fig. 4(I)**) showed a significant upregulation of the anti-inflammatory cytokine IL-10 and a significant decrease in the pro-inflammatory cytokine IL-1 β in the DexMA@P@CM+NIR group ($***p < 0.001$), demonstrating that the scaffold effectively inhibited inflammatory responses. iNOS/CD206 ratio analysis (**Fig. 4(J)**) further demonstrated a decrease in the proportion of pro-inflammatory M1 macrophages and a significant increase in the proportion of anti-inflammatory M2 macrophages in the DexMA@P@CM+NIR group. Immunofluorescence staining results (**Fig. 4(K)**) were consistent with this finding, demonstrating an increase in the M2 marker CD206 signal and a decrease in the M1

marker iNOS signal. Flow cytometry analysis (**Fig. 4(L), (M)**) provided further evidence: in the DexMA@P@CM+NIR group, the proportion of M2 macrophages was the highest (approximately 19.47%), while the proportion of M1 macrophages was the lowest, indicating that the scaffold reshapes the immune microenvironment by promoting M2 polarization and inhibiting M1 polarization [47]. These results clearly reveal that the composite scaffold can promote the transformation of macrophages to M2 type through photothermal effect and synergy with functional components, thereby reshaping the local immune microenvironment [44].

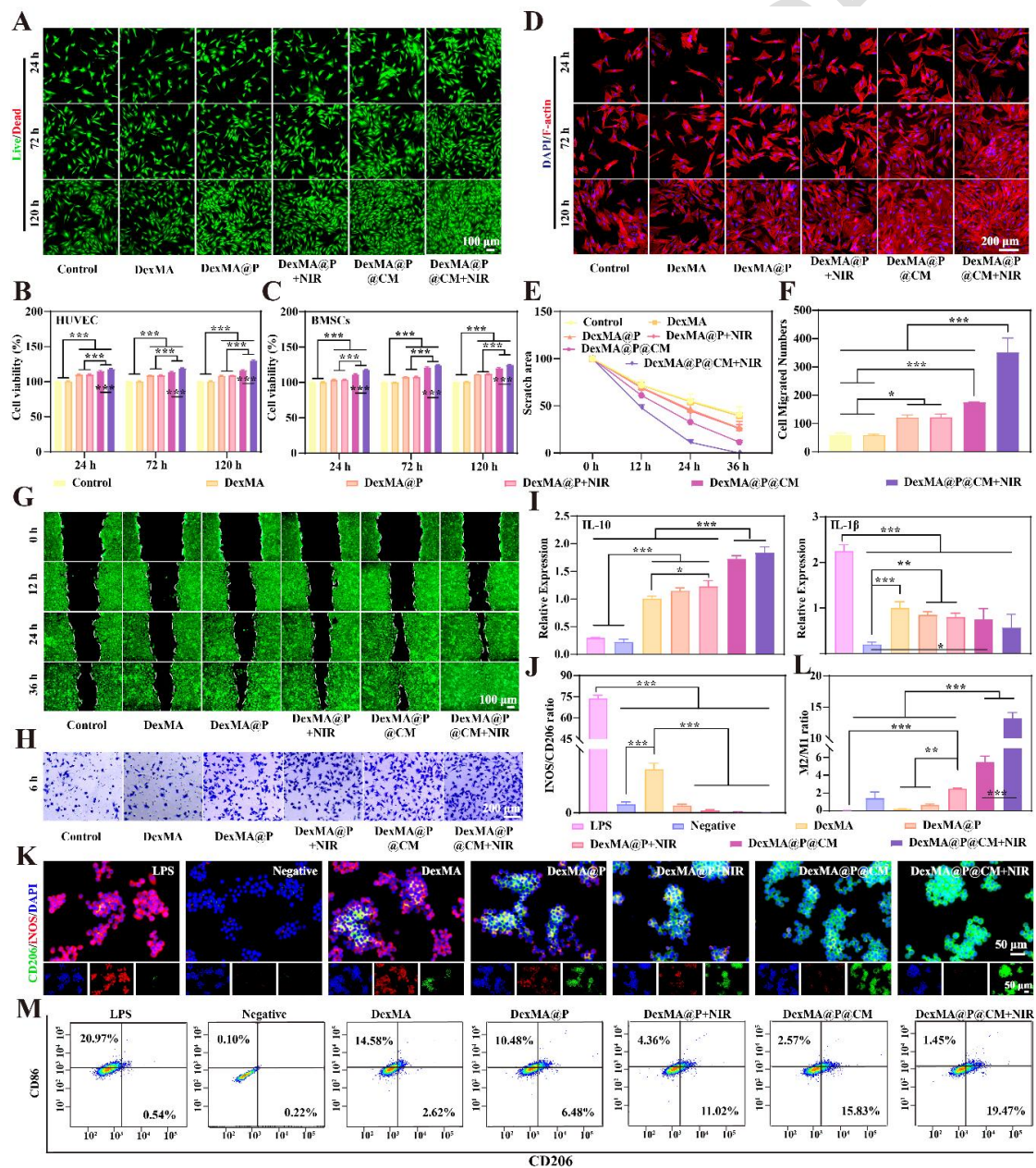


Fig. 4. Evaluation of cell compatibility and immunomodulatory properties of 3D-printed composite scaffolds. (A) Fluorescent staining images of BMSCs at different time points (24, 72, 120 h). (B) Cell viability of HUVECs at different time points. (C) Cell viability of BMSCs at different time points. (D) Fluorescent staining images of the cytoskeleton of BMSCs at different time points. Quantitative analysis of HUVEC (E) cell scratch area at different time points; (G) cell migration images. (F) Number of cells migrating into the lower chamber in the Transwell assay. (H) Transwell images after 6 h of interaction between hydrogel and HUVECs. (I) mRNA expression levels of anti-inflammatory factor IL-10 and pro-inflammatory cytokine IL-1 β . (J) Quantitative analysis of iNOS/CD206 expression. (K) Immunofluorescence staining images of iNOS and CD206. (L) Quantitative analysis of M1/M2 ratio via flow cytometry. (M) Flow cytometry results for CD86 and CD206. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.5 Verification of angiogenesis and osteogenesis functions regulated by photothermal synergy.

In complex microenvironments such as diabetic bone defects, angiogenesis and bone regeneration often exhibit synchronously coupled processes [48]. Constructing functional scaffolds with dual regulatory capabilities is crucial for tissue regeneration [49]. To systematically validate the synergistic effects of the DexMA@P@CM composite scaffold on angiogenesis and osteogenesis, this study conducted a multi-dimensional assessment at the cellular functional and molecular mechanistic levels. As shown in **Fig. 5**, in the tube formation experiment (**Fig. 5(A)**), HUVECs formed only limited tubular networks in the DexMA and DexMA@P scaffolds. However, in the DexMA@P@CM group, the tubular structure was more complex and branched, and the network formation ability was significantly enhanced, especially after near-infrared (NIR) light irradiation. Quantitative results further confirmed that this group significantly surpassed the other groups in terms of node number, total tube length, and number of branch points (**Fig. 5(B)(C)(D)**), indicating that the introduction of Cu₂O@MXene and photothermal stimulation synergistically promoted angiogenesis. To further explore the underlying mechanism, immunofluorescence analysis of angiogenesis-related factors was performed. Results showed that the expression levels of CD31, VEGF, and its receptor VEGFR2 were significantly elevated in the DexMA@P@CM+NIR group (**Fig. 5(E)-(H)**), suggesting that the composite scaffold may promote endothelial cell function by activating the VEGF/VEGFR2 signaling pathway. Furthermore, Western blot analysis revealed that the DexMA@P@CM+NIR group had the highest expression of CD31 and VEGF proteins (**Fig. 5(O)(P)**), further supporting its pro-angiogenic ability. This phenomenon may be attributed to the following factors: First, the Cu₂O component is converted into a localized warm stimulus under NIR irradiation, enhancing endothelial cell activity and migration [50];

second, the released Cu^{2+} acts as a pro-angiogenic signaling factor, inducing upregulation of VEGF expression [51]; and third, the MXene layer improves the surface charge distribution and hydration properties of the scaffold, thereby enhancing cell adhesion and proliferation [52].

In terms of osteogenesis, ALP staining and enzyme activity assays (**Fig. 5(I)(J)**) demonstrated that the DexMA@P@CM+NIR group significantly induced early osteogenic differentiation of BMSCs under illumination, as evidenced by enhanced ALP activity. Alizarin Red S staining and quantification of mineral deposition (**Fig. 5(K)(L)**) revealed that the DexMA@P@CM scaffold induced the most calcium nodule formation under illumination, reflecting its promotion of late osteogenesis. This effect may be related to the following factors: the HAP component continuously releases calcium and phosphate ions, providing a substrate for bone matrix deposition [53]; and the NIR-induced temperature increase and dynamic changes in intracellular Ca^{2+} , which activate osteogenesis-related transcriptional pathways [54]. Further immunofluorescence staining to assess the expression of osteogenic marker proteins (**Fig. 5(M)(N)**) revealed significantly enhanced fluorescence intensity of RUNX2 and OCN in the DexMA@P@CM+NIR group, closely correlating with the level of osteogenic differentiation. Western blotting analysis further confirmed significantly elevated expression levels of key osteogenic proteins, including COL-I, β -catenin, RUNX2, ALP, and OCN (**Fig. 5(Q)(R)**), suggesting that the composite scaffold may synergistically promote bone formation by activating the Wnt/ β -catenin pathway. Overall, the DexMA@P@CM composite scaffold exhibited significant effects on both angiogenesis and osteogenic differentiation, with the effects being even more pronounced under NIR illumination. This mechanism involves the coordinated regulation of multiple factors, including photothermal effects, functional ion release, and activation of cell signaling pathways.

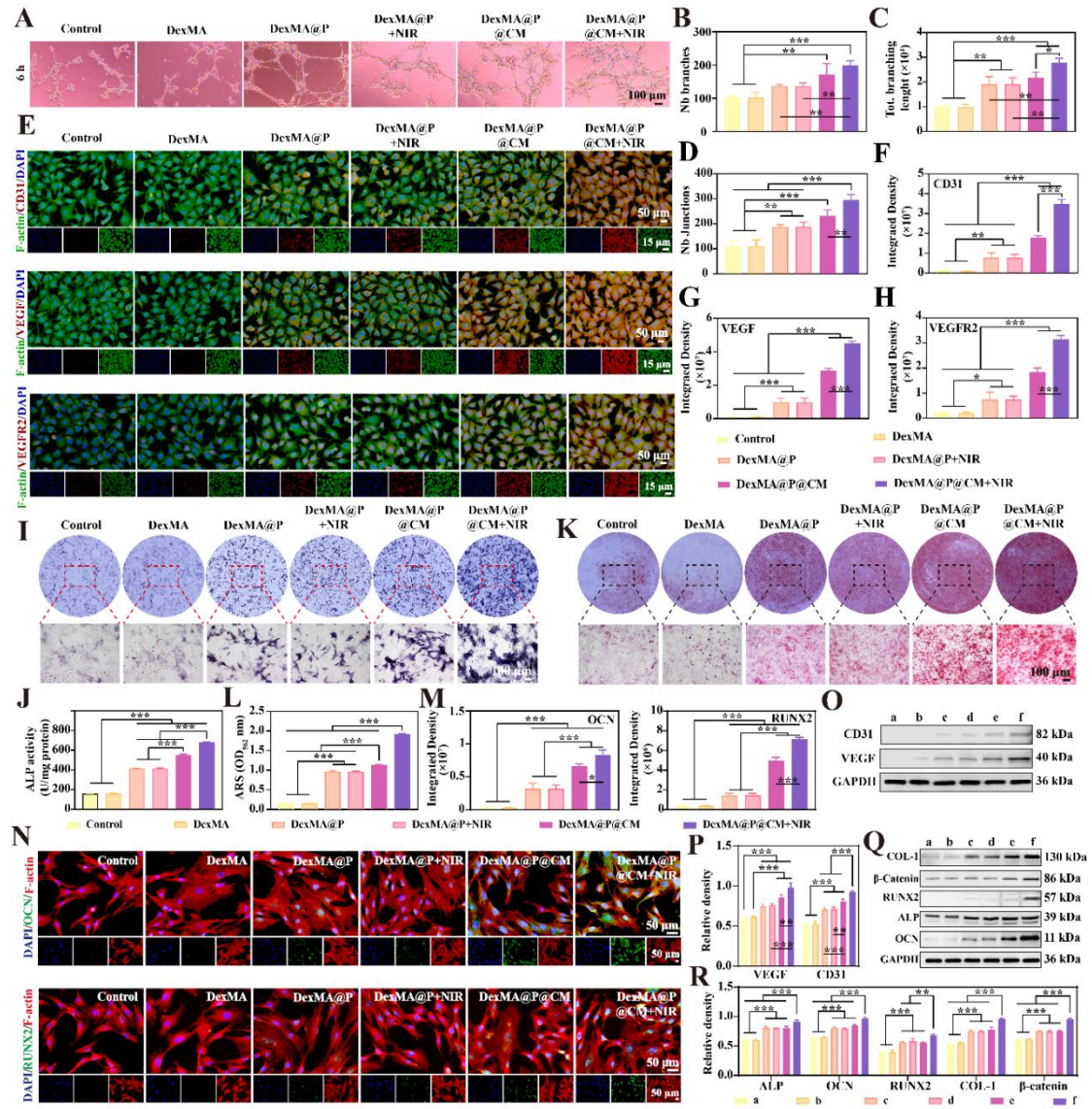


Fig. 5. Evaluation of the angiogenesis and osteogenic properties of 3D-printed composite scaffolds. (A) Representative images of HUVECs after 6 h of tube formation under different scaffold conditions. (B) Quantitative analysis of number of branches, (C) total length, and (D) number of junctions. (E) Immunofluorescence staining images of CD31, VEGF, and VEGFR2 after the scaffolds were applied to HUVECs. (F–H) Quantitative analysis of fluorescence intensity of angiogenesis-related proteins CD31, VEGF, and VEGFR2. (I, J) ALP staining images and quantitative analysis of ALP enzyme activity. (K) Alizarin Red S staining images. (L) Quantitative analysis of absorbance values after ARS dye extraction corresponding to the Alizarin Red staining images. (M, N) Immunofluorescence staining images of the osteogenesis-related proteins OCN and RUNX2 and quantitative analysis of mean fluorescence intensity. (O) Western blot analysis of the tube formation markers CD31 and VEGF. (P) Quantitative analysis of CD31 and VEGF expression levels corresponding to the Western blot results. (Q) Western blot analysis of osteoblast markers COL-I, β -catenin, RUNX2, ALP, and OCN. (R) Quantitative analysis of relative grayscale values of COL-I, β -catenin, RUNX2, ALP, and OCN protein expression (Among them, a in (O)(P)(Q)(R) is Control, b is DexMA, c is DexMA@P, d is DexMA@P+NIR, e is DexMA@P@CM, f is DexMA@P@CM+NIR).

and f is DexMA@P@CM+NIR.). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.6 Molecular regulatory mechanisms mediated by 3D-printed composite scaffolds.

To further elucidate the underlying mechanisms of the composite scaffold's promotion of osteogenesis, angiogenesis, and immune regulation, we performed transcriptome sequencing analysis on BMSCs after scaffold treatment to comprehensively analyze the gene expression remodeling and pathway regulation characteristics induced at the transcriptional level. Principal component analysis (PCA, **Fig. 6(A)**) revealed a clear separation between the control and DexMA@P@CM+NIR groups along the PC1 and PC2 dimensions, reflecting significant differences in global gene expression patterns following material intervention. A Venn diagram (**Fig. 6(B)**) revealed 11,980 genes shared between the two groups, of which 166 genes were specifically expressed in the DexMA@P@CM+NIR group, suggesting that the scaffold induces specific transcriptional regulatory programs. Furthermore, a volcano plot (**Fig. 6(C)**) further identified 71 significantly upregulated and 274 significantly downregulated differentially expressed genes (DEGs). The upregulated genes, such as *Cxcl1*, *Mmp9*, and *Mmp13*, are closely associated with immune chemotaxis, matrix remodeling, and osteogenesis, demonstrating the scaffold's ability to reprogram the microenvironment [55]. Inter-sample correlation analysis (**Fig. 6(D)**) and a heatmap of differentially expressed genes (**Fig. 6(E)**) confirmed the good reproducibility of the data and systematic changes at the genomic level across the intervention groups. Notably, the expression of multiple osteogenesis-related transcription factors (such as *Runx2* and *Sp7*) and angiogenesis regulators (such as *Vegfa* and *Flt1*) was significantly upregulated in the intervention group, suggesting that the material promotes tissue regeneration by synergistically activating bone-vascular coupling pathways [56]. GO enrichment analysis (**Fig. 6(F)**) further revealed that DexMA@P@CM+NIR significantly impacted multiple key biological processes, including positive regulation of MAPK signaling, Wnt signaling, bone tissue development, cell-cell adhesion, and inflammatory response regulation. This suggests that the material intervention has broad regulatory capabilities in inducing stem cell fate determination, maintaining microenvironmental immune homeostasis, and matrix remodeling. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment results (**Fig. 6(G)**) also support this view, with differentially expressed genes concentrated in signaling pathways closely related to stem cell activity, osteogenic differentiation, and immune regulation, such as PI3K-Akt, MAPK, Wnt, TGF- β , VEGF, and NF- κ B. Further validation of key functional pathways using GSEA enrichment analysis (**Fig. 6(H)–(K)**) revealed that DexMA@P@CM+NIR significantly activated the VEGF signaling

pathway ($p=0.0488$), Wnt signaling pathway ($p=0.0436$), and PI3K-Akt signaling pathway ($p=0.0336$), while inhibiting the NF- κ B signaling pathway ($p=0.0237$). This further confirms that the scaffold material may exert its therapeutic advantages in angiogenesis, osteogenic and inflammatory regulation through multiple synergistic signaling pathways. Notably, the Wnt and PI3K-Akt signaling pathways play a core regulatory role in promoting MSC osteogenic differentiation, while VEGF and NF- κ B respectively dominate angiogenesis and immune environment remodeling. The biological functions exhibited by these two pathways were also confirmed at the molecular level [57-60].

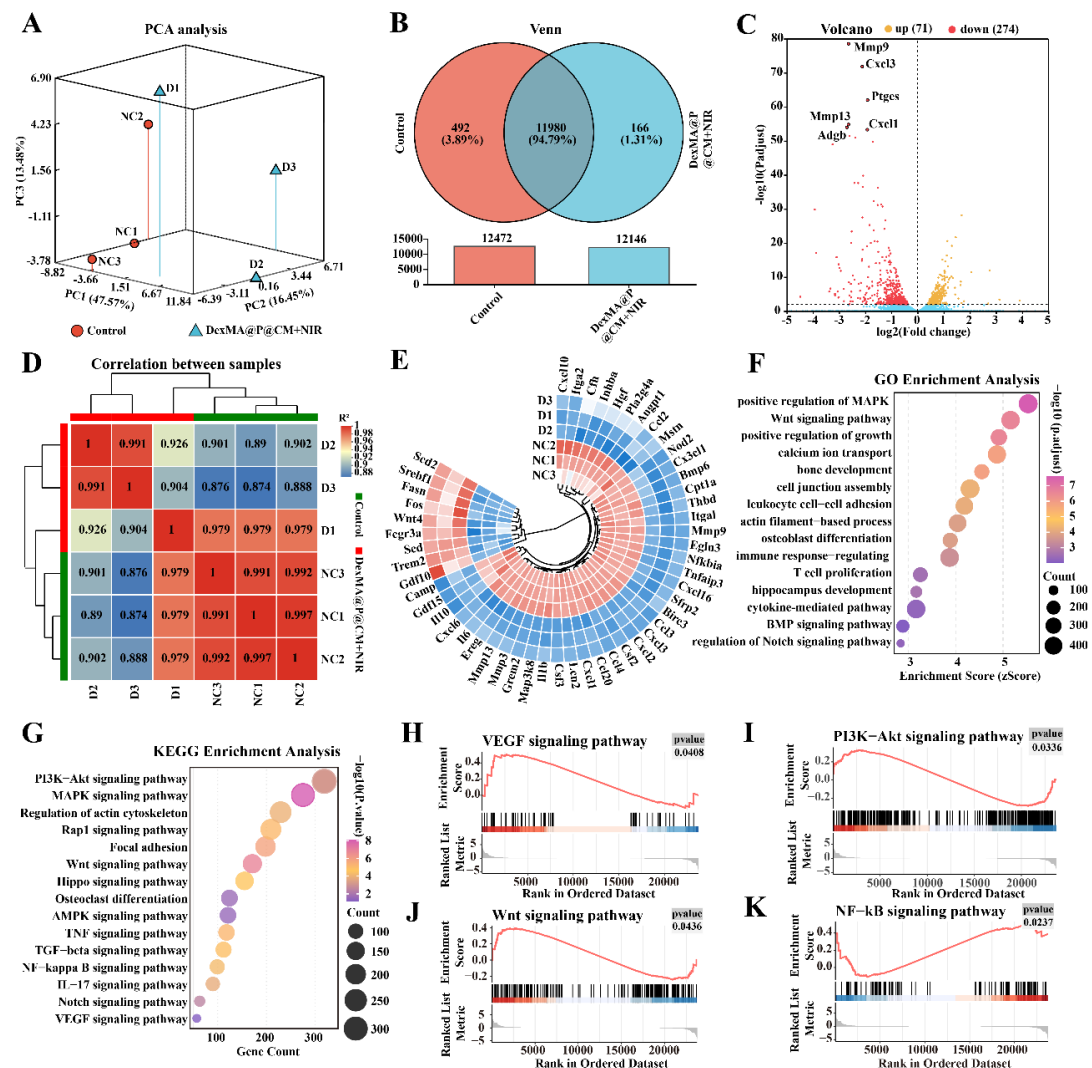


Fig. 6. Transcriptome sequencing reveals the molecular regulatory mechanism of the DexMA@P@CM+NIR scaffold in BMSCs. (A) Principal component analysis (PCA) plot. (B) Venn diagram of gene expression intersection and specificity distribution between the two groups. (C) Volcano plot of significantly differentially expressed genes (DEGs) between the two groups, with red indicating

downregulated genes, yellow indicating upregulated genes, and gray indicating non-differential genes. (D) Expression correlation heatmap. (E) Circular clustering heatmap of differentially expressed genes. (F) GO enrichment analysis. (G) KEGG pathway enrichment. GSEA enrichment analysis of the (H) VEGF signaling pathway, (I) PI3K-Akt signaling pathway, (J) Wnt signaling pathway, and (K) NF- κ B signaling pathway.

3.7 Establishing a diabetic infectious skull defect Model.

In previous in vitro experiments, we have demonstrated that the DexMA@P@CM composite scaffold possesses excellent osteoinduction, angiogenesis, and immunomodulatory capabilities at the cellular level. However, infected bone defects are often accompanied by persistent bacterial invasion and inflammatory microenvironmental disturbances, which significantly hinder bone tissue regeneration [61]. Therefore, achieving both antibacterial and osteogenic repair in vivo is crucial. To this end, this study systematically evaluated the bone regeneration and antibacterial properties of the DexMA@P@CM+NIR scaffold in an established rat infected skull defect model (**Fig. 7**). Micro-CT three-dimensional reconstruction images (**Fig. 7(A)**) showed that at 6 and 12 weeks post-surgery, the DexMA@P@CM+NIR group exhibited extensive, dense, and continuous new bone formation within the defect area, while the control and DexMA groups exhibited significantly less new bone mass and incomplete bone bridging. Quantitative analysis (**Fig. 7(B)**) further confirmed that the bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N) in the DexMA@P@CM+NIR group reached their highest levels at week 12, suggesting that the scaffold effectively promotes bone defect repair and bone microarchitecture remodeling. In an infectious environment, antibacterial properties are a prerequisite for successful bone repair [62]. Plate experiments (**Fig. 7(C)**) showed that the DexMA and DexMA@P groups had little inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*, while the DexMA@P@CM+NIR group virtually eliminated the bacterial colonies, demonstrating a near-complete bactericidal effect. This result is closely related to its photothermal properties. Infrared thermal imaging (**Fig. 7(D)**) showed that under 808 nm laser irradiation, the surface temperature of the DexMA@P@CM+NIR group gradually increased and reached a peak of approximately 51.2°C after 5 min of NIR irradiation, providing a short-term thermal condition for bacterial inactivation. However, because thermal effects depend on the complete temperature–time history and tissue-specific sensitivity, the safety of this irradiation protocol for surrounding tissues requires further evaluation. This demonstrates that the efficient photothermal conversion capability of Cu₂O@MXene not only enables rapid local sterilization but also improves local blood flow and cell activity through

microthermal stimulation, creating a favorable microenvironment for bone regeneration.

Hematoxylin and eosin (HE) and Masson staining further verified bone tissue regeneration and structural remodeling (**Fig. 7(E)**). Six weeks after surgery, significant new bone islands and collagen deposition were observed in the defect area of the DexMA@P@CM+NIR group, suggesting early initiation of bone repair. By week 12, the area of new bone in this group had further increased, integrating well with the host bone, and the medullary cavity structure was gradually restored. In contrast, the control group and the single-component group still had large areas of unhealed bone and significant residual fibrous tissue, indicating limited repair capacity. Further analysis using Goldner trichrome staining (**Fig. 7(F)**) revealed that the DexMA@P@CM+NIR group showed a significant increase in green mineralized bone area at 6 weeks, while at 12 weeks the mineralized area almost covered the entire defect area, accompanied by significant trabecular remodeling and bone plate formation. This indicates that the system not only promotes early osteogenesis but also continuously drives the later mineralization maturation process. Combining the antibacterial and photothermal results, it can be inferred that the DexMA@P@CM+NIR composite scaffold works synergistically through the dual mechanisms of "photothermal sterilization + osteogenesis promotion" in an infected environment: on the one hand, antibacterial and infection control are achieved through photothermal effect and Cu^{2+} release, reducing the adverse effects of the inflammatory microenvironment [44]; on the other hand, relying on the mineralization environment provided by PDA-HAP and the signal regulation of MXene, it promotes the activation of osteogenesis-related pathways (such as Wnt/ β -catenin and TGF- β), thereby accelerating bone tissue reconstruction [63].

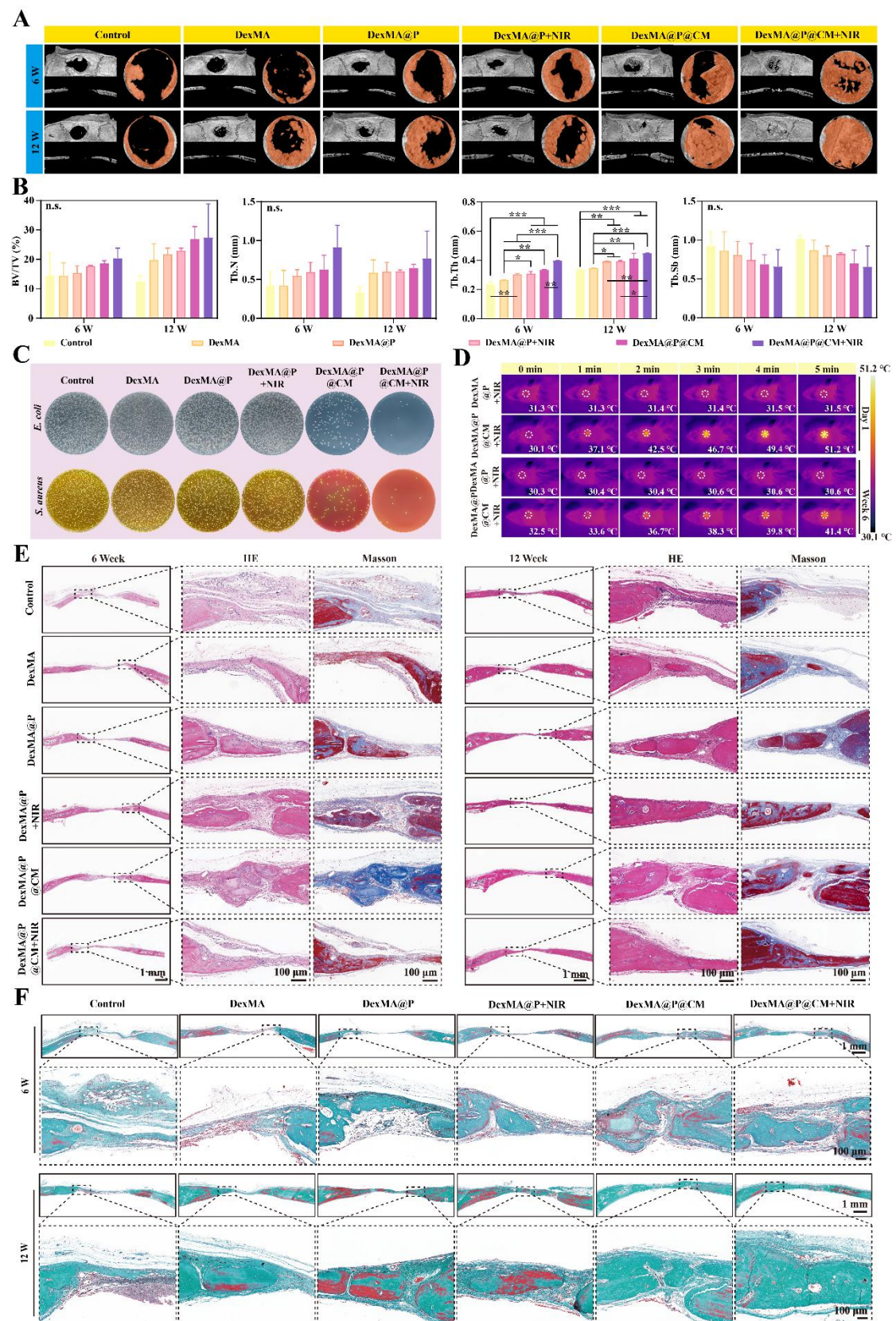


Fig. 7. Composite scaffolds promote bone reconstruction in an infectious bone defect model. (A) Micro-CT reconstructed image; the orange area represents newly formed bone tissue. **(B)** Quantitative

analysis of the corresponding Micro-CT images, including statistical comparisons of bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp). (C) Antibacterial activity of the scaffold against two typical bacteria (*E. coli* and *S. aureus*) in a rat infection model, expressed as colony-forming units (CFUs) on the plates of each group. (D) Thermal imaging of different scaffolds under near-infrared laser irradiation (808 nm, 1.0 W/cm²). (E) HE and Masson staining images of the injury site after 6 and 12 weeks of composite scaffold treatment. (F) Goldner stained images of the injury site 6 and 12 weeks after composite stent treatment. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

3.8 Longitudinal analysis of repair outcomes *in vivo*

In the microenvironment of infected bone defects, persistent bacterial colonization and the release of toxins such as lipopolysaccharide (LPS) can chronically activate the innate immune response, leading to persistent overactivation of the NF- κ B inflammatory pathway and resulting in the excessive secretion of inflammatory factors such as IL-1 β , IL-6, and TNF- α [64, 65]. These inflammatory factors not only directly inhibit the differentiation of bone marrow mesenchymal stem cells (BMSCs) into the osteogenic lineage but also damage vascular endothelial cell function, forming a vicious cycle of "inflammation-osteogenic inhibition-vascular degeneration [66]." Therefore, without effective control of the infection and inflammatory microenvironment, simply providing scaffold support is insufficient to achieve effective bone regeneration. This study first evaluated the ability of different materials to regulate osteogenic differentiation using Osterix (OSX) immunofluorescence. As shown in **Fig. 8(A)(B)**, at 12 weeks post-surgery, the number of OSX-positive cells in the defect area of the DexMA@P@CM+NIR group was significantly increased, while the Control and non-combined groups showed only scattered weak expression. The fundamental reason for this phenomenon lies in the fact that the DexMA@P@CM+NIR system effectively reduces the local bacterial load through photothermal antibacterial action, thereby weakening the inhibitory effect of inflammatory signals on osteoblast differentiation at its source [44]. Previous studies have shown that Runx2-OSX axis signaling is inhibited in a high IL-1 β /TNF- α microenvironment [67]. This system, by reducing inflammatory factor levels, indirectly restores and enhances the activity of the Runx2/OSX osteogenic differentiation pathway, leading to a significant upregulation of Osterix expression. This also explains why, although DexMA@P@CM or DexMA@P+NIR, when used alone, can partially improve the microenvironment, their osteogenic induction ability is still significantly weaker than that of the photothermal + antibacterial dual synergistic system.

Furthermore, COL-I immunofluorescence results (**Fig. 8 (C)(D)**) showed that the

DexMA@P@CM+NIR group exhibited the strongest type I collagen deposition at both 6 and 12 weeks. Type I collagen is a core structural protein in bone matrix formation, and its expression level reflects the functional output capacity of osteoblasts [68]. Its significant upregulation was not the result of a single osteogenic stimulus, but rather stemmed from a synergistic dual mechanism: on the one hand, the relief of the inflammatory microenvironment reduced the inhibitory effect of inflammatory factors on osteoblast ECM synthesis; on the other hand, the DexMA-based hydrogel provided a favorable microenvironment for cell adhesion and support, and enhanced cell-matrix interaction through CM modification, thereby further increasing the matrix secretion activity of osteoblasts [11, 69]. As the time increased from 6 to 12 weeks, COL-I changed from localized enhancement to a large-scale uniform distribution, indicating that this system not only promoted early bone matrix deposition but also effectively promoted the transformation of new bone from woven bone to mature lamellar bone. Angiogenesis is another key bottleneck in the repair of infected bone defects. CD31 immunofluorescence results (**Fig. 8 (E)(F)**) showed that the DexMA@P@CM+NIR group had formed a large number of new blood vessels at 6 weeks, which were further enhanced at 12 weeks, showing a continuous distribution of tubular structures and significant co-localization with bone formation areas. This result indicates that the system effectively activated angiogenesis during bone repair, and angiogenesis is considered a key basis for the supply of oxygen, nutrients and osteogenic signaling molecules, playing a decisive supporting role in the osteogenic process [70]. This reflects that the scaffold achieved good angiogenesis-osteogenic coupling regulation capability *in vivo*.

At the level of inflammation regulation, the immunohistochemical results of IL-1 β , IL-6, and TNF- α (**Fig. 8 (G)(H)**) further confirm the above inferences mechanistically. The DexMA@P@CM+NIR group showed the lowest positive area at both time points, and its reduction was significantly better than other groups. In particular, it could significantly inhibit the inflammatory cascade response in the early 6 weeks. Combining the aforementioned photothermal antibacterial properties and the functions of the material components, it can be inferred that this scaffold achieves local photothermal sterilization under near-infrared excitation, effectively reducing the bacterial load in the defect area, weakening the continuous activation of the Toll-like receptor/NF- κ B inflammatory signaling axis from the source, thereby reducing the expression levels of IL-1 β , IL-6, and TNF- α , and alleviating the inhibitory effect of the inflammatory microenvironment on osteoblast differentiation and angiogenesis.

Compared with existing bone-repair scaffolds, the present system was specifically designed for the infected diabetic microenvironment. Metallic and PEEK implants provide reliable mechanical support but generally lack intrinsic antibacterial and

regenerative activity, whereas bioceramic scaffolds are primarily osteoconductive and have limited capacity to regulate infection and inflammation [71, 72]. Cell- or growth-factor-loaded scaffolds may enhance regeneration but face challenges in biological stability and clinical translation [73]. In contrast, the DexMA/P-HAP/Cu₂O@MXene scaffold integrates customizable 3D printing with staged photothermal antibacterial treatment, osteogenic induction, immunomodulation, and angiogenic stimulation. In particular, sequential temperature control at 50°C and 41°C enables early infection control followed by mild photothermal stimulation of tissue regeneration, distinguishing this system from conventional single-temperature photothermal scaffolds.

Importantly, the scaffold was designed as a cell-free platform intended to support in situ regeneration through its porous structure and the delivery of osteogenic, immunomodulatory, and angiogenic signals. However, its efficacy was evaluated solely in a rat calvarial defect model featuring a simple geometry and a 5 mm diameter, a setup that failed to adequately replicate the actual clinical conditions of diabetic bone defects regarding size, thickness, mechanical environment, or chronic pathological characteristics. Furthermore, the study did not directly track the origins of infiltrating host cells or their distribution patterns relative to depth. Additionally, the specific contribution of the 3D-printed structure to tissue regeneration remains unclear, as no cast scaffolds of identical composition were included as controls. Therefore, future research will compare printed scaffolds with cast scaffolds, track host cell infiltration, evaluate the long-term biosafety of Cu₂O@MXene and near-infrared (NIR)-induced heat generation, and validate regenerative efficacy in large-animal models of critical-sized bone defects.

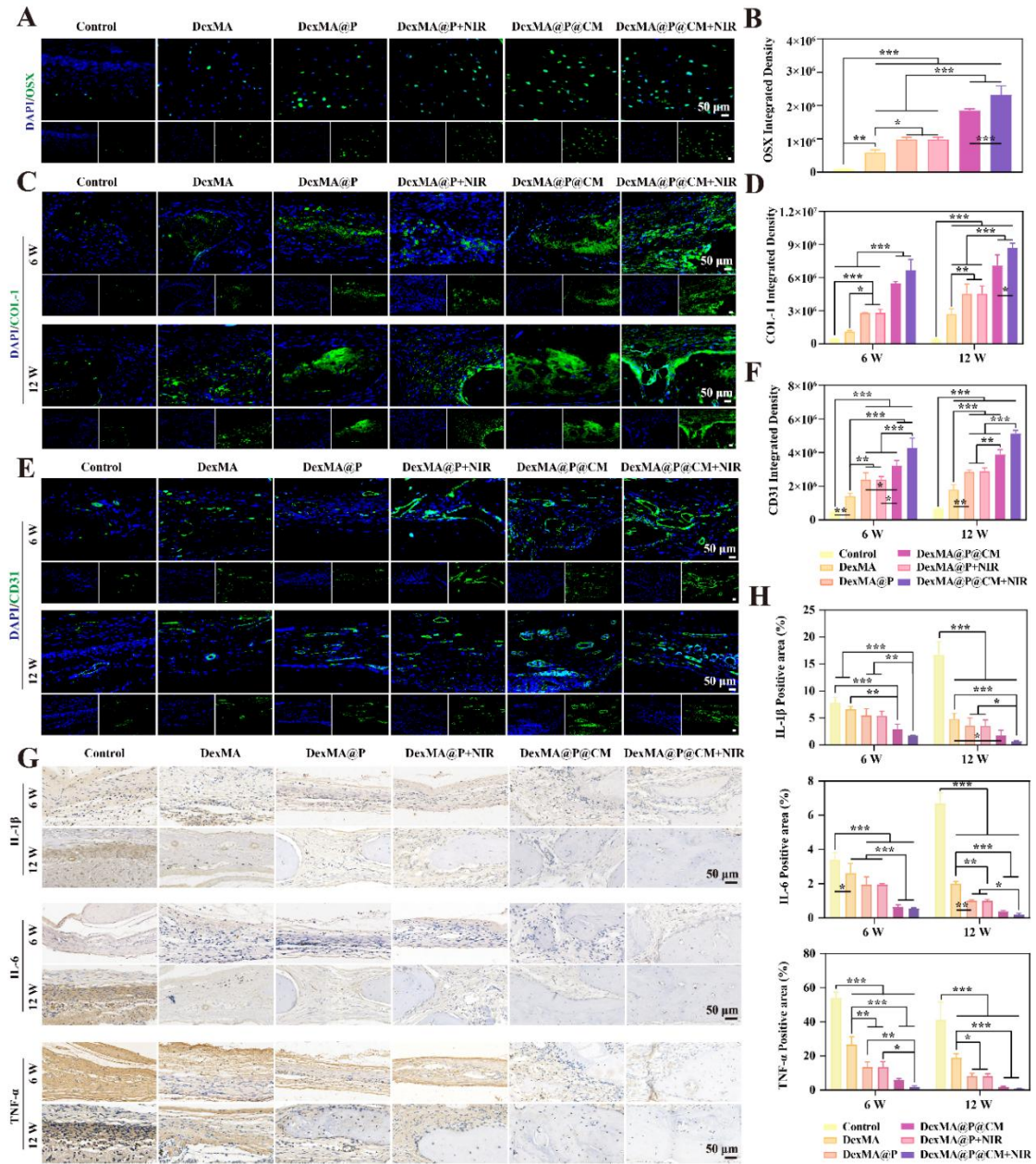


Fig. 8. *In vivo* evaluation of osteogenesis, angiogenesis, and inflammatory modulation at different time points post-implantation. (A) OSX immunofluorescence staining image of the defect area at 12 weeks postoperatively. (B) Quantitative analysis of the integrated density of OSX positive signal in each group at 12 weeks. (C) Col-I immunofluorescence staining images of the defect area at 6 and 12 weeks postoperatively. (D) Quantitative statistical results of Col-I fluorescence integrated density at different time points. (E) CD31 immunofluorescence staining images of the defect area at 6 and 12 weeks postoperatively. (F) Quantitative analysis of the integrated density of CD31 positive signal in each group. (G) Immunohistochemical staining images of inflammatory factors (IL-1 β , IL-6, and TNF- α) in the defect area at 6 and 12 weeks postoperatively. (H) Quantitative analysis of the proportions of IL-1 β , IL-6, and TNF- α positive areas in different groups at 6 and 12 weeks. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Conclusion

This study designed and constructed a DexMA/P-HAP/Cu₂O@MXene composite scaffold that combines structural printability, bioactivity, and intelligent responsiveness to address the multiple challenges of repairing diabetic-associated infected bone defects. The scaffold utilizes DexMA to construct a three-dimensional framework, exhibiting excellent mechanical stability and formability. PDA-modified hydroxyapatite (P-HAP) enhances the inorganic-matrix bonding and osteogenic induction. The Cu₂O@MXene nanoplateform achieves a near-infrared-triggered dual-temperature photothermal response, enabling sequential switching between sterilization (50°C) and osteogenic regulation (41°C). The synergistic release of Cu²⁺ ions contributes to its multiple functions: antibacterial, angiogenic, and immunomodulatory. Mechanism studies have shown that the scaffold can significantly clear ROS *in vitro*, inhibit the expression of inflammatory factors, induce macrophage polarization to M2 type, and activate VEGF signaling *in vivo* to promote angiogenesis. At the same time, it upregulates the expression of bone markers such as OSX and Col-I, accelerates new bone formation and defect repair, and constructs a "photothermal-immune-vascular-osteogenesis" multi-pathway synergistic reconstruction mechanism. In the complex environment of diabetes, the scaffold exhibits excellent anti-infection, osteogenesis and tissue integration capabilities. Collectively, this modular scaffold provides a promising acellular strategy for treating diabetic infected bone defects. It can be further expanded into a precise treatment carrier that can be dynamically monitored and regulated by integrating drug sustained-release systems or biosensor elements, providing new strategic support for bone regeneration treatment related to diabetes and other chronic diseases.

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Conflict of interest

The authors declare no competing financial interest.

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Funding acquisition: Lin Zhou

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Ethics approval and consent to participate

This study adhered to the Principles of Laboratory Animal Care (National Institutes of Health Publication No. 85-23, revised 1996) and was approved by the National Institutional Animal Care Committee. The animal ethics approval number is 202501007.

Consent for publication

Not applicable.

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Appendix

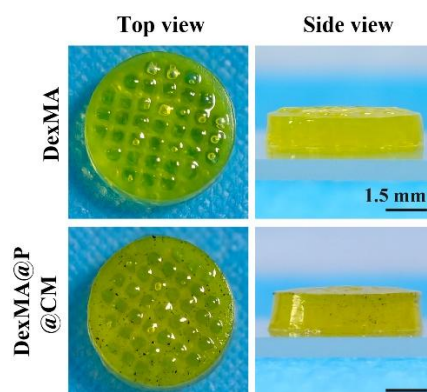


Fig. A1. Representative macroscopic morphology of the fabricated scaffolds. Top and side views of the DexMA and DexMA@P@CM scaffolds fabricated by DLP-based vat photopolymerization. The cylindrical scaffolds were designed with dimensions of 5.0 mm $\times$ 5.0 mm $\times$ 1.5 mm (length $\times$ width $\times$ height). Scale bars: 1.5 mm.