

ACCEPTED PAPER · MAIN TEXT

TGP-377 nanoparticle-loaded HAMA hydrogel-infused PLGA/ β -TCP composite scaffold enhances early bone repair with angiogenesis-related responses

Paper version: Accepted Paper

Accepted Papers are manuscripts accepted for publication, encompassing all changes made following the peer review process, along with a standard cover page indicating the paper version and an “Accepted Paper” watermark, but excluding any other editing, typesetting or other changes made by AccScience Publishing and/or authors post-acceptance.

Article ID: IJB026260277

Citation: Sun J, Chen C, Yao C, *et al.* TGP-377 nanoparticle-loaded HAMA hydrogel-infused PLGA/ β -TCP composite scaffold enhances early bone repair with angiogenesis-related responses. *Int J Bioprint.* 2026. doi: 10.36922/IJB026260277

Copyright: © 2026 Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher’s Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

AccScience Publishing

RESEARCH ARTICLE

Volume X Issue X (2026)

doi: 10.36922/IJB026260277

TGP-377 nanoparticle-loaded HAMA hydrogel-infused PLGA/ β -TCP composite scaffold enhances early bone repair with angiogenesis-related responses

Jie Sun ^{1†}, Cao Chen ^{1†}, Chen Yao ^{1†}, Minrui Ji ², Ruize Wei ³, Bo Zhang ¹, Zeping Gui ^{4*}, and Yafeng Zhang ^{1*}

¹ Department of Orthopaedics, Affiliated Hospital of Nantong University, Medical College of Nantong University, Nantong 226001, China

² Hand Surgery Research Center, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Medical School of Nantong University, Nantong 226001, China

³ Medical College of Nantong University, Nantong 226001, China

⁴ Department of Urology, The Second Affiliated Hospital with Nanjing Medical University, Nanjing, China

[†]*These authors contributed equally to this work.*

***Corresponding authors:**

Zeping Gui (gzp@njmu.edu.cn)

Yafeng Zhang (fcus2004@hotmail.com)

Citation: Sun J, Chen C, Yao C, *et al.* TGP-377 nanoparticle-loaded HAMA hydrogel-infused PLGA/ β -TCP composite scaffold enhances early bone repair with angiogenesis-related responses. *Int J Bioprint.* 2026. doi: 10.36922/IJB026260277

Received: June 28, 2026

Revised: July 30, 2026

Accepted: August 5, 2026

Published online: August 5, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Bone defects stemming from trauma, infection, osteoporosis, tumor resection, and congenital or metabolic bone diseases often demand effective strategies for repair. Current bone graft options, such as autologous or allogeneic bone, face limitations related to donor morbidity, immune rejection, and poor osseointegration. Bone tissue engineering, which integrates biocompatibility, osteogenic capacity, and angiogenic potential, therefore offers a promising approach for bone defect repair. This study evaluated the therapeutic potential of a novel composite scaffold, the TGP-377 nanoparticles-loaded HAMA hydrogel-infused PLGA/ β -TCP (THPT) scaffold, in supporting early bone repair and angiogenesis-related responses. The scaffold was designed to provide sustained local release of TGP-377 and to support concurrent osteogenic and angiogenesis-related responses. In vitro, TGP-377 exhibited pronounced osteogenic and angiogenic effects, boosting MC3T3-E1 cell proliferation and osteogenic differentiation, alongside HUVEC proliferation and migration. In a rabbit femoral defect model, THPT increased mineralized tissue-related micro-CT indices and osteogenic and angiogenesis-related marker expression at 4 weeks. Moreover, the scaffold demonstrated favorable biocompatibility, with no pathological changes observed in major organs. These findings support further investigation of THPT as a local bioactive scaffold for early bone-defect repair.

Keywords: Bone defect repair; TGP-377; PLGA/ β -TCP scaffold; Osteogenesis; Angiogenesis

1. Introduction

Bone defects represent common and clinically challenging conditions in orthopedics, trauma surgery, and musculoskeletal oncology, resulting from trauma, infection, osteoporosis, tumor resection, and congenital or metabolic bone diseases¹. Critical-sized bone defects remain difficult to repair because of mechanical instability, insufficient intrinsic regenerative capacity, and prolonged healing. Although autologous bone grafting remains the gold standard for bone repair, its clinical application is limited by donor-site morbidity, restricted graft availability, additional surgical trauma, and postoperative complications². Therefore, the development of artificial bone substitutes integrating biocompatibility, biodegradability, mechanical support, and osteoregenerative activity has become a major focus in bone tissue engineering³⁻⁷.

Bone regeneration is a highly coordinated biological process involving the close interaction between angiogenesis and osteogenesis^{8,9}. Newly formed blood vessels supply oxygen, nutrients, and routes for cell migration into the defect, and reciprocally regulate osteoprogenitor recruitment, osteoblast differentiation, and extracellular matrix deposition through multiple signaling pathways¹⁰. Insufficient vascularization is a major cause of delayed bone healing and poor-quality regeneration^{11,12}. Therefore, designing scaffolds that concurrently support angiogenesis and osteogenesis has become a key objective in bone tissue engineering^{13,14}.

Among current biomaterial systems, polymer/bioceramic composite scaffolds have attracted considerable attention because they combine structural support with biological activity. Poly (lactic-co-glycolic acid) (PLGA) represents a biodegradable synthetic polymer extensively applied in bone repair, owing to its outstanding biocompatibility, adjustable degradation kinetics, and superior processability¹⁵⁻¹⁷. β -Tricalcium phosphate (β -TCP), a representative calcium phosphate ceramic, closely resembles the inorganic phase of natural bone in composition while demonstrating good biocompatibility, osteoconductivity, and osteoinductive potential¹⁸. Incorporating β -TCP into PLGA not only improves the scaffold's mechanical performance and surface bioactivity but also neutralizes the acidic microenvironment produced during PLGA degradation, thereby creating a more favorable interface for bone regeneration^{19,20}. Accordingly, PLGA/ β -TCP (PT) composite scaffolds are regarded as promising candidates for bone defect repair²¹⁻²⁴. However, their intrinsic properties alone are often insufficient to support rapid vascular ingrowth and efficient bone regeneration and efficient bone regeneration, particularly in critical-sized defects^{8,25}.

Recent advances have demonstrated that integrating structural scaffolds with bioactive molecule delivery systems can enhance regenerative outcomes by creating a favorable microenvironment^{26,27}.

Hydrogels have emerged as attractive carriers for controlled delivery because of their extracellular matrix-like characteristics, high water content, and capacity for molecular loading. Among them, methacrylated hyaluronic acid (HAMA) hydrogel exhibits excellent biocompatibility, tunable properties, and potential for sustained local delivery of bioactive agents²⁸⁻³⁰. TGP-377 is a reported small molecule that selectively upregulates VEGFA expression³¹. VEGFA is a central regulator of angiogenesis and also serves a critical function in bone repair by mediating endothelial–osteogenic crosstalk, osteoprogenitor recruitment, and new bone formation^{12,32-34}. Although various angiogenic and osteogenic factors, such as VEGF and BMP-2, have been incorporated into biomaterial systems to enhance bone regeneration, their clinical translation remains limited by several challenges, including high production costs, protein instability, short biological half-life, and potential safety concerns associated with uncontrolled biological activity. TGP-377 represents a different regulatory strategy by stimulating endogenous VEGFA expression rather than directly supplying exogenous growth factors. As a small-molecule regulator, TGP-377 may provide advantages in terms of chemical stability, manufacturing feasibility, and compatibility with controlled-release biomaterial platforms.

In this study, we developed a TGP-377 nanoparticles-loaded HAMA hydrogel-infused PLGA/ β -TCP composite scaffold (THPT) for vascularized bone defect repair. The PT scaffold provides structural support, while the TGP-377-loaded HAMA hydrogel serves as a functional delivery phase to establish a sustained pro-angiogenic microenvironment. We hypothesized that THPT scaffolds could enhance angiogenesis-related responses associated with VEGFA upregulation and support early osteogenic regeneration. To test this hypothesis, the biological effects of TGP-377 were evaluated *in vitro*, and the regenerative potential of THPT scaffolds was assessed using a rabbit femoral bone defect model through micro-CT, histological, and molecular analyses. (Figure 1)

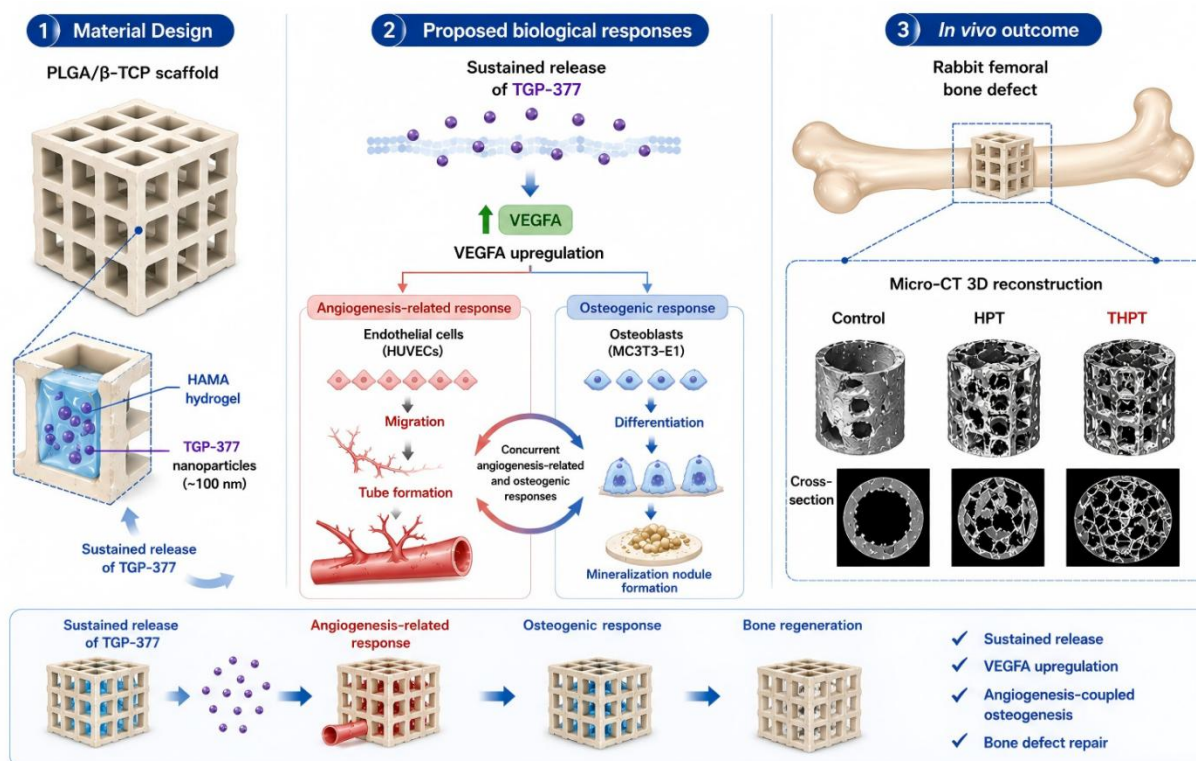


Figure 1. Angiogenesis-coupled osteogenesis mediated by a multifunctional PLGA/ β -TCP scaffold for bone defect repair.

2. Materials and methods

2.1. PLGA/ β -TCP scaffold fabrication and characterization

PLGA (lactide:glycolide molar ratio 65:35, molecular weight 40,000–75,000) underwent dissolution in 1,4-dioxane (Aladdin, China) to yield a 10% (w/v) homogeneous solution. β -TCP powder (Shanghai Bio-lu Biomaterials Co., China) was subsequently incorporated into the PLGA solution at a β -TCP:PLGA mass ratio of 1:4, and the mixture was thoroughly stirred using a magnetic stirrer to obtain a uniform PT paste. A low-temperature rapid-prototyping 3D printer (CLRF-2000-II, Nantong University, China) was employed to fabricate the composite scaffolds at

−30 °C. Upon completion of printing, the scaffolds underwent lyophilization in a freeze dryer (BoYiKang FD-1-50, China) for 24 h at a vacuum level of 20–40 Pa.

The porosity of the PT scaffolds was determined using the liquid displacement method with anhydrous ethanol as the displacement medium. Briefly, dried PT scaffolds were completely immersed in a graduated cylinder containing a known initial volume of anhydrous ethanol (V1). Vacuum-assisted infiltration was performed to facilitate complete penetration of ethanol into the interconnected pores and remove trapped air until no visible bubbles were observed. After complete infiltration, the total volume of the ethanol and scaffold was recorded as V2. The ethanol-saturated scaffold was then carefully removed without squeezing, and the remaining ethanol volume was recorded as V3. The porosity of the PT scaffold was calculated according to the following equation: Porosity (%) = $[(V1 - V3) / (V2 - V3)] \times 100\%$. At least three independent PT scaffolds were analyzed, and the results were expressed as mean $\pm$ standard deviation.

The pore size of the PT scaffold was determined from scanning electron microscopy (SEM) images. Representative SEM images were calibrated according to the scale bar, and clearly identifiable complete pores were measured using image analysis software. The equivalent pore diameter was calculated for individual pores, and the mean pore diameter and pore size distribution were determined from multiple pores in representative regions.

2.2. Fabrication and characterization of TGP-377-encapsulated nanoparticles

As previously reported³⁵, TGP-377-loaded nanoparticles were fabricated via a double emulsion solvent evaporation approach. In brief, 200 μ L of TGP-377 dissolved in DMSO (10 mg/mL) was mixed with 1 mL of dichloromethane incorporating 100 mg of PLGA. Using an ultrasonic homogenizer, the mixture underwent emulsification for 30 s in 3 mL of 7% (w/v) poly (vinyl alcohol) (PVA, molecular weight 14,160) solution under ice-bath conditions. The resulting primary emulsion was then poured into 50 mL of a 1% (w/v) PVA solution and underwent sonication for an additional 60 s. The mixture underwent stirring for no less than 24 h at ambient conditions until dichloromethane was thoroughly evaporated. The resultant nanoparticles underwent purification through three consecutive wash-centrifugation cycles (distilled water, 15,000 rpm, 5 min) and were finally resuspended in deionized water for subsequent experiments. The TGP-377-loaded nanoparticles underwent characterization via dynamic light scattering (DLS)

on a Mastersizer 3000 analyzer (Malvern Instruments Ltd., UK). Nanoparticle encapsulation efficiency and drug loading rate reached 73% and 14%, respectively.

2.3. Preparation of sodium methacrylate hyaluronate photocurable hydrogel

Sodium hyaluronate (HA) underwent dissolution in deionized water at 2–5% (w/v) and was stirred in an ice bath for 30 min. Methacrylic anhydride (MA) was subsequently introduced, and the reaction proceeded at a pH of 8.0–8.5 over a 24 h period. The resulting solution was dialyzed against deionized water for three days with water changes three times per day and subsequently lyophilized to obtain methacrylated hyaluronic acid (HAMA) solid. For use, the HAMA solid underwent dissolution in deionized water at 3% (w/v), and a photocurable HAMA solution was prepared by adding the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) at 0.1% (w/v).

2.4. Preparation of HPT and THPT

To ensure consistent terminology throughout this study, PLGA/ β -TCP scaffolds are referred to as PT scaffolds, HAMA hydrogel-infused PT scaffolds as HPT scaffolds, and TGP-377 nanoparticles-loaded HAMA hydrogel-infused PT scaffolds as THPT scaffolds. Briefly, the drug-free HAMA precursor solution or HAMA precursor solution containing TGP-377-loaded PLGA nanoparticles was introduced into the interconnected pores of the PT scaffolds by vacuum-assisted infiltration. The infiltrated scaffolds were then irradiated with 405-nm light for approximately 30 s to photocrosslink the HAMA phase in situ. Following photocrosslinking, the entire composite constructs were frozen at -80°C and lyophilized for 24h to obtain the final dried HPT and THPT scaffolds. The dried composite scaffolds were stored under -80°C until subsequent use.

The mechanical properties of the PT, HPT, and THPT scaffolds were evaluated using a TMS-PRO texture analyzer (FTC, USA) in texture profile analysis (TPA) mode. A 10-mm-diameter cylindrical steel probe was used for compression testing. The trigger force was set at 5 g (approximately 0.049 N), and the samples were compressed to 90% deformation. The pre-test, test, and post-test speeds were all set at 1 mm/s (60 mm/min). Each sample underwent two consecutive compression cycles, and the corresponding load–displacement curves were recorded. Mechanical parameters, including first-cycle hardness, initial modulus, and resilience, were obtained from the instrument-generated data. Three independent specimens were analyzed for each group.

2.5. Scanning electron microscopy of PT and THPT

PT and THPT scaffolds were cross-sectioned with a sharp blade. The scaffold morphologies and porous structures were examined by scanning electron microscopy (Supra 55, Zeiss, Germany) at 15 kV accelerating voltage following sputter-coating with gold.

2.6. Particle size measurement of nanoparticles

The nanoparticle suspension was diluted to 0.1 mg/mL with deionized water, and the size distribution was assessed by repeated measurements using DLS. This method provides an accurate representation of the actual particle size distribution in an aqueous medium.

2.7. ^1H NMR detection

Structural characterization was performed by Bruker AVANCE III HD 400M NMR spectroscopy at 25°C with deuterated water as solvent and chemical shift units expressed in ppm.

2.8. Hydrogel rheological properties testing

The hydrogels underwent rheological characterization on a rheometer fitted with a parallel-plate geometry (35 mm diameter). The sol–gel transition was tracked by continuously recording the storage modulus (G') and loss modulus (G'') throughout the gelation process. Measurements were conducted at a steady temperature of 20 °C, and the evolution of the moduli over time was recorded on logarithmic coordinates. The gelation time was identified at the crossover point of G' and G'' , marking the transition from a liquid-like state ($G'' > G'$) to a solid-like state ($G' > G''$).

2.9. Infrared spectroscopy of hydrogel

Lyophilized samples from each group were ground and pressed into KBr pellets, whereas the solid hydrogels were directly analyzed by attenuated total reflectance (ATR) mode. Spectra were acquired across the 4000–400 cm^{-1} range. The analysis focused on verifying methacryloyl grafting via the ester carbonyl peak near 1720 cm^{-1} and on confirming the HA backbone through characteristic bands near 1600 cm^{-1} and 1040 cm^{-1} .

2.10. Release of TGP-377

To compare the release kinetics of TGP-377 from scaffolds with and without nanoparticle/hydrogel mediation, two formulations were prepared: TGP-377 directly loaded onto

the PT scaffold (TGP-377/PT) and THPT. Each sample, containing the same theoretical loading of TGP-377, was submerged in 5 mL of PBS (pH 7.4, 0.1% Tween-80) at 37 °C under gentle shaking. At designated time points, 1 mL of supernatant was withdrawn and substituted with an equivalent volume of fresh release medium. The TGP-377 concentration in collected samples was measured via high-performance liquid chromatography (HPLC) equipped with a reverse-phase C18 column and UV detection at 254 nm. Cumulative drug release was computed as the percentage of TGP-377 released relative to the total loaded amount. The cumulative release profiles of TGP-377/PT and THPT were fitted using the zero-order, first-order, Higuchi, and Weibull models. The Korsmeyer–Peppas model was additionally applied to data points corresponding to cumulative drug release $\leq 60\%$ to evaluate the early-stage release behavior. Model fitting was assessed using the coefficient of determination (R^2), adjusted R^2 , root-mean-square error (RMSE), and corrected Akaike information criterion (AICc). The model with the lowest AICc, together with a higher adjusted R^2 and lower RMSE, was considered to provide the most appropriate fit. Release kinetic analysis was performed using GraphPad Prism 9.5.

2.11. Cell culture

MC3T3-E1 cells and human umbilical vein endothelial cells (HUVECs) were procured from the National Collection of Authenticated Cell Cultures (Shanghai, China). MC3T3-E1 cells underwent culture in α -MEM comprising 10% fetal bovine serum (FBS; Gibco, USA) along with 1% penicillin/streptomycin (100 U/mL penicillin and 100 μ g/mL streptomycin; Suzhou NCM Biotech Co., Ltd., China) under 37 °C with 5% CO₂. HUVECs were kept in DMEM comprising 10% FBS and the same antibiotics under identical conditions.

2.12. Cell biocompatibility

To assess the influence of HPT and THPT on MC3T3-E1 cell and HUVEC proliferation and cytotoxicity, a live/dead staining assay was employed. For the Transwell assay, MC3T3-E1 cells or HUVECs (1.0×10^4 cells/mL) were plated into the lower chamber of a 24-well Transwell plate (Corning, USA), with the blank control, HPT, or THPT positioned in the upper chamber. At days 1, 3, and 5, cells underwent staining with 500 μ L of Calcein-AM/PI working solution (Beyotime, China) for 30 min and were then detected under a fluorescence microscope (ZEISS, Germany). For the direct contact assay, 1×10^6 MC3T3-E1 cells or HUVECs were placed on the surfaces of the blank control, HPT, and THPT in 24-well plates and maintained for 3 days. Afterward, the

cells underwent staining with Calcein-AM/PI working solution for 30 min, and cell adhesion and proliferation on the various surfaces were observed under the microscope.

2.13. Cell viability

MC3T3-E1 cell and HUVEC viability was examined via the CCK-8 assay (Beyotime Biotechnology, Shanghai, China). Cells underwent seeding into 96-well plates and were cultured to roughly 80% confluence, followed by treatment with TGP-377 or no treatment. At 24, 48, and 72 h post-treatment, the culture medium was discarded and substituted with 100 μ L of fresh medium comprising 10 μ L of CCK-8 solution. After a subsequent 2 h incubation at 37 $^{\circ}$ C, absorbance at 450 nm was recorded using a microplate spectrophotometer.

2.14. 5-Ethynyl-20-deoxyuridine (EdU) assay

Cell proliferation was evaluated with an EdU assay kit (RiboBio, Guangzhou, China) per the supplier's protocols. MC3T3-E1 cells and HUVECs were plated in 96-well plates and underwent the same treatment as mentioned in the CCK-8 assay. In brief, cells underwent incubation with 100 μ L of EdU solution for 2 h at 37 $^{\circ}$ C, succeeded by three phosphate-buffered saline (PBS) washes. Subsequently, cells underwent fixation in 4% paraformaldehyde and permeabilization with 0.5% Triton X-100. Next, 100 μ L of Apollo staining solution was applied to each well and maintained for 30 min at ambient conditions. Nuclei were then counterstained using Hoechst solution for 30 min. A fluorescence microscope (Leica DMR3000, Leica Microsystems, Bensheim, Germany) was employed to capture fluorescence images.

2.15. Wound healing assay

MC3T3-E1 cells and HUVECs underwent trypsinization and were plated into 6-well plates. Upon reaching approximately 80% confluence, linear scratches were made across the cell monolayer with a sterile pipette tip. Detached cells were eliminated by PBS washing, and images were acquired at the initial time point (0 h) using a microscope. The cells then underwent incubation under standard culture conditions, and additional Images were acquired at 24 h for MC3T3-E1 cells and at 12 h for HUVECs.

2.16. Cell cycle analysis

MC3T3-E1 cells and HUVECs underwent treatment for 48 h, followed by trypsinization, centrifugation, and resuspension in 50 μ L of PBS. To achieve fixation, 900 μ L of pre-cooled 70% ethanol was introduced dropwise under gentle vortexing, after which the samples were kept at -20°C for 3 days. Before analysis, cells were pelleted by centrifugation, rinsed three times with PBS, and exposed to propidium iodide (PI) for 20 min in the dark. The stained cells then underwent centrifugation, after which the supernatant was removed, and the pellet was resuspended in 400 μ L PBS. Cell cycle distribution was subsequently assessed via a flow cytometer (Attune NxT, Invitrogen).

2.17. Transwell analysis

Cell migration was assessed via a Transwell assay employing 8- μ m pore size inserts. Cells underwent harvesting, resuspension in serum-free basal medium, and adjustment to 5×10^4 cells/mL. Subsequently, 300 μ L of the cell suspension was placed into the upper chamber, while 700 μ L of complete medium comprising 20% FBS was introduced into the lower chamber as a chemoattractant. The assembled chambers underwent incubation at 37°C in a 5% CO_2 humidified atmosphere for 24 h (MC3T3-E1 cells) or 12 h (HUVECs). Following incubation, the upper chamber medium was aspirated, and the inserts underwent gentle rinsing with PBS. Cells that had traversed to the lower surface underwent fixation in 4% paraformaldehyde for 30 min at ambient conditions, washed with PBS, and subsequently stained with crystal violet for 5–10 min. A cotton swab was employed to gently clear non-migrated cells from the upper side. Migrated cells were then visualized and recorded utilizing a fluorescence microscope (Leica DMR3000, Leica Microsystems, Bensheim, Germany).

2.18. Western blot (WB) assay of cell protein

Total protein was procured from MC3T3-E1 cells or HUVECs with RIPA lysis buffer comprising 1% protease inhibitor. Lysates were combined with $1\times$ loading buffer, underwent denaturation by boiling, and were kept at -20°C until further use. Proteins underwent separation on 6–12% gradient SDS–PAGE gels and were then blotted onto PVDF membranes. Membranes were blocked using 5% non-fat milk at ambient conditions for 1.5 h and subsequently underwent overnight incubation at 4°C with the primary antibodies listed below: RUNX2 (1:1000, Cell Signaling Technology, USA), OPN (1:1000, Proteintech, China), VEGFA (1:1000, Abcam, USA), ALP (1:1000, Zenbio, China), and HIF-1 α (1:1000, Cell Signaling Technology, USA). Post-rinsing,

membranes were exposed overnight at 4 °C to IRDye 800-conjugated secondary antibody (1:10,000, Rockland, Gilbertsville, PA, USA). Protein bands were acquired through the Odyssey imaging system and subjected to quantification via ImageJ software, with normalization to GAPDH or β -actin (1:10,000, Proteintech, China).

2.19. Alkaline phosphatase (ALP) and Alizarin Red S staining for mineralization

MC3T3-E1 cells were plated in 6-well plates at 5×10^3 cells/cm² and underwent osteogenic induction for 14 or 21 days. Osteogenic differentiation was triggered by supplementing α -MEM with 50 μ g/mL L-ascorbic acid (Sigma-Aldrich, USA), 10 mM β -glycerophosphate (Sigma-Aldrich), and 10 nM dexamethasone (Sigma-Aldrich). ALP activity was determined via a BCIP/NBT color development kit (C3206, Beyotime, China) per the supplier's protocols. Mineralization was examined through Alizarin Red S staining using a modified kit for calcium detection (G3280, Solarbio, China). Stained plates underwent imaging with a digital camera, and representative microscopic photographs were obtained using a light microscope (BX41, Olympus). Five arbitrarily chosen fields per well ($\times 10$ magnification) underwent quantitative analysis via ImageJ software (version 1.53n, National Institutes of Health, USA). ALP staining was performed on day 14, and Alizarin Red S staining was performed on day 21.

2.20. Tube formation assay

Matrigel (KeyGenBioTECH, Nanjing, Jiangsu, China) was retrieved from the -80 °C freezer one day prior and transferred to the 4 °C refrigerator for overnight thawing. The Matrigel was then blended with basal medium at a 1:1 ratio, and 40 μ L of this mixture was dispensed onto the center of a 24-well plate, spread in a circular pattern along the well wall, and the plate underwent incubation for 1 h until solidification occurred. Digested HUVECs underwent resuspension in DMEM comprising 10% FBS, were counted, and seeded at roughly 5×10^3 cells per well. Cells were kept at 37 °C in a 5% CO₂ humidified incubator. Images were captured at 6 h from three distinct angles under high magnification.

2.21. Rabbit femoral bone defect model

Six-month-old New Zealand White rabbits (3.0 ± 0.5 kg) underwent surgery to establish a femoral bone defect model. The animals were arbitrarily split into three groups: Control (no scaffold), simple scaffold (HPT), and scaffold loaded with TGP-377 (THPT), with five rabbits per group (n

= 5). Bilateral femoral defects were created in each rabbit. Prior to surgery, animals underwent anesthesia induction through ear vein injection of 3% sodium pentobarbital. The surgical area around the knee joint was shaved and disinfected with iodine. A medial parapatellar arthrotomy was performed, followed by patellar dislocation to expose the distal femur. Cylindrical bone defects (3 mm in diameter and 3 mm in depth) were created using a sterile drill under constant saline irrigation (0.9%) to avoid thermal injury to the adjacent bone tissue. Scaffolds were implanted into the defects, and the wounds were closed in layers. Postoperatively, enrofloxacin (25 mg/kg) was administered for infection prophylaxis. At 4 weeks after surgery, the rabbits were deeply anesthetized with sodium pentobarbital (30 mg/kg, intravenous administration). An adequate depth of anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes. An additional dose of sodium pentobarbital was then administered intravenously to achieve a total dose of at least 100 mg/kg. Death was confirmed by the permanent cessation of spontaneous respiration and heartbeat and the absence of corneal reflexes before tissue collection. Femoral specimens were collected and immersed in 10% formalin for subsequent analyses. The animal experiment protocol was approved by the Experimental Animal Care and Use Committee of Nantong University (approval number S20240110-101). The study is reported in accordance with the ARRIVE 2.0 guideline

2.22. Micro-CT scanning and analysis

Micro-computed tomography (micro-CT) analysis was conducted utilizing a SkyScan-1276 system (Bruker Micro-CT, Kontich, Belgium) to assess bone defect healing in the rabbit model. Femurs were obtained and kept at -80°C before scanning. Samples underwent scanning at a pixel resolution of $15\text{ }\mu\text{m}$ with a 1-mm aluminum filter at 85 kV and 200 μA . A cylindrical volume of interest (VOI) corresponding to the original defect region was selected while excluding surrounding native bone. Three-dimensional reconstruction was performed using CTVox software, and quantitative morphometric parameters, including bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular separation (Tb.Sp), were calculated using CTAn software. Considering the radiopacity of β -TCP, scaffold-related mineral signals were carefully interpreted with reference to the background characteristics of the PT scaffold, following previously reported approaches for PLGA/ β -TCP composite scaffolds.

2.23. WB assay of tissue protein

Bone defect specimens underwent snap-freezing in liquid nitrogen and were subsequently ground into a fine powder using a mortar and pestle at ambient conditions kept by liquid nitrogen. The powdered tissue underwent lysis using RIPA buffer comprising 1% protease inhibitor. Following lysis, 1× loading buffer was added, and proteins were denatured by boiling. Protein samples were kept at −20 °C until use. Identical amounts of protein underwent separation on 6–12% gradient SDS-PAGE gels and were then transferred onto PVDF membranes. Membranes underwent blocking with 5% non-fat milk for 1.5 h at ambient conditions, followed by overnight incubation at 4 °C with primary antibodies against RUNX2 (1:1000, Proteintech, China), Col I (1:1000, Cell Signaling Technology, USA), ALP (1:1000, Zenbio, China) and VEGFA (1:1000, Abcam, USA). After washing, membranes underwent incubation with IRDye800-conjugated secondary antibody (1:10000, Rockland, USA) at 4 °C overnight. Protein bands were detected via the Odyssey imaging system and analyzed using ImageJ software, with β -actin (1:10000, Proteintech, China) serving as the internal reference for normalization.

2.24. Real-time reverse transcription quantitative PCR (RT-qPCR)

Total cellular RNA underwent isolation with an RNA Isolation Kit (Beyotime, R0026) per the supplier's protocols. RNA concentration and purity were determined with a Nanodrop One spectrophotometer (Thermo, USA). Subsequently, 1 μ g of RNA underwent reverse transcription utilizing SweScript RT II Enzyme Mix (Servicebio, G3330) per the supplier's protocols. Quantitative real-time PCR (RT-qPCR) was carried out on a LightCycler 480 system (Roche Diagnostics, USA) with 2× Universal SYBR Green Fast qPCR Mix (Abclonal, RK21203). Gapdh was employed as the internal reference gene. The primer sequences used were as follows: *ALPL*, forward 5'-TGGACCTCGTGGACATCTG-3' and reverse 5'-CAGGAGTTCAGTGCGGTTC-3'; *COL1A1*, forward 5'-GGAAACGATGGTGCTACTGG-3' and reverse 5'-CCGACAGCTCCAGGGAAG-3'; *RUNX2*, forward 5'-GCAGTTCCCAAGCATTTCATC-3' and reverse 5'-GTGTAAGTAAAGGTGGCTGGATA-3'; *VEGFA*, forward 5'-TGCCCACCGAGGAGTTCA-3' and reverse 5'-GGCCCTGGTGAGGTTTGTAT-3'; and *GAPDH*, forward 5'-AAACTCACTGGCATGGCCTT-3' and reverse 5'-TTAGCAGCTTTCTCCAGGCG-3'.

2.25. HE staining

Femoral specimens from each rabbit group were retrieved and preserved in 4% paraformaldehyde at ambient conditions for 24 h. The samples then underwent careful decalcification, followed by paraffin embedding and sectioning at a thickness of 5 μm . After rehydration, the sections underwent staining with hematoxylin and eosin (HE) (Servicebio Technology Co., Ltd, Wuhan, China) to evaluate bone defect repair. Similarly, heart, liver, spleen, lung, and kidney tissues from the various experimental groups were paraffin-embedded, sectioned, and HE-stained to assess tissue response and overall biocompatibility of the THPT scaffold treatment.

2.26. Statistical analysis

All quantitative data are presented as the mean $\pm$ standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. Comparisons between two independent groups were performed using an unpaired two-tailed Student’s t-test. Comparisons among three or more groups were performed using one-way analysis of variance (ANOVA), followed by Tukey’s multiple-comparison test when the overall ANOVA indicated statistical significance. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism 9.5.

3. Results

3.1. Characterization of THPT

PLGA nanoparticles have been extensively employed as drug carriers owing to their superior biocompatibility, minimal toxicity, film-forming ability, and degradability, with applications in pharmaceuticals and biomedical materials³⁶⁻³⁸. In this study, the fabrication process of the THPT scaffold is illustrated in **Figure 2A**. Firstly, TGP-377 nanoparticles were synthesized and characterized. TEM revealed that both the empty nanoparticles and TGP-377-loaded nanoparticles exhibited uniform, spherical morphologies under high magnification (**Figure 2B**). Particle size analysis showed that the empty nanoparticles had an average diameter of 126.97 ± 1.55 nm, whereas TGP-377-loaded nanoparticles measured approximately 117 nm (**Figure 2C**). The properties of HAMA hydrogels, both empty and TGP-377-loaded, were subsequently examined.

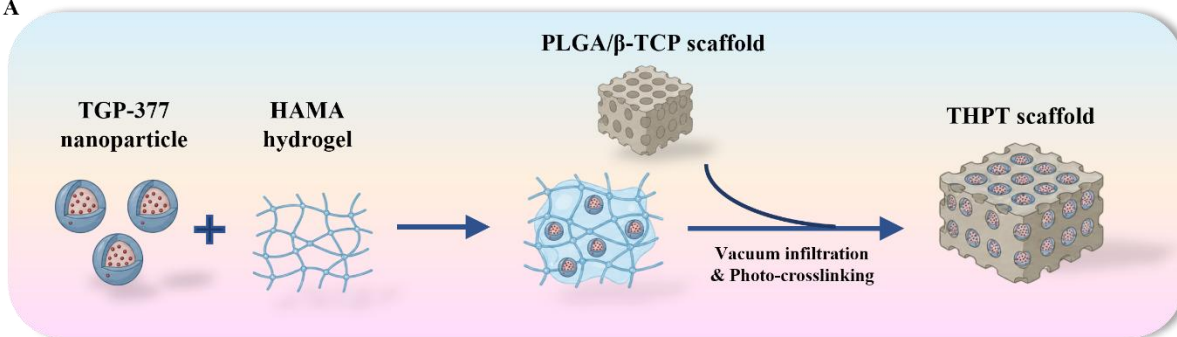
Photopolymerization testing demonstrated that the HAMA hydrogel underwent a transition from a flowing liquid state to a solid when irradiated with 405 nm UV light (**Figure 2D**). SEM revealed a three-dimensional porous network in both empty and TGP-377-loaded hydrogels, which is favorable for cell migration and tissue regeneration (**Figure 2E**). Proton nuclear magnetic resonance (^1H NMR) spectroscopy verified the presence of methacrylate (MA) groups, with characteristic peaks at 5.7 and 6.1 ppm corresponding to the two protons of the double bond ($\text{C}=\text{CH}_2$) (**Figure 2F**). Fourier-transform infrared (FT-IR) spectroscopy further verified hydrogel synthesis: hyaluronic acid exhibited characteristic COO^- asymmetric stretching and $\text{C}-\text{O}-\text{C}$ vibrations at 1405 cm^{-1} and 1030 cm^{-1} , respectively, and a new peak at 1740 cm^{-1} assigned to the $\text{C}=\text{O}$ group of MA (**Figure 2G**). Rheological analysis confirmed that the HAMA hydrogel undergoes a liquid-to-solid transition upon light exposure (**Figure 2H**).

In our study, the porosity of the PT scaffold, as determined by the liquid displacement method, was $53.77 \pm 14.88\%$, while SEM-based image analysis showed a mean pore diameter of $543.53 \pm 165.92\text{ }\mu\text{m}$. Compression testing revealed distinct mechanical behaviors among the PT, HPT, and THPT scaffolds (**Figure 2I-2J**). The representative load–displacement curves showed that the PT scaffold exhibited the highest first-cycle peak load, with a fracture force of $86.06 \pm 7.88\text{ N}$, together with the highest hardness and initial stiffness, reflecting the rigid structural support provided by the PLGA/ β -TCP framework. Following incorporation of the HAMA hydrogel and TGP-377-containing components, both HPT and THPT exhibited moderately reduced first-cycle hardness and initial modulus compared with PT, while no obvious abrupt fracture event was observed under the applied compression conditions. In contrast, resilience increased progressively from PT to HPT and THPT, with the THPT scaffold exhibiting significantly higher resilience than the PT scaffold. These changes suggest that incorporation of the hydrogel phase reduced the rigidity of the PLGA/ β -TCP framework while imparting a more compliant and resilient mechanical response. This behavior may be attributed to the flexible hydrogel matrix distributed within the rigid porous framework, which could partially buffer externally applied deformation and mitigate abrupt structural failure. Importantly, despite the reduction in hardness and stiffness, the HPT and THPT scaffolds retained substantial resistance to compression, indicating that hydrogel incorporation did not eliminate the mechanical support provided by the PLGA/ β -TCP framework. Overall, the composite design achieved a balance between the structural support of the rigid PLGA/ β -TCP

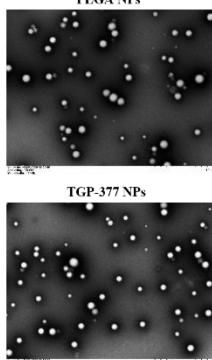
framework and the mechanical flexibility imparted by the hydrogel phase, which may be advantageous for adapting to the dynamic mechanical environment of bone defects.

Drug release studies demonstrated that TGP-377 exhibited sustained release behavior. In the TGP-377/PT scaffold, approximately $77.29 \pm 1.19\%$ of TGP-377 was released within 21 days, whereas in the THPT scaffold, only $55.49 \pm 0.37\%$ was released after 21 days (**Figure 2K**). Kinetic analysis showed that the TGP-377/PT release profile was best described by the Weibull model ($R^2 = 0.9754$, adjusted $R^2 = 0.9683$, RMSE = 3.31%, and AICc = 29.66). For THPT, the Higuchi model showed the lowest AICc (30.10), while the Weibull model provided a comparable fit with a slightly higher R^2 (0.9458) and lower RMSE (3.49%) (**Figure A1**, in Appendix). These findings suggest that incorporation of the TGP-377-loaded nanoparticles into the HAMA hydrogel substantially retarded drug release. The slower release from THPT is attributed to the HAMA hydrogel forming a gel network that gradually releases the drug, prolonging its availability. This sustained release is critical for fracture healing, as bone regeneration and angiogenesis-related responses require extended time^{39,40}. The prolonged release profile may provide a sustained local supply of TGP-377; however, whether the released fraction retains the full biological activity of freshly prepared TGP-377 remains to be determined. Moreover, continuous VEGFA stimulation enhances angiogenesis-related responses, increases capillary formation, improves nutrient and oxygen delivery, and accelerates bone tissue regeneration^{41,42}.

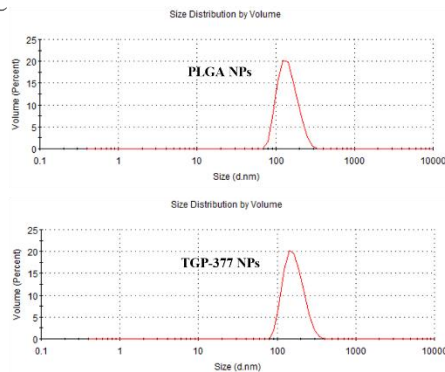
A



B



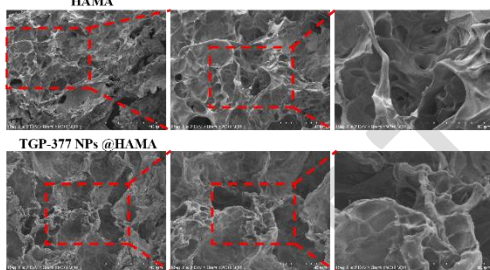
C



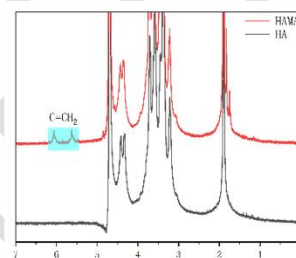
D



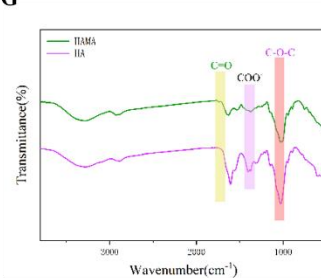
E



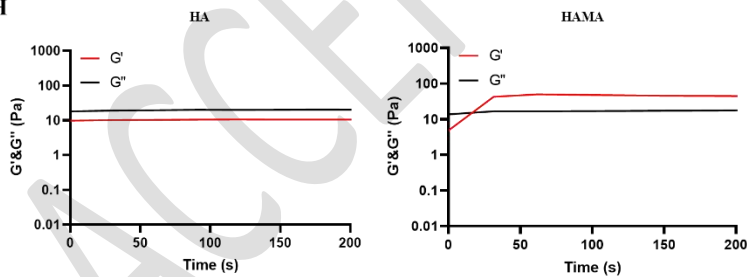
F



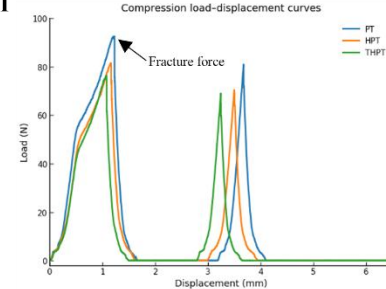
G



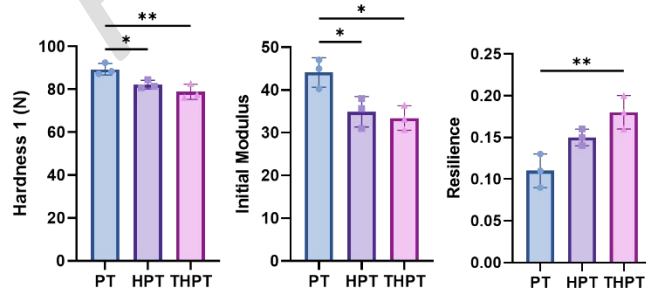
H



I



J



K

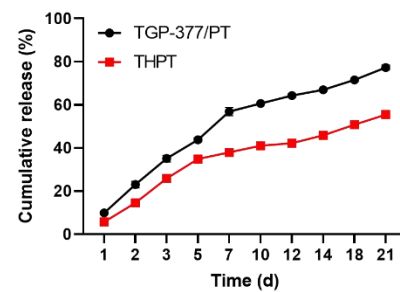


Figure 2. Fabrication and physicochemical characterization of the THPT scaffold. (A) Schematic illustration of THPT scaffold fabrication. (B) Representative microscopic images of the PLGA Nanoparticles and TGP-377 Nanoparticles. (C) Particle size distribution analysis of the nanoparticles. (D) Photographs showing the photopolymerization process under 405 nm light irradiation. (E) SEM images showing the porous microstructure of the HAMA hydrogel. (F) ¹H NMR spectrum confirming the successful modification of MA. (G) FTIR spectra of HA and HAMA, showing characteristic chemical groups after modification. (H) Rheological testing. (I, J) Compression behavior and mechanical parameters of PT, HPT, and THPT scaffolds (n=3). (K) Cumulative TGP-377 release from TGP-377/PT and THPT scaffolds over 21 days (n=3).

Figure 3A shows the gross morphology of PT and THPT, indicating that both scaffolds maintained an intact and stable overall structure. SEM images further revealed the surface microstructure of PT and THPT (**Figure 3B**). Compared with PT, THPT exhibited a more favorable porous and roughened surface morphology owing to the incorporation of hydrogel, which may provide additional anchoring sites for cell attachment and facilitate subsequent cell spreading. These structural characteristics are important for tissue-engineered scaffolds, as surface morphology and pore architecture can directly influence cell–material interactions.

Biocompatibility and cell adhesion are critical for tissue engineering applications. MC3T3-E1 cells and HUVECs underwent seeding and co-culture on HPT or THPT for cytotoxicity and cellular response evaluation in two ways (**Figure 3C**). Cell counting and CCK-8 assays further demonstrated that all groups supported cell proliferation over 5 days, with no evidence of toxicity (**Figure 3D-F**). Observations under the microscope indicated sparse cell attachment on HPT scaffolds, whereas THPT scaffolds supported robust cell adhesion, spreading, and growth (**Figure 3G**), highlighting the positive influence of TGP-377 incorporation on cellular interactions.

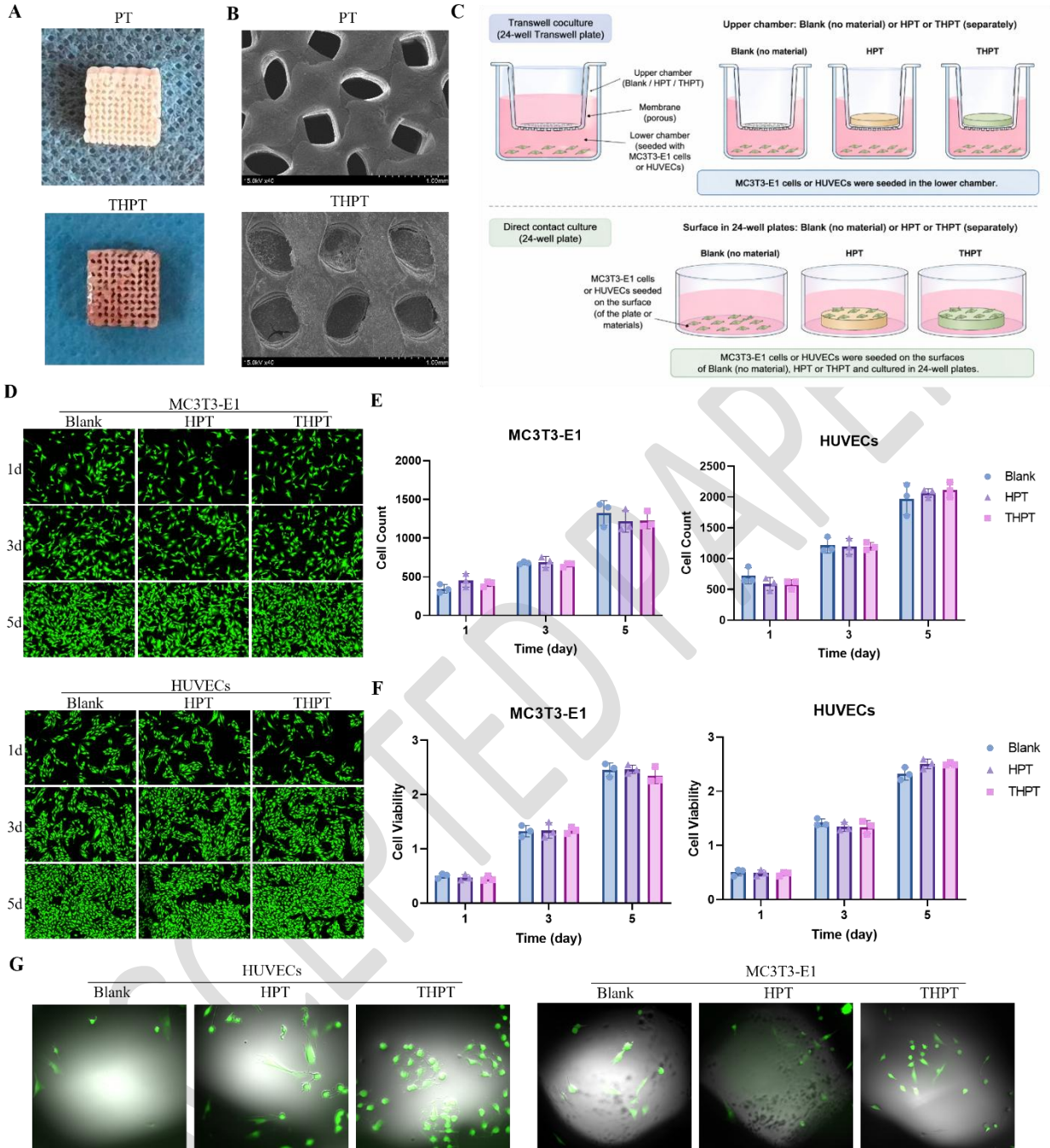


Figure 3. In vitro biocompatibility evaluation of HPT and THPT scaffolds. (A) Gross photographs of PT and HPT scaffolds. (B) SEM images showing the porous microstructures of PT and HPT scaffolds. (C) Schematic illustration of Transwell coculture systems. (D, E) Live-cell staining images of MC3T3-E1 cells and HUVECs cultured with different groups (n=3). (F) Quantitative analysis of cell viability in the Transwell coculture systems (n=3). (G) Images

showing HUVECs and MC3T3-E1 cells' adhesion and spreading on HPT and THPT scaffolds. Data are denoted as mean $\pm$ SD.

3.2. TGP-377 facilitated MC3T3-E1 cell proliferation and osteogenic differentiation

The effect of TGP-377 on osteoblast proliferation was assessed via the CCK-8 assay to determine the optimal concentration. Treatment with 500 nM TGP-377 for 48 and 72 h significantly enhanced MC3T3-E1 cell proliferation compared with the NC group, whereas 250 nM and 1000 nM showed less pronounced effects (**Figure 4A**), indicating that 500 nM is the optimal concentration for promoting proliferation and was used in subsequent experiments. Transwell migration assays revealed that TGP-377 significantly increased MC3T3-E1 cell migration through the inserts relative to the NC group (**Figure 4B–C**). Consistently, the wound healing assay demonstrated accelerated scratch closure in TGP-377-treated cells compared with controls (**Figure 4D–E**), confirming enhanced migratory capacity. Flow cytometry further showed that TGP-377 promoted cell cycle progression, increasing the fraction of cells in S and G2 phases, indicative of elevated DNA replication and proliferative activity (**Figure 4F–G**). These results were corroborated by the EdU assay, which revealed a higher fraction of cells undergoing DNA synthesis in the TGP-377 group (**Figure 4H–I**).

To evaluate osteogenic differentiation, MC3T3-E1 cells underwent culture in osteogenic induction medium. ALP staining after 14 days showed increased ALP activity in TGP-377-treated cells, indicating enhanced early-stage osteogenic differentiation (**Figure 4J**). By day 21 of induction, Alizarin Red S staining revealed more abundant calcium nodule deposition and extracellular matrix mineralization in the TGP-377 group than in controls (**Figure 4K**). Collectively, these findings indicate that TGP-377 enhances both proliferation and osteogenic differentiation of MC3T3-E1 cells.

Finally, we further confirmed the effect of TGP-377 on osteogenesis-related protein expression. With GAPDH serving as an internal control, RUNX2, OPN, and ALP expression levels were evaluated. WB analysis demonstrated that TGP-377 markedly upregulated these osteogenic markers versus the control group (**Figure 4L–O**), suggesting that TGP-377 promotes osteogenic differentiation by boosting the expression of key osteogenesis-related proteins. Notably, TGP-377

also increased VEGFA expression in MC3T3-E1 cells (**Figure 4L, 4P**). Although the precise mechanism underlying VEGFA production and secretion by osteoblasts remains incompletely understood, VEGFA is known to influence multiple aspects of osteoblast function. Proposed mechanisms include: (i) coupling angiogenesis with osteogenesis by modulating osteoblast responses to vascular growth; (ii) acting as an autocrine regulator of osteoblast differentiation and activity; and (iii) inducing adjacent cells to express regulatory factors, thereby indirectly modulating osteoblast function.

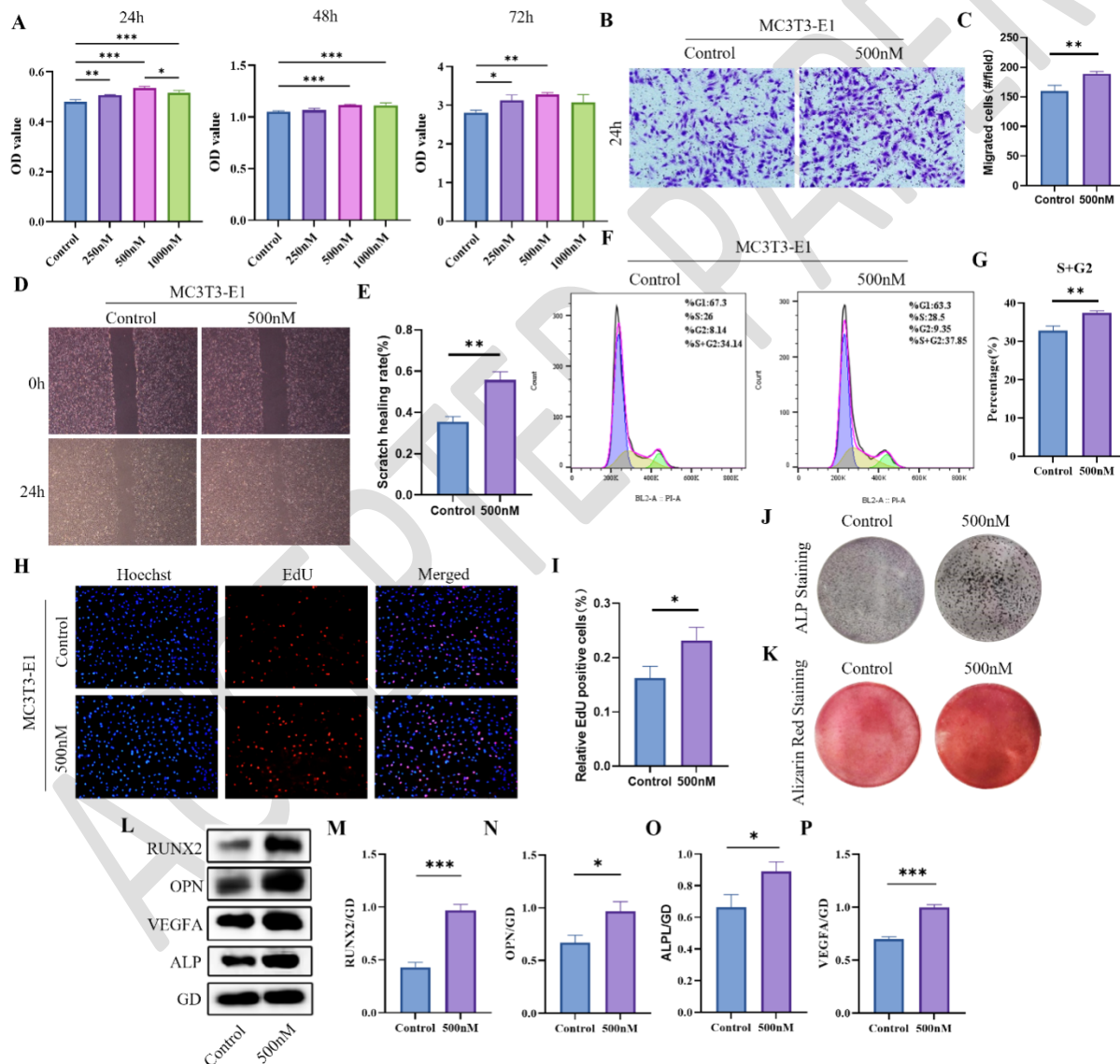


Figure 4. TGP-377 enhances proliferation and osteogenic differentiation in MC3T3-E1 cells.

(A) CCK-8 assays revealing how different concentrations of TGP-377 affect MC3T3-E1 cell

proliferation (n=4). (B-C) Transwell migration assays and quantitative analysis demonstrating enhanced migration after TGP-377 treatment (n=3). (D-E) Wound healing assays indicating accelerated scratch closure (n=3). (F-G) Flow cytometry analysis of cell cycle distribution showing increased S and G2 phase populations (n=3). (H-I) EdU staining confirming enhanced cell proliferation (n=3). (J-K) ALP staining and Alizarin Red S staining showing promoted osteogenic differentiation and mineralization. (L-P) WB analysis of osteogenesis-related proteins (RUNX2, ALP, OPN) and VEGFA expression (n=3). Data are denoted as mean $\pm$ SD. *P < 0.05, **P < 0.01, ***P < 0.001.

3.3. TGP-377 promoted the proliferation and angiogenic capacity of HUVECs

The CCK-8 assay showed that treatment with 500 nM TGP-377 for 48 h and 72 h significantly enhanced HUVEC proliferation versus the Control group, whereas the 250 nM and 1000 nM treatments were less effective, indicating 500 nM as the optimal concentration for promoting proliferation (**Figure 5A**). All subsequent experiments were conducted using this concentration. Transwell migration assays demonstrated that TGP-377 significantly increased HUVEC migration through the inserts (**Figure 5B–C**), and the scratch assay confirmed accelerated wound closure relative to the Control group (**Figure 5D–E**), indicating enhanced migratory capacity.

Flow cytometry analysis revealed that TGP-377 promoted the transition of HUVECs into the S and G2 phases, indicative of increased DNA replication and cell proliferation (**Figure 5F–G**). EdU assays further confirmed an elevated proportion of HUVECs undergoing DNA synthesis (**Figure 5H–I**). WB analysis showed that TGP-377 markedly upregulated the expression of angiogenic proteins VEGFA and HIF-1 α relative to the Control (**Figure 5J–L**), suggesting that TGP-377 enhances endothelial angiogenic capacity. HIF-1 α is known to regulate multiple target genes associated with angiogenesis and bone regeneration, coordinating the coupling of these processes. Finally, the Matrigel tube formation assay demonstrated that TGP-377 significantly promoted the formation of vascular structures by HUVECs after 6 h of treatment (**Figure 5M–P**).

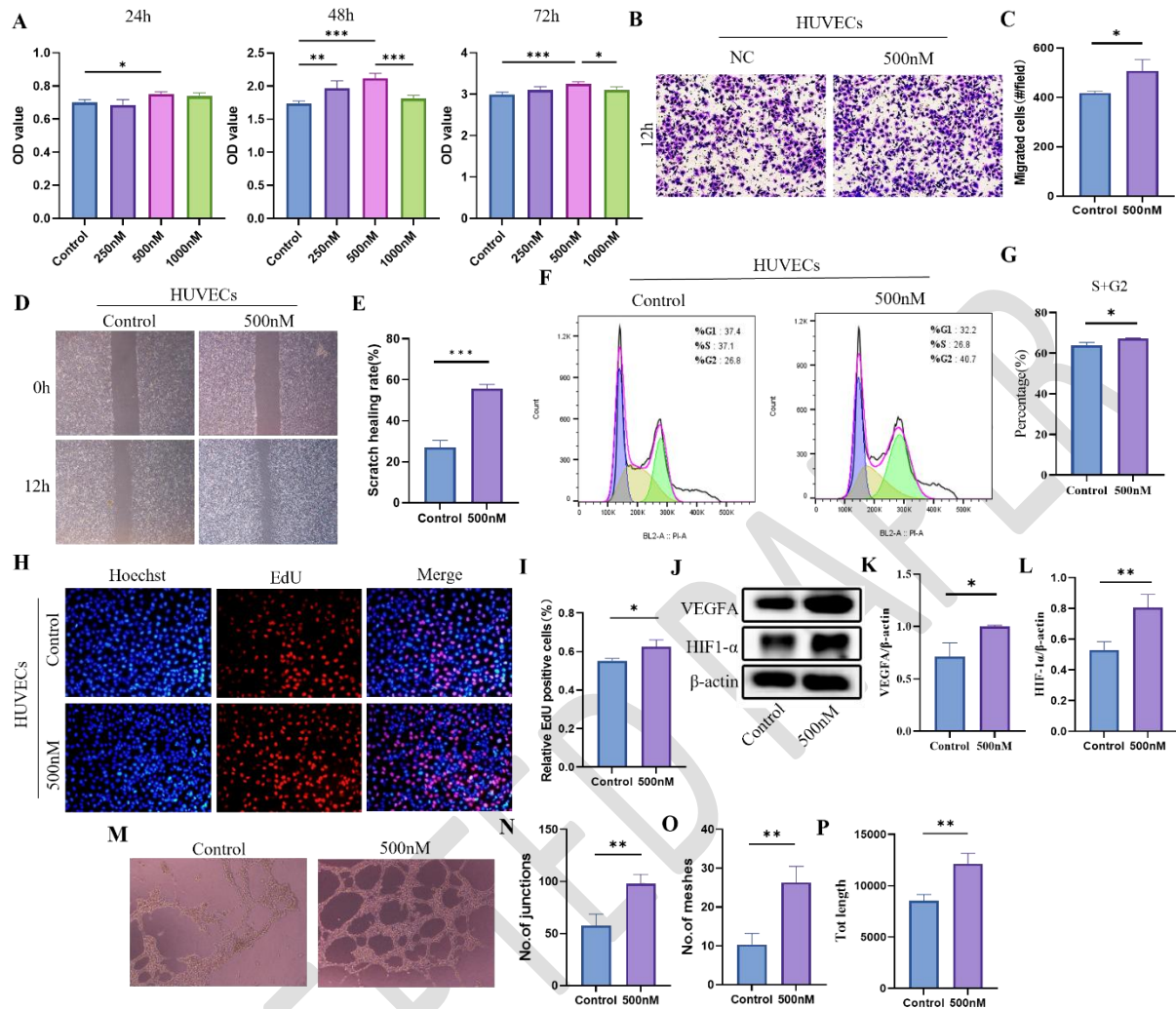


Figure 5. TGP-377 enhances proliferation and angiogenic capacity of HUVECs. (A) CCK-8 assays indicating how varying concentrations of TGP-377 affect HUVEC proliferation (n=4). (B-C) Transwell migration assays and quantitative analysis demonstrating enhanced migration of HUVECs after TGP-377 treatment (n=3). (D-E) Wound healing assays indicating accelerated scratch closure (n=3). (F-G) Flow cytometry analysis of cell cycle distribution showing increased S and G2 phase populations (n=3). (H-I) EdU staining confirming enhanced cell proliferation (n=3). (J-L) WB analysis of angiogenesis-related proteins (VEGFA and HIF-1α) (n=3). (M-P) Tube formation assay demonstrating enhanced angiogenic potential of HUVECs (n=3). Data are denoted as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

3.4. THPT enhanced early bone repair and angiogenesis-related marker expression in rabbit femoral defects

To assess drug-loaded scaffolds' efficacy *in vivo*, a rabbit femoral bone defect model was established (**Figure 6A**). Both imaging and histomorphometric analyses were performed, including micro-CT for three-dimensional assessment of bone formation and HE staining for histological evaluation. HE staining revealed that the THPT group's regenerated bone tissue was substantially greater than that of the Control and HPT groups, with less fibrous tissue, demonstrating the scaffold's strong osteoinductive properties (**Figure 6B**). The scaffold provided an optimal environment for osteoblast proliferation and angiogenesis. Micro-CT further confirmed these observations: the Control group showed minimal bone regeneration, the HPT group exhibited moderate new bone formation, and the THPT group displayed abundant mineralized bone (**Figure 6C**). Quantitative analysis of bone parameters indicated significantly higher bone volume fraction (BV/TV) and trabecular number (Tb.N), along with lower trabecular separation (Tb.Sp) in the THPT group compared with the other groups (**Figure 6D–F**), suggesting full bone regeneration along the scaffold's pores.

The THPT scaffold significantly enhanced osteogenesis and angiogenesis at the defect site. WB and RT-qPCR analyses of regenerated bone tissue showed that osteogenic markers (Col-I, ALP, RUNX2) were notably upregulated in the THPT group versus Control and HPT (**Figure 6G–J, 6L–N**), while angiogenic marker VEGFA was also significantly increased (**Figure 6G, 6K, 6O**). This indicates that TGP-377 promoted both osteoblast differentiation and vascular formation, creating a synergistic microenvironment for bone regeneration. Concurrent vascular and bone tissue formation enhanced nutrient and oxygen delivery, sustaining ongoing osteoblast activity.

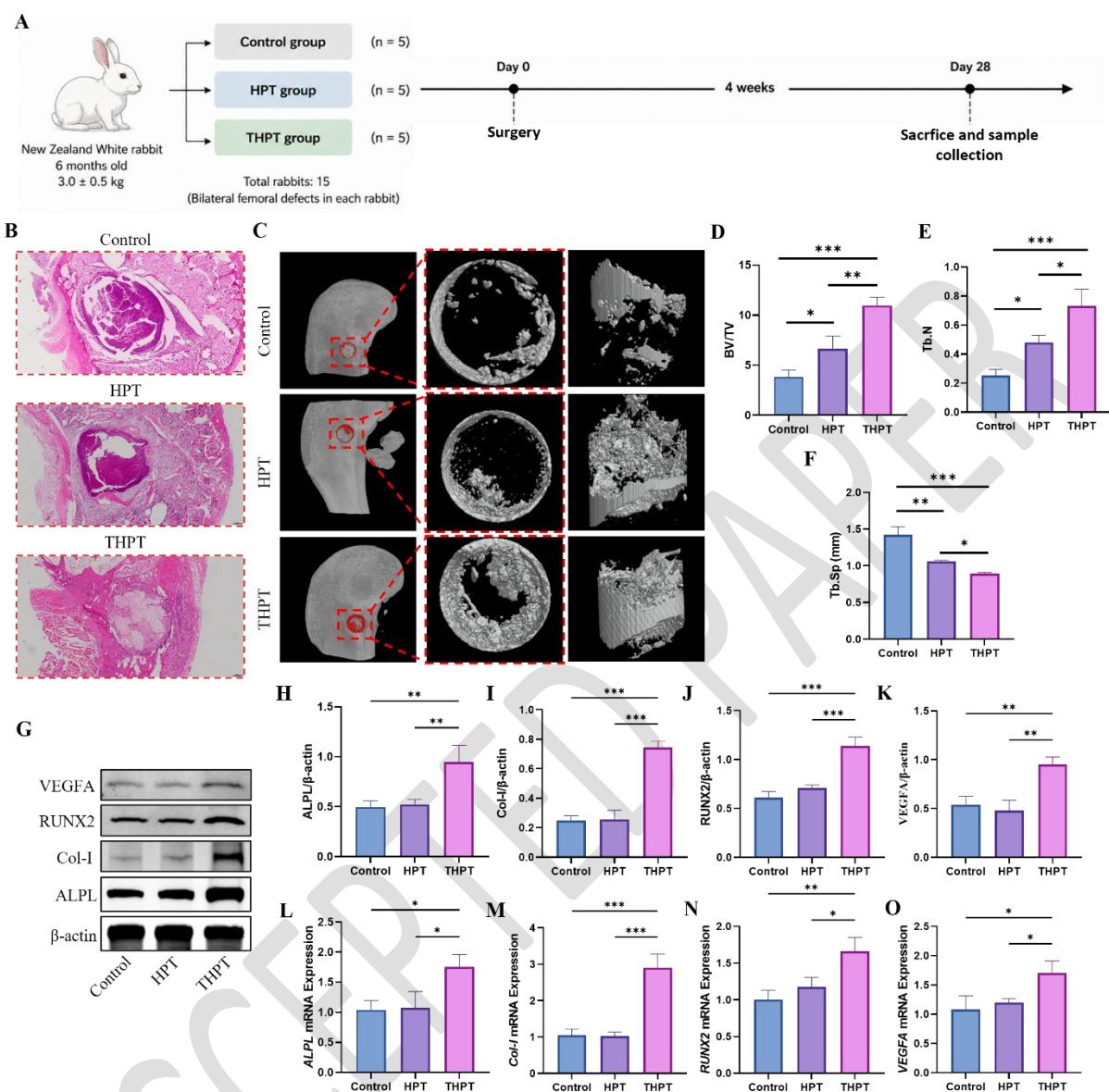


Figure 6. In vivo bone regeneration evaluation in a rabbit femoral defect model. (A) Schematic of the animal experiment. New Zealand white rabbits with bilateral femoral defects were divided into Control, HPT, and THPT groups and analyzed after 4 weeks. (B) Representative H&E staining of defect areas. (C) Representative micro-CT images and 3D reconstructions showing mineralized tissue within the defect regions. (D–F) Quantitative micro-CT analysis of BV/TV, Tb.N, and Tb.Sp (n=5). (G) Representative WB images of osteogenesis- and angiogenesis-related proteins. (H–K) Quantification of protein expression levels (n=3). (L–O) RT-qPCR analysis of ALPL, COL1A1, RUNX2, and VEGFA mRNA expression. Data are denoted as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.

Additionally, the THPT scaffold provided suitable mechanical properties alongside sustained TGP-377 release, creating an ideal environment for bone tissue formation and maturation. The scaffold provided a porous structural framework that supported tissue ingrowth and bone regeneration during the observation period. However, the *in vivo* degradation kinetics and replacement of the scaffold by newly formed bone were not directly quantified in the present study.. Biocompatibility evaluations revealed no notable differences in blood routine or biochemical parameters across the groups, indicating that HPT and THPT scaffolds did not induce systemic physiological changes (**Figure 7B–D**). HE staining of major organs (heart, liver, spleen, lungs, kidneys) revealed no pathological changes (**Figure 7A**), confirming the scaffolds' good *in vivo* biocompatibility.

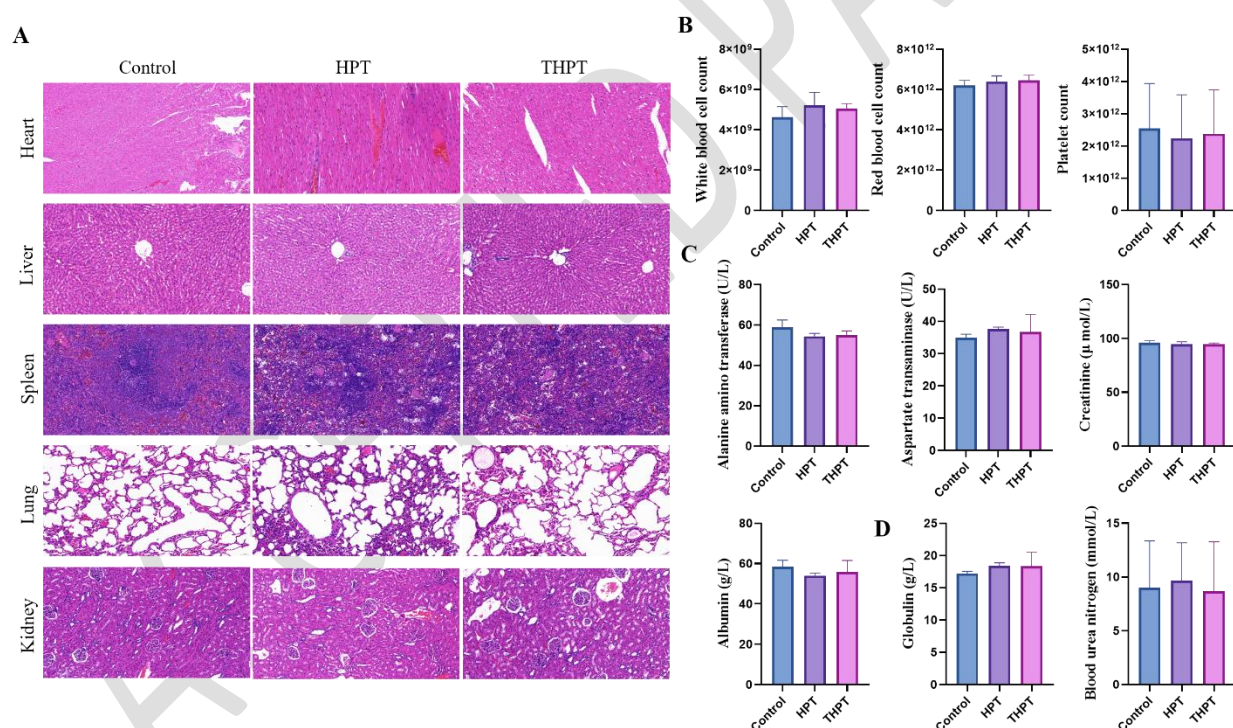


Figure 7. In vivo biosafety evaluation of THPT. (A) HE staining images of major organs, encompassing the heart, liver, spleen, lung, and kidney, from each group. (B) Routine blood test results (n=3). (C) Liver function analysis (n=3). (D) Kidney function analysis (n=3). Data are denoted as mean $\pm$ SD.

4. Discussion

In this study, we developed a multifunctional THPT scaffold by integrating TGP-377-loaded PLGA nanoparticles and a HAMA hydrogel within a porous PLGA/ β -TCP framework. The composite retained interconnected porosity and compressive support while gaining a more compliant mechanical response and a markedly prolonged TGP-377 release profile. In vitro, TGP-377 enhanced the proliferation, migration, and osteogenic differentiation of MC3T3-E1 cells and promoted the proliferation, migration, VEGFA/HIF-1 α expression, and tube formation of HUVECs. In a rabbit femoral defect model, THPT increased mineralized tissue formation, improved trabecular microarchitecture, enhanced osteogenic and angiogenic marker expression, and produced no evident systemic toxicity over the 4-week observation period. Together, these findings indicate that the THPT design can couple local angiogenic stimulation with osteogenic support and thereby improve early bone-defect repair.

Bone repair scaffolds are increasingly designed as multifunctional microenvironment-regulating systems rather than passive defect fillers. Stage-regulative constructs that coordinate osteoimmunomodulation, increased angiogenesis-related marker expression, and osteogenesis, as well as locally delivered small molecules that simultaneously stimulate angiogenesis and osteogenesis, have demonstrated the value of integrating structural and biological functions within a single platform^{43,44}. The THPT scaffold follows this principle: the PLGA/ β -TCP phase provides a porous structural framework, the HAMA hydrogel fills the interconnected pores and improves local cargo retention, and the nanoparticle formulation slows TGP-377 release. The reduction in stiffness after hydrogel incorporation was accompanied by increased resilience, suggesting that the composite achieved a balance between rigid support and deformation tolerance. Such a hybrid architecture may be advantageous for irregular defects because it combines scaffold stability with a hydrated, cell-accessible microenvironment.

TGP-377 was originally identified as a small molecule capable of increasing VEGFA expression through RNA-targeted regulation³¹. The present results extend this concept to bone regeneration: TGP-377 increased VEGFA expression in both osteoblastic and endothelial cells and enhanced HUVEC migration and tube formation in vitro. In bone-defect tissue, THPT treatment was accompanied by increased VEGFA expression. These findings are consistent with a VEGFA-related pro-angiogenic response, although direct vessel visualization and quantitative vascular

assessment were not performed. These observations are biologically plausible because VEGFA is a central mediator of vascularized bone repair. Previous studies showed that VEGFA inhibition reduces angiogenesis-related responses, callus mineralization, and bone formation, whereas VEGFA supplementation promotes angiogenesis and defect bridging⁴⁵; VEGF blockade also impairs vascular invasion and endochondral ossification⁴⁶. In addition, osteoblast-derived VEGFA contributes to vascular recruitment and osteoblast differentiation⁴¹, and deletion of VEGFA in early osteoblast-lineage cells compromises periosteal angiogenesis, woven-bone formation, and fracture healing⁴⁶. Therefore, the coordinated increases in endothelial activity and osteogenic markers observed here support the interpretation that TGP-377 may improve bone repair by creating a VEGFA-enriched microenvironment that facilitates angiogenesis–osteogenesis coupling.

An effective bone substitute must provide an osteoconductive framework while supporting cell recruitment, matrix deposition, and functional tissue maturation⁴⁷. Reciprocal interactions between osteogenic cells and newly formed vessels are essential because vascular networks deliver oxygen and nutrients and provide signals that sustain extracellular matrix synthesis and mineralization^{48,49}. Mechanical stability is also necessary to maintain the defect space and provide an appropriate physical environment for bone formation^{50,51}. In this study, free TGP-377 increased RUNX2, OPN, ALPL, and VEGFA expression in MC3T3-E1 cells in vitro, whereas THPT increased ALPL, COL-I, RUNX2, and VEGFA expression in rabbit bone-defect tissues. Micro-CT further showed higher BV/TV and Tb.N and lower Tb.Sp in the THPT group. Because residual β -TCP is radiopaque and may remain incompletely degraded at 4 weeks, these micro-CT measurements should be interpreted as mineralized tissue-related indices rather than unequivocal new-bone volume. Their agreement with H&E histology nevertheless strengthens the conclusion that THPT promoted early bone regeneration.

Recent biomaterial research has emphasized multifunctional platforms that combine architecture, controlled delivery, and biological regulation. A microgel–hydrogel hybrid system, for example, demonstrated how integrated structural and functional phases can improve mechanical stability and vascularized tissue formation⁵². Within bone repair, Zheng et al. reported an HA15-loaded β -TCP/PLGA scaffold that promoted osteogenesis and vascular regeneration in a rabbit radial defect model⁵³, while Cao et al. combined a PRP-loaded GelMA/AlgMA hydrogel with laponite and a

PCL framework to achieve sustained growth-factor release, vascular ingrowth, and bone regeneration⁵⁴. The present THPT system shares the concept of coupling a mechanically supportive framework with a bioactive delivery phase, but differs by using a small-molecule regulator to enhance endogenous VEGFA expression rather than directly delivering exogenous angiogenic proteins. This approach may offer advantages in chemical stability, manufacturing feasibility, and compatibility with sustained-release biomaterials, although these potential advantages require direct comparative and translational validation.

This study has several limitations. First, although TGP-377 treatment was associated with increased VEGFA expression and enhanced osteogenic and endothelial cellular responses, VEGFA/VEGFR inhibition, cell-specific knockdown, and rescue experiments were not performed. Therefore, the causal contribution of VEGFA-related signaling to the angiogenic and osteogenic effects of THPT remains to be established. Second, direct visualization and quantitative assessment of blood vessels, including CD31/ α -SMA immunostaining, microvessel-density analysis, and vascular perfusion imaging, were not conducted. Accordingly, the increased tissue VEGFA expression observed in this study should be interpreted as an angiogenesis-related molecular response rather than definitive evidence of mature or functional neovascularization. Third, the biological activity of TGP-377 recovered from the THPT release medium was not directly evaluated; therefore, the sustained-release data and the cellular effects observed with free TGP-377 represent complementary but separate lines of evidence. Fourth, the *in vivo* experiment included a single TGP-377 dose, one early 4-week endpoint, and a relatively small animal cohort. In addition, the absence of a bare PLGA/ β -TCP group prevented separate evaluation of the independent contribution of the HAMA hydrogel to bone repair. Fifth, residual radiopaque β -TCP may have contributed to the micro-CT mineralized signals and could not be completely distinguished from newly formed bone using the current analysis. Quantitative histomorphometry, long-term scaffold degradation, wet-state mechanical properties, bone-scaffold replacement, and restoration of functional mechanical strength were also not assessed. Finally, the pharmacokinetics, immune response, long-term local and systemic safety, and efficacy of THPT in larger or load-bearing defect models remain unknown. Future studies should incorporate mechanistic intervention experiments, direct assessment of released TGP-377 bioactivity, multiple doses and observation time points, quantitative vascular and bone histomorphometry, improved

discrimination between residual scaffold and newly formed bone, and long-term evaluation in appropriately powered large-animal models before clinical translation is considered.

5. Conclusion

In this study, a THPT scaffold integrating a porous PLGA/ β -TCP framework with HAMA hydrogel containing TGP-377-loaded PLGA nanoparticles was developed. Free TGP-377 enhanced the proliferation, migration, and osteogenic differentiation of MC3T3-E1 cells and increased the proliferation, migration, and tube-forming activity of HUVECs in vitro. In a rabbit femoral defect model, THPT increased mineralized tissue-related micro-CT indices and osteogenic and angiogenesis-related marker expression at 4 weeks compared with the HPT and Control groups. No obvious abnormalities were observed in the assessed organ histology or blood biochemical parameters during the 4-week observation period. These findings support further investigation of THPT as a local bioactive scaffold for early bone-defect repair. Mechanistic intervention studies, direct evaluation of the bioactivity of released TGP-377, longer observation periods, and quantitative discrimination between residual scaffold and newly formed bone are required before translational conclusions can be drawn.

Acknowledgments

We thank Bullet Edits Limited for the linguistic editing and proofreading of the manuscript.

Funding

This study was supported by Research Project of Jiangsu Provincial Health Commission (M2021063), Jiangsu Provincial Research Hospital (YJXYY202204-YSB10), and Science and Technology Project of Nantong City (grant number MSZ2025139).

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Zeping Gui, Yafeng Zhang

Data curation: Jie Sun, Cao Chen

Formal analysis: Jie Sun, Cao Chen

Funding acquisition: Chen Yao, Yafeng Zhang

Investigation: Jie Sun, Minrui Ji, Bo Zhang

Methodology: Jie Sun, Chen Yao, Minrui Ji, Ruize Wei

Project administration: Yafeng Zhang

Supervision: Zeping Gui, Yafeng Zhang

Visualization: Jie Sun, Cao Chen

Writing—original draft: Jie Sun

Writing—review & editing: Zeping Gui, Yafeng Zhang

Ethics approval and consent to participate

The experiment protocol was approved by the Experimental Animal Care and Use Committee of Nantong University (code S20240110-101).

Consent for publication

Not applicable.

Availability of data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Further disclosure

AI-assisted technology was used only for the preparation of schematic illustrations (Figure 1) in this manuscript. All generated content was carefully reviewed, revised, and approved by the authors. No AI tools were used to generate, analyze, or interpret experimental data, or to draw scientific conclusions.

References

1. Dong Y, Fei Y, Hu Y, Cai C, Li M, Luo Z. Microenvironment engineering with injectable hydrogel-based biofunctional scaffolds for augmenting bone defect regeneration. *J Nanobiotechnology*. Apr 18 2026;doi:10.1186/s12951-026-04378-2
2. Hou Z, Wang Y, Feng C, et al. Comprehensive overview of biomaterials for bone regeneration in cleft lip and palate. *J Mater Chem B*. Oct 8 2025;13(39):12402-12422. doi:10.1039/d5tb01383f
3. Kaiser F, Schroter L, Ignatius A, Gbureck U. Magnesium phosphate minerals and cements as bone substitute materials. *Acta Biomater*. Jan 9 2026;doi:10.1016/j.actbio.2026.01.019
4. Lei W, Wu Y, He P, et al. Osteoclastogenesis-characterized osteoinductive biphasic calcium phosphate ceramic for bone regeneration in rabbit maxillary sinus lift. *J Mater Chem B*. May 21 2025;13(20):5880-5897. doi:10.1039/d4tb02743d
5. Wang L, Wu TH, Hu X, et al. Biomimetic polydopamine-laced hydroxyapatite collagen material orients osteoclast behavior to an anti-resorptive pattern without compromising osteoclasts' coupling to osteoblasts. *Biomater Sci*. Nov 9 2021;9(22):7565-7574. doi:10.1039/d1bm01119g
6. Huang L, Zhang S, Bian M, et al. Injectable, anti-collapse, adhesive, plastic and bioactive bone graft substitute promotes bone regeneration by moderating oxidative stress in osteoporotic bone defect. *Acta Biomater*. May 2024;180:82-103. doi:10.1016/j.actbio.2024.04.016
7. Xu C, Xia Y, Zhuang P, et al. FePSe(3) -Nanosheets-Integrated Cryogenic-3D-Printed Multifunctional Calcium Phosphate Scaffolds for Synergistic Therapy of Osteosarcoma. *Small*. Sep 2023;19(38):e2303636. doi:10.1002/smll.202303636
8. Han S, Yang H, Ni X, et al. Programmed release of vascular endothelial growth factor and exosome from injectable chitosan nanofibrous microsphere-based PLGA-PEG-PLGA hydrogel for enhanced bone regeneration. *Int J Biol Macromol*. Dec 31 2023;253(Pt 1):126721. doi:10.1016/j.ijbiomac.2023.126721

9. Yin S, Zhang W, Zhang Z, Jiang X. Recent Advances in Scaffold Design and Material for Vascularized Tissue-Engineered Bone Regeneration. *Adv Healthc Mater.* May 2019;8(10):e1801433. doi:10.1002/adhm.201801433
10. Mahapatra C, Kumar P, Paul MK, Kumar A. Angiogenic stimulation strategies in bone tissue regeneration. *Tissue Cell.* Dec 2022;79:101908. doi:10.1016/j.tice.2022.101908
11. Min Z, Shichang Z, Chen X, Yufang Z, Changqing Z. 3D-printed dimethyloxallyl glycine delivery scaffolds to improve angiogenesis and osteogenesis. *Biomater Sci.* Aug 2015;3(8):1236-44. doi:10.1039/c5bm00132c
12. Kumar A, Sood A, Singhmar R, Mishra YK, Thakur VK, Han SS. Manufacturing functional hydrogels for inducing angiogenic-osteogenic coupled progressions in hard tissue repairs: prospects and challenges. *Biomater Sci.* Sep 27 2022;10(19):5472-5497. doi:10.1039/d2bm00894g
13. Xu Z, Wang B, Huang R, et al. Efforts to promote osteogenesis-angiogenesis coupling for bone tissue engineering. *Biomater Sci.* May 28 2024;12(11):2801-2830. doi:10.1039/d3bm02017g
14. Kazemian-Najafabadi Z, Hashemi-Najafabadi S, Baghaban-Eslaminejad M, Bagheri F. In Vivo Investigation of Osteogenesis and Angiogenesis of Modified Polycaprolactone-Hydroxyapatite-Zinc Oxide Electrospun Nanofibers Loaded with Metformin. *Ann Biomed Eng.* Sep 2025;53(9):2095-2110. doi:10.1007/s10439-025-03761-8
15. Jin S, Xia X, Huang J, et al. Recent advances in PLGA-based biomaterials for bone tissue regeneration. *Acta Biomater.* Jun 2021;127:56-79. doi:10.1016/j.actbio.2021.03.067
16. Wu J, Wang X, Wang Y, Xun Z, Li S. Application of PLGA in Tumor Immunotherapy. *Polymers (Basel).* Apr 30 2024;16(9)doi:10.3390/polym16091253
17. Wang Y, Zhao L, Zhou L, Chen C, Chen G. Sequential release of vascular endothelial growth factor-A and bone morphogenetic protein-2 from osteogenic scaffolds assembled by PLGA microcapsules: A preliminary study in vitro. *Int J Biol Macromol.* Mar 31 2023;232:123330. doi:10.1016/j.ijbiomac.2023.123330
18. Ni X, Feng J, Liang M, et al. Enhancing Bone Repair with beta-TCP-Based Composite Scaffolds: A Review of Design Strategies and Biological Mechanisms. *Orthop Res Rev.* 2025;17:313-340. doi:10.2147/orr.S525959

19. Xu S, Liu J, Zhang L, Yang F, Tang P, Wu D. Effects of HAp and TCP in constructing tissue engineering scaffolds for bone repair. *J Mater Chem B*. Aug 14 2017;5(30):6110-6118. doi:10.1039/c7tb00790f
20. Huang L, Song Z, Wang J, et al. Absorbable calcium and phosphorus bioactive membranes promote bone marrow mesenchymal stem cells osteogenic differentiation for bone regeneration. *Open Life Sci*. 2024;19(1):20220854. doi:10.1515/biol-2022-0854
21. Go EJ, Kang EY, Lee SK, et al. An osteoconductive PLGA scaffold with bioactive beta-TCP and anti-inflammatory Mg(OH)(2) to improve in vivo bone regeneration. *Biomater Sci*. Feb 4 2020;8(3):937-948. doi:10.1039/c9bm01864f
22. Zhang G, Chen X, Cheng X, Ma W, Chen C. BMSC seeding in different scaffold incorporation with hyperbaric oxygen treats seawater-immersed bony defect. *J Orthop Surg Res*. Apr 13 2021;16(1):249. doi:10.1186/s13018-021-02368-8
23. Zhu Y, Hu J, Luo B, et al. Resonant acoustic mixing-assisted fabrication and evaluation of FDM-printed PLA/beta-TCP scaffolds for bone tissue engineering. *Front Bioeng Biotechnol*. 2025;13:1691481. doi:10.3389/fbioe.2025.1691481
24. Khojasteh A, Fahimipour F, Eslaminejad MB, et al. Development of PLGA-coated beta-TCP scaffolds containing VEGF for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl*. Dec 1 2016;69:780-8. doi:10.1016/j.msec.2016.07.011
25. Zheng W, Bai Z, Huang S, Jiang K, Liu L, Wang X. The Effect of Angiogenesis-Based Scaffold of Mesoporous Bioactive Glass Nanofiber on Osteogenesis. *Int J Mol Sci*. Oct 21 2022;23(20)doi:10.3390/ijms232012670
26. Lv Z, Sun B, Li R, et al. A temporally functional composite hydrogel scaffold for cranial defect repair via sequential modulation of angiogenesis and osteogenesis. *Theranostics*. 2026;16(3):1445-1465. doi:10.7150/thno.119835
27. Sheehy EJ, Miller GJ, Amado I, et al. Mechanobiology-informed regenerative medicine: Dose-controlled release of placental growth factor from a functionalized collagen-based scaffold promotes angiogenesis and accelerates bone defect healing. *J Control Release*. Jun 10 2021;334:96-105. doi:10.1016/j.jconrel.2021.03.031
28. Lu J, Gao Z, He W, Lu Y. Harnessing the potential of hyaluronic acid methacrylate (HAMA) hydrogel for clinical applications in orthopaedic diseases. *J Orthop Translat*. Jan 2025;50:111-128. doi:10.1016/j.jot.2024.11.004

29. Li S, Cui Y, Liu H, et al. Dual-functional 3D-printed porous bioactive scaffold enhanced bone repair by promoting osteogenesis and angiogenesis. *Mater Today Bio*. Feb 2024;24:100943. doi:10.1016/j.mtbio.2024.100943
30. Shen M, Liu H, Shen Z, et al. 3D printing of methacrylated hyaluronic acid/alpha-TCP composite scaffold for bone defect repair. *J Biomater Sci Polym Ed*. Nov 2025;36(16):2451-2471. doi:10.1080/09205063.2025.2503930
31. Haniff HS, Knerr L, Liu X, et al. Design of a small molecule that stimulates vascular endothelial growth factor A enabled by screening RNA fold-small molecule interactions. *Nat Chem*. Oct 2020;12(10):952-961. doi:10.1038/s41557-020-0514-4
32. Gao J, Ren J, Ye H, et al. Thymosin beta 10 loaded ZIF-8/sericin hydrogel promoting angiogenesis and osteogenesis for bone regeneration. *Int J Biol Macromol*. May 2024;267(Pt 1):131562. doi:10.1016/j.ijbiomac.2024.131562
33. Liu L, Zhou N, Fu S, et al. Endothelial cell-derived exosomes trigger a positive feedback loop in osteogenesis-angiogenesis coupling via up-regulating zinc finger and BTB domain containing 16 in bone marrow mesenchymal stem cell. *J Nanobiotechnology*. Nov 19 2024;22(1):721. doi:10.1186/s12951-024-03002-5
34. Jin L, Long Y, Zhang Q, Long J. MiRNAs regulate cell communication in osteogenesis-angiogenesis coupling during bone regeneration. *Mol Biol Rep*. Oct 2023;50(10):8715-8728. doi:10.1007/s11033-023-08709-6
35. Sun J, Chen QZ, Hong AZ, et al. Dissolvable microneedle-assisted transdermal administration of diacerein nanoparticles achieved satisfactory therapeutic effects in tendon-bone insertion repair by reducing oxidative stress and inflammation. *Mater Today Bio*. Aug 2025;33:101999. doi:10.1016/j.mtbio.2025.101999
36. Khan MS, Rehman U, Alqahtani T, et al. Advances in PLGA-based polymeric nanocarriers for colorectal cancer therapy: overcoming chemoresistance through controlled delivery strategies. *Mol Cancer*. Nov 13 2025;24(1):288. doi:10.1186/s12943-025-02435-2
37. Mir M, Ahmed N, Rehman AU. Recent applications of PLGA based nanostructures in drug delivery. *Colloids Surf B Biointerfaces*. Nov 1 2017;159:217-231. doi:10.1016/j.colsurfb.2017.07.038

38. Ren P, Sun W, Wang D, et al. Sustained release PLGA microspheres loaded with a bone-affinity Bmp2 enhance fracture healing and mitigate heterotopic ossification. *Bioact Mater.* Jul 2026;61:796-814. doi:10.1016/j.bioactmat.2026.02.050
39. Rinderknecht H, Nussler AK, Steinestel K, Histing T, Ehnert S. Smoking Impairs Hematoma Formation and Dysregulates Angiogenesis as the First Steps of Fracture Healing. *Bioengineering (Basel).* Apr 24 2022;9(5)doi:10.3390/bioengineering9050186
40. Murahashi Y, Yano F, Nakamoto H, et al. Multi-layered PLLA-nanosheets loaded with FGF-2 induce robust bone regeneration with controlled release in critical-sized mouse femoral defects. *Acta Biomater.* Feb 2019;85:172-179. doi:10.1016/j.actbio.2018.12.031
41. Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone.* Oct 2016;91:30-8. doi:10.1016/j.bone.2016.06.013
42. Buettmann EG, McKenzie JA, Migotsky N, et al. VEGFA From Early Osteoblast Lineage Cells (Osterix+) Is Required in Mice for Fracture Healing. *J Bone Miner Res.* Sep 2019;34(9):1690-1706. doi:10.1002/jbmr.3755
43. Yuan Y, Xu Y, Mao Y, et al. Three Birds, One Stone: An Osteo-Microenvironment Stage-Regulative Scaffold for Bone Defect Repair through Modulating Early Osteo-Immunomodulation, Middle Neovascularization, and Later Osteogenesis. *Adv Sci (Weinh).* Feb 2024;11(6):e2306428. doi:10.1002/advs.202306428
44. Cao H, Li L, Li L, et al. New use for old drug: Local delivery of puerarin facilitates critical-size defect repair in rats by promoting angiogenesis and osteogenesis. *J Orthop Translat.* Sep 2022;36:52-63. doi:10.1016/j.jot.2022.05.003
45. Street J, Bao M, deGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci U S A.* Jul 23 2002;99(15):9656-61. doi:10.1073/pnas.152324099
46. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med.* Jun 1999;5(6):623-8. doi:10.1038/9467
47. Rolvien T, Barbeck M, Wenisch S, Amling M, Krause M. Cellular Mechanisms Responsible for Success and Failure of Bone Substitute Materials. *Int J Mol Sci.* Sep 23 2018;19(10)doi:10.3390/ijms19102893

48. Zhang S, Chen J, Yu Y, Dai K, Wang J, Liu C. Accelerated Bone Regenerative Efficiency by Regulating Sequential Release of BMP-2 and VEGF and Synergism with Sulfated Chitosan. *ACS Biomater Sci Eng*. Apr 8 2019;5(4):1944-1955. doi:10.1021/acsbiomaterials.8b01490
49. Bornert F, Clauss F, Hua G, et al. Mechanistic Illustration: How Newly-Formed Blood Vessels Stopped by the Mineral Blocks of Bone Substitutes Can Be Avoided by Using Innovative Combined Therapeutics. *Biomedicines*. Aug 3 2021;9(8)doi:10.3390/biomedicines9080952
50. Sallent I, Capella-Monsonis H, Procter P, et al. The Few Who Made It: Commercially and Clinically Successful Innovative Bone Grafts. *Front Bioeng Biotechnol*. 2020;8:952. doi:10.3389/fbioe.2020.00952
51. Zhang S, Jiang G, Prabhakaran MP, Qin X, Ramakrishna S. Evaluation of electrospun biomimetic substrate surface-decorated with nanohydroxyapatite precipitation for osteoblasts behavior. *Mater Sci Eng C Mater Biol Appl*. Oct 1 2017;79:687-696. doi:10.1016/j.msec.2017.05.113
52. Wang X, Liu X, Li K, et al. A Microgel-Hydrogel Hybrid for Functional Compensation and Mechanical Stability in 3D Printed Cell-Dense Vascularized Liver Tissue. *Adv Mater*. Jul 2025;37(28):e2413940. doi:10.1002/adma.202413940
53. Zheng C, Attarilar S, Li K, et al. 3D-printed HA15-loaded beta-Tricalcium Phosphate/Poly (Lactic-co-glycolic acid) Bone Tissue Scaffold Promotes Bone Regeneration in Rabbit Radial Defects. *Int J Bioprint*. 2021;7(1):317. doi:10.18063/ijb.v7i1.317
54. Cao B, Lin J, Tan J, et al. 3D-printed vascularized biofunctional scaffold for bone regeneration. *Int J Bioprint*. 2023;9(3):702. doi:10.18063/ijb.702

Appendix

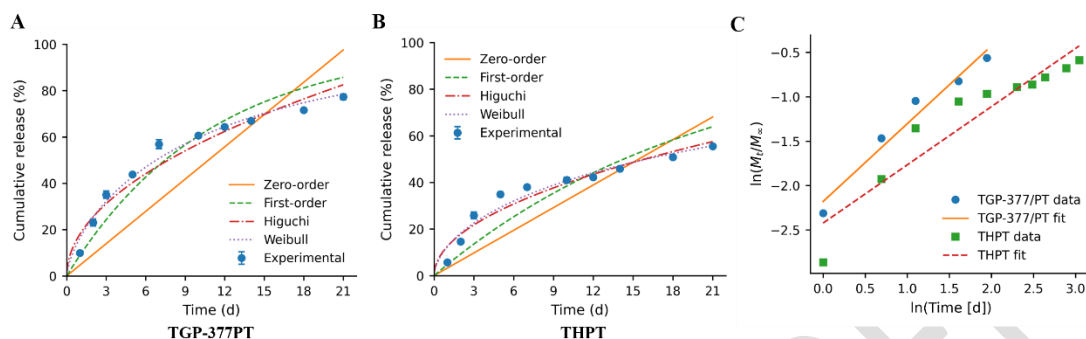


Figure A1. Release kinetic analysis of TGP-377 from TGP-377/PT and THPT scaffolds. (A, B) Experimental cumulative release profiles of TGP-377 from TGP-377/PT and THPT scaffolds, respectively, fitted using the zero-order, first-order, Higuchi, and Weibull models. Experimental data are presented as the mean $\pm$ SD from three independent samples ($n = 3$). (C) Korsmeyer–Peppas analysis of the TGP-377 release profiles. The model was fitted using data points corresponding to cumulative drug release $\leq 60\%$. Symbols represent the experimental data, and lines represent the corresponding fitted curves.