

RESEARCH ARTICLE

Selective laser melting-fabricated porous tantalum scaffold integrated with nano-MgO/alginate hydrogel for enhanced osteogenesis and immunomodulation in critical-size bone defect repair

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Abstract

The effective repair of critical-sized bone defects remains a major challenge in bone tissue engineering. While porous tantalum (pTa) scaffolds fabricated by selective laser melting offer excellent mechanical compatibility and structural stability, their inherent biological inertness limits their osteogenic potential. This study aims to develop a functionally enhanced composite scaffold by filling three-dimensional-printed pTa with an alginate hydrogel incorporated with nano-magnesium oxide particles (pTa@SA-MgO). The hydrogel acted as a carrier for controlled Mg²⁺ ion release. Comprehensive *in vitro* and *in vivo* evaluations revealed that this composite scaffold significantly promoted osteogenic activities, including MC3T3-E1 cell proliferation, migration, adhesion, and differentiation (as evidenced by increased alkaline phosphatase activity, mineralized nodule formation, and upregulation of *Runx2*, *Ocn*, *Opn*, and *Col1a1* gene expression). Moreover, it modulated the immune microenvironment by enhancing M2 macrophage polarization and suppressing M1 macrophage polarization. In a rabbit femoral defect model, the pTa@SA-MgO scaffold demonstrated superior bone regeneration compared to controls, with greater bone volume and enhanced new bone formation. This research successfully integrates a sustained ion release strategy with a high-performance metallic scaffold, providing new insights into the design of bone repair materials with synergistic mechanical support and bioactive functionality.

Keywords: Three-dimensional-printed porous tantalum; Nano-magnesium oxide; Sodium alginate hydrogel; Bone defect

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1. Introduction

The repair of critical-sized bone defects has long remained one of the major challenges in the field of bone tissue engineering.¹ Autografts and allografts are currently the most commonly used clinical treatments; however, their application in the repair of

critical-sized bone defects is significantly limited by donor shortage, surgical trauma, immune rejection, and the risk of infection.^{2,3}

With the rapid development of regenerative medicine and materials science, bone tissue engineering has provided new strategies to address these challenges.⁴ A wide range of scaffold materials (including metals, ceramics, polymers, hydrogels, and their composites), stem cells (primarily bone marrow mesenchymal stem cells, adipose-derived stem cells, and periosteal stem cells), and bioactive factors (e.g., cytokines, drugs, and metal ions) have been extensively investigated for the repair of critical-sized bone defects.⁵⁻⁷

Additive manufacturing technologies have become an ideal approach for the fabrication of tissue engineering scaffolds due to their advantages in personalized customization and the precise fabrication of complex porous architectures.⁸ Metallic materials exhibit excellent overall mechanical properties and are particularly suitable for the repair of critical-sized bone defects in load-bearing sites.^{9,10} Among various metallic materials, titanium alloys have attracted considerable attention, and porous titanium alloy scaffolds fabricated by selective laser melting and electron beam melting have been widely studied for bone defect repair.¹¹ Tantalum (Ta), owing to its unique physicochemical properties and excellent biocompatibility, has been widely applied in bone repair and joint reconstruction.^{12,13} Our previous studies demonstrated that, compared to Ti6Al4V, Ta implants induce less stimulation of the surrounding bone tissue and elicit a lower inflammatory response *in vivo*, resulting in significantly enhanced peri-implant bone regeneration.¹⁴

The elastic modulus of porous Ta (pTa) matches that of natural bone, effectively reducing the stress-shielding effect after implantation while also demonstrating excellent corrosion resistance and structural stability.¹⁵ In particular, with the maturation of selective laser melting (SLM) technology, researchers are able to precisely control the pore size, porosity, and geometric architecture of tantalum scaffolds through three-dimensional (3D) printing, thereby improving cell adhesion, nutrient transport, and osseointegration. However, a single material is rarely able to simultaneously satisfy all the requirements of an ideal bone tissue engineering scaffold, including biomechanical performance, osteogenic and angiogenic activity, as well as immunomodulatory capacity.¹⁶ Introducing biodegradable fibers or hydrogel fillers into the pores of 3D-printed porous scaffolds to form composite scaffolds—where the fillers can serve as carriers for cells, cytokines, drugs, and other bioactive factors with osteogenic and immunomodulatory functions—has emerged as a promising strategy for

achieving large-scale bone tissue regeneration.^{17,18} Composite scaffold materials not only optimize the physicochemical properties of scaffolds but also endow them with multiple biological functions, thereby achieving superior overall performance.¹⁹ Extensive research has focused on the fabrication of composite scaffolds by incorporating various fillers into 3D-printed porous scaffolds, including calcium phosphates, silicates, metals, bioactive molecules, collagen, hydrogels, and other organic materials.²⁰

Among various bioactive agents, metal ions—including Mg^{2+} , Sr^{2+} , Ga^{3+} , and Zn^{2+} —have attracted widespread attention due to their stability, reliability, and cost-effectiveness.²¹ Early studies primarily attempted to endow metallic scaffolds with osteogenic, angiogenic, and antibacterial functions by constructing surface coatings containing various metal ions.²² Most of these metal ions are incorporated into calcium phosphates to form bioactive coatings on metallic scaffolds, from which the ions are released into the peri-implant microenvironment via coating degradation to exert their biological effects.²³ Although this strategy improves the biological activity of metallic scaffolds, the amount of metal ions loaded within the coating is extremely limited, and the coating thickness is typically only a few to several tens of micrometers. Consequently, release kinetics studies have shown that the concentration of bioactive metal ions released from coating-functionalized porous metal scaffolds is often far below the optimal range required to exert maximal biological effects.²⁴ For example, magnesium-containing coatings typically exhibit an average daily Mg^{2+} release of only several parts per million (ppm), which is substantially lower than the optimal concentration of approximately 50 ppm.²⁵

Among various carriers for bioactive factors, hydrogels exhibit excellent biocompatibility, and their structural and compositional diversity provides suitable spatial architectures and customizable release kinetics for bioactive agent loading.²⁶ Owing to their wide availability, good biocompatibility, injectability, and gelation capability, hydrogels have been widely employed in controlled release systems for drugs, ions, and growth factors.²⁷ Their three-dimensional porous network structure not only enables the loading of nano-scale bioactive components but also provides a favorable microenvironment for tissue regeneration.²⁸

Nano-magnesium oxide (MgO), as a novel bioactive inorganic material, has gained increasing attention due to its excellent biocompatibility, stability, and controllable ion release characteristics.²⁹ Accumulated evidence indicates that optimal Mg^{2+} concentrations exert regulatory effects

on osteoblast behaviors including adhesion, spreading, and differentiation. These ions elevate alkaline phosphatase (ALP) activity and stimulate the expression of key osteogenic markers such as Runx2, collagen type I (COL I), and osteocalcin (OCN). Furthermore, Mg^{2+} facilitates vascularization and bone regeneration by tuning the local microenvironmental pH and regulating intracellular signaling pathways.³⁰ In addition, MgO exhibits intrinsic antibacterial and anti-inflammatory properties, which contribute to improving the immune microenvironment at the implantation site and thereby facilitate bone repair.^{31–33}

On the other hand, embedding nano-MgO particles into alginate hydrogels enables sustained Mg^{2+} ion release, thereby prolonging the duration of bioactivity and avoiding irritation or potential toxicity caused by burst ion release.³⁴ Therefore, integrating MgO-loaded hydrogels with pTa scaffolds is expected to simultaneously provide mechanical support, controlled ion release, and bioactive regulation.³⁵

Based on these considerations, this study constructed—for the first time—a composite scaffold composed of a nano-MgO-loaded hydrogel integrated with a 3D-printed pTa scaffold.³⁶ Alginate hydrogel served as a carrier for nano-MgO, enabling the sustained release of nano-MgO particles into the peri-implant region through alginate degradation, where MgO subsequently degraded in the physiological environment to generate Mg^{2+} ions.³⁷ By adjusting the loading amount of MgO, the release kinetics of Mg^{2+} ions from the composite scaffold were regulated to achieve concentrations within the optimal range for maximal biological efficacy.³⁸ This study aims to combine an active ion regulation strategy with high-performance metallic scaffolds to develop a novel bone repair material featuring both mechanical stability and tunable biological functionality, thereby providing experimental evidence and theoretical support for the functional design and clinical translation of bone tissue engineering scaffolds.

2. Materials and methods

2.1. Materials preparation

The spherical tantalum powder, with a purity of more than 99.95 wt%, was sourced from Stardust Technology Co., Ltd (China). pTa samples featuring a diamond-shaped pore architecture and 75% porosity, with an average pore size of around 500 μm , were fabricated using SLM (Renishaw AM400, Renishaw, United Kingdom). The printing process was carried out on a Ti-6Al-4V substrate in an atmosphere of high-purity argon, with oxygen levels in the chamber kept under 100 ppm. SLM processing was conducted with a laser power set at 260 W, a point distance of 50 μm , an exposure time of 100 μs , a layer thickness of 30 μm , and a hatch spacing of 65 μm . The samples were

subjected to a heat treatment at 1,100 °C for 2 h under a vacuum of 10^{-2} Pa after printing, followed by sandblasting to remove Ta powder that had not melted. The samples were then subjected to ultrasonic cleaning in acetone, absolute ethanol, and ultrapure water for 15 min each and subsequently dried for further use.

The above SLM fabrication parameters were adopted directly from our previous studies. The SLM manufacturing process, printing quality, relative density, and microstructure of tantalum scaffolds have been systematically characterized and validated in our previously published work; therefore, repetitive analyses were not performed in this study.^{39,40}

Samples were prepared in different sizes according to experimental requirements: discs with a diameter of 10 mm and a thickness of 3 mm for release and cell experiments; cylinders with a diameter of 4 mm and a thickness of 8 mm for animal experiments; and cylinders with a diameter of 10 mm and a height of 15 mm for mechanical testing.

2.2. Sample preparation

At room temperature, 0.2 g of sodium alginate (SA) was dissolved in 10 mL of deionized water under magnetic stirring. Subsequently, MgO particles (0 g, 0.8 g, 1.2 g, and 1.6 g) were added to the solution under vigorous stirring. After complete dispersion, 200–400 μL of calcium chloride solution was added to induce pre-crosslinking, and the mixture was further stirred for 10 min at room temperature until a composite hydrogel was formed. The composite hydrogel was then injected into the pTa scaffolds to form composite scaffolds.

Scaffold extracts were obtained by immersing the composite scaffolds in culture medium at a 0.2 g/mL solid–liquid ratio and incubating them on a shaker for three days. The extracts were centrifuged at a speed of 1,000 r/min for 5 min, and the supernatant was filtered through a 0.22 μm membrane to yield sterile scaffold extracts for further experimentation.

2.3. Material characterization

The elemental composition and distribution on the scaffold surface were analyzed using scanning electron microscopy (Hitachi 3500, Hitachi, Japan) equipped with energy-dispersive X-ray spectroscopy (EDS; Oxford X-Max, Oxford Instruments, United Kingdom). The elemental composition of dense disc samples was determined using X-ray fluorescence spectroscopy (Analytical Zetium, Malvern Panalytical, the Netherlands). Phase composition was analyzed by X-ray diffraction (XRD; Rigaku D/Max 2500, Rigaku Corporation, Japan) using Cu K α radiation ($\lambda = 1.5406$ Å). Surface elemental composition and

chemical states were characterized by X-ray photoelectron spectroscopy (Thermo Scientific Escalab 250XI, Thermo Fisher Scientific, USA).

The pTa, pTa@SA, and pTa@SA-MgO scaffolds were immersed in phosphate-buffered saline (PBS; HyClone, Cytiva, USA) at a solid-to-liquid ratio of 0.2 g/mL and incubated at 37°C under gentle shaking. At predetermined time points, the supernatant was collected and replaced with an equal volume of fresh PBS to continue incubation. The collected solutions were filtered through a 0.22 µm syringe filter and acidified prior to analysis. The concentration of Mg²⁺ ions was quantified using inductively coupled plasma optical emission spectrometry, and the cumulative Mg²⁺ release was calculated accordingly.

To evaluate the effect of the composite scaffolds on the pH of the surrounding medium under a simulated physiological environment, the scaffolds were immersed in 5 mL of PBS (pH 7.2; Cytiva, USA). The supernatant was collected on days 1, 2, 3, 5, 7, 10, and 14, and an equal volume of fresh PBS was added after each sampling. The pH value of the collected supernatant was measured using a pH meter (PHS-3E, INESA Scientific Instrument Co., Ltd., China).

2.4. *In vitro* cell experiments

2.4.1. Cell culture

Mouse preosteoblast MC3T3-E1 and monocyte/macrophage RAW264.7 cells from the Cell Bank of the Chinese Academy of Sciences (China) were used for *in vitro* experiments. MC3T3-E1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; HyClone, Cytiva, USA) or in F-12 (F-12; HyClone, Cytiva, USA), and RAW264.7 cells were cultured in high-glucose DMEM. Both media were supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, USA) and 1% penicillin-streptomycin (No. BMC1030, Abbkine Scientific Co., Ltd., China). Cells were incubated at 37 °C in a 5% CO₂ incubator with sufficient humidity, and used for experiments after two passages.

2.4.2. Cell proliferation assay

MC3T3-E1 cells were cultured in DMEM/F-12 containing 10% FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO₂ incubator. Cells were passaged/cryopreserved every 2–3 days, and passaged using 0.25% trypsin (No. 25200056, Gibco, Thermo Fisher Scientific, USA). For proliferation detection, Cell Counting Kit-8 (CCK-8; No. CK04, Dojindo Molecular Technologies, Inc., Japan) assay was adopted. Cells (5 × 10³/well) were seeded in 48-well plates and cultured with sample extracts (prepared per ISO 10993-12:2012, 3 cm²/mL, three days of incubation).

Medium was renewed every two days, and optical density (OD) values were detected via microplate reader at day 1, 4 and 7.

2.4.3. Osteoblast viability assay

On days 1, 4, and 7 after exposure to the extracts, cells were gently rinsed twice with PBS and stained using a Calcein-AM/PI Live/Dead Cell Staining Kit (No. CA1630., Dojindo, Japan), followed by imaging under a fluorescence microscope (TXM-500C, Shanghai Zhongchen Digital Technology Equipment Co., China). The staining solution was prepared by adding 2 µL of Calcein acetoxymethyl ester and 3 µL of propidium iodide to 1 mL of PBS.

2.4.4. Cell spreading assay

MC3T3-E1 cells (2 × 10⁴ cells/well) were seeded in 48-well plates and cultivated with extracts from pTa, pTa@SA, and pTa@SA-MgO. Cells collected at 2 h and 24 h were rinsed twice with PBS and fixed with precooled 4% paraformaldehyde in the dark for 15–30 min. After PBS washing, 0.1% Triton X-100 (No. T8200, Beijing Solarbio Science & Technology Co., Ltd., China) was used for permeabilization for 30 min, followed by triple PBS washing.

Each well received 100 µL AbFluor594-Phalloidin (Abbkine, China) for 30 min staining at room-temperature, followed by triple PBS washing. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Coolaber, China) for 5 min, washed again, and maintained in PBS. Cellular spreading was visualized using a TXM-500C inverted fluorescence microscope.

2.4.5. Wound healing assay

MC3T3-E1 cells (3–5 × 10⁵/well) were cultured overnight in 6-well plates to full confluence. Uniform scratches were made using sterile 200 µL pipette tips; detached cells were removed by PBS washing, and cells were incubated with different scaffold extracts.

Reference marks were made on the bottom of the plate to ensure that images were captured at the same locations at each time point. Images were immediately acquired at 0 h as the baseline and subsequently at 6 h and 24 h during continued incubation. At least three randomly selected fields of view were recorded per well, with a minimum of three technical replicates and three biological replicates for each group.

The acquired images were analyzed using ImageJ software (version 1.54p, National Institutes of Health, USA). The region of interest was defined based on the wound boundary at 0 h, and the wound width or cell-free area was quantified using the “Wound Healing Tool/MRI

Wound Healing” plugin to calculate the wound closure rate.

2.4.6. Immunofluorescence staining

MC3T3-E1 cells were seeded into 48-well plates at a density of 2×10^4 cells per well and cultured overnight in medium supplemented with 10% FBS and 1% penicillin-streptomycin. The medium was then replaced with osteogenic induction extracts from pTa, pTa@SA, or pTa@SA-MgO, which were refreshed every two days during the culture period.

Alkaline phosphatase immunofluorescence staining was performed on day 7. Briefly, the cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.2% Triton X-100 for 20 min, and subsequently blocked with 10% goat serum (Beyotime, China) for 20 min. The cells were then incubated with a primary antibody against ALP (ab83369, Abcam, United Kingdom) overnight at 4 °C. After washing with PBS, a fluorescently labeled secondary antibody (Alexa Fluor 488; ab150077, Abcam, United Kingdom) was applied and incubated at room temperature for 60 min in the dark. Following PBS washes, cell nuclei were counterstained with DAPI (Beyotime, China) for 10 min. Finally, fluorescence images were acquired using a fluorescence microscope, and quantitative analysis was performed using ImageJ software version 1.51.

2.4.7. Osteogenic differentiation assay

Osteogenic medium was formulated with scaffold extracts supplemented with Sigma additives (50 µg/mL ascorbic acid, 10 mM β-glycerophosphate disodium, and 10 nM dexamethasone). MC3T3-E1 cells (8×10^3 /well) were seeded in 12-well plates. After overnight cultivation, original medium was substituted with the three types of induction extracts. Medium volume was increased to 2 mL on day 3 and 3 mL on day 5, renewed every two days until day 10, and then refreshed daily under light protection. ALP staining using a Solarbio BCIP/NBT kit (PR1100, Beijing Solarbio Science & Technology Co., Ltd., China) was performed on days 7 and 14, and images were captured

using an XD202 optical microscope (XD202, China).

2.4.8. Alizarin red S staining

After overnight incubation, MC3T3-E1 cells (5,000 cells/well in 12-well plates) received 1 mL of osteogenic extracts from the three scaffolds. Medium volume was adjusted to 2 mL on day 3 and 3 mL on day 5, refreshed every two days until day 10 and then replaced daily under light protection. Mineralized nodules were stained with 2% alizarin red S (Beyotime, China) at days 14 and 21 and photographed using XD202 optical microscope.

2.4.9. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qPCR) was used to detect osteogenic gene expression in MC3T3-E1. Cells (3×10^4 /well) were cultured overnight in 6-well plates before replacement of the medium extracts from the three scaffolds (2 mL/well). Medium volume was increased to 3 mL on day 5 and 4 mL on day 7, renewed every two days, and refreshed daily starting from day 10. Cell samples collected on days 7 and 14 were subjected to total RNA isolation using the AG21022 kit (AG21022, Accurate Biotechnology Co., Ltd., China). RNA quantification was measured using a NanoDrop One (Thermo Scientific, USA), and reverse transcription into complementary DNA was performed using Takara RR047A reagents (RR047A, Takara Bio Inc., Japan) on an Eppendorf thermal cycler. qPCR was performed using Takara TB Green Premix (RR820A, Takara Bio Inc., Japan) on an ABI 7500 Fast Dx instrument (Applied Biosystems, Thermo Fisher Scientific, USA). *Gapdh* served as the housekeeping gene for normalization of *Runx2*, *Ocn*, *Opn*, and *Col1a1*; all primers were custom-synthesized by Accurate Biology. The synthesized primers are shown in Table 1.

2.4.10. Macrophage immunofluorescence staining

RAW264.7 macrophage cells were cultured in high-glucose DMEM supplemented with 10 vol% FBS, 1% penicillin-streptomycin, and 1% sodium pyruvate (SL6070, Beijing Coolaber Science & Technology Co., Ltd., China). Cells

Table 1. Primer sequences for quantitative real-time polymerase chain reaction used to detect osteogenic gene expression in MC3T3-E1 cells

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')
<i>Gapdh</i>	GCCTCCTCCAATTCAACCCTT	CCAAATCCGTTACACCCGAC
<i>Ocn</i>	GAACAGACAAGTCCACACAGC	TCAGCAGAGTGAGCAGAAAGAT
<i>Opn</i>	ATCCTTGCTTGGGTTTGACG	ACTGCCAATCTCATGGTCGT
<i>Runx2</i>	AGCGGACGAGGCAAGAGTTT	AGGCGGGACACCTACTCTCAT
<i>Col1a1</i>	CTCCCAGAACATCACCTATCACT	GGAGGTCTTGGTGGTTTGTATT

were collected using a cell scraper and passaged or cryopreserved every 2–3 days.

RAW264.7 cells (3×10^4 /dish) were seeded in 15 mm confocal dishes. After overnight culture, original medium was replaced with 2 mL of different scaffold extracts for a 48 h incubation period. Cells were rinsed with PBS, fixed with pre-cooled 4% paraformaldehyde on ice in the dark, then permeabilized using 0.3% Triton X-100, and finally blocked with 10% goat serum. Diluted primary antibodies against arginase 1 (Arg-1; 16001-1-AP, Proteintech Group, Inc., USA) and C-C motif chemokine receptor (CCR7; 1:200; No. 25898-1-AP, Proteintech, USA) were incubated with cells overnight at 4 °C. After PBS rinsing, matching Alexa Fluor 488/594-conjugated secondary antibodies (1:200; 25898-1-AP, Abcam, United Kingdom) were applied and kept in the dark for 2 h at ambient temperature. Nuclei were DAPI-stained after washing, and fluorescent images were captured by a Zeiss CLSM510 confocal microscope (Zeiss, Germany).

2.4.11. Expression of angiogenesis- and macrophage polarization-related genes

To quantify M1/M2 marker gene expression in RAW264.7 macrophages, qPCR was employed. Cells were cultured in 10 cm dishes to 80% confluence, then treated with 10 mL extracts of pTa, pTa@SA, and pTa@SA-12MgO scaffolds. The medium was adjusted to 15 mL on day 4, with daily renewal of fresh extracts. After seven days of incubation, experiments were carried out as described in Section 2.4.9. *Gapdh* was used as the internal control gene. *Arg1* and *Nrros* were M2 markers, while *Nos2* and *Il6* were M1 markers. All primers were provided by Accurate Biology, with sequences listed in Table 2.

2.5. In vivo experiments

2.5.1. Establishment of the rabbit bone defect model

All *in vivo* animal operations were ethically reviewed and approved by the Animal Welfare and Ethics Committee of

Zhongshan Hospital, Dalian University (No. DW2025-008-01). Based on the findings of preliminary *in vitro* assays, pTa@SA-MgO scaffolds were set as the experimental group, and conventional pTa scaffolds with stable comprehensive properties were adopted as the control group.

Five-month-old male Japanese white rabbits ($n = 18$) were selected as experimental animals, with six rabbits allocated to each group. Before surgical operation, all rabbits were weighed and acclimatized until their physiological status stabilized. General anesthesia was performed via intravenous injection through the marginal ear vein of 3% pentobarbital sodium at a dosage of 25 mg/kg. After successful anesthesia, the surgical site was shaved and disinfected with povidone-iodine solution. A surgical incision was made to expose the femoral tissue, and a standardized bone defect with a diameter of 3 mm was fabricated using a matched drill bit. Sterilized cylindrical scaffolds (3 mm diameter, 8 mm height) including pTa, pTa@SA, and pTa@SA-MgO were implanted into the prepared bone defects. Each group contained six rabbits, which were divided into two observation time points (6 weeks and 12 weeks), with three rabbits sacrificed for sampling at each time point.

After implantation, surgical incisions were sutured, and intramuscular gentamicin was continuously administered for three days to avoid postoperative infection. The postoperative health status of all experimental rabbits was monitored daily. At 6 and 12 weeks post-implantation, three rabbits in each group were euthanized, and femoral specimens containing scaffold implants were collected for subsequent biological and morphological analysis.

2.5.2. Micro-computed tomography analysis

Femoral samples were harvested after euthanasia and fixed in 75 vol% ethanol. Micro-computed tomography (micro-CT; Inveon CT, Siemens, USA) was applied to evaluate peri-implant bone regeneration 6 and 12 weeks after implantation. Scanning parameters were as follows: 80

Table 2. Primer sequences for quantitative real-time polymerase chain reaction used to quantify M1 and M2 marker gene expression in RAW264.7 macrophages

Gene	Forward primer sequence (5'–3')	Reverse primer sequence (3'–5')
<i>Gapdh</i>	GCCTCCTCCAATTCAACCCTT	CCAAATCCGTTACACCGAC
<i>Arg1</i>	AGCCAGGGACTGACTACCTT	TTGGGAGGAGAAGGCGTTTG
<i>Nrros</i>	AAGACTGAGCACTGACCCTC	TGTCAGACCAACGTTGAGGAG
<i>Nos2</i>	CTGCCAGGGTCACAACCTTTACA	AACAGCTCAGTCCCTTCACC
<i>Il6</i>	GTCCTTCCTACCCCAATTTCCA	TGGTCTTGGTCCTTAGCCAC

kV, 500 μ A, 1,000 ms exposure, 1-frame average, binning factor 1, pixel size of 9.41 μ m, and 360° rotation with 180 steps. Images were reconstructed in three dimensions using Inveon Acquisition Workplace software (scaling factor = 2; version 1.2, Siemens Preclinical Solutions, USA). Bone volume fraction (BV/TV) and trabecular number (Tb.N) were then quantitatively analyzed.

2.5.3. Fluorochrome labeling

Fluorochrome labeling was implemented before animal euthanasia to dynamically monitor the *in vivo* osteogenesis process. Specifically, 30 mg/kg alizarin red S (ARS; Coolaber, China) was intramuscularly injected two weeks prior to sample collection. This reagent was pre-dissolved in PBS and sterilized via membrane filtration before use. One week before euthanasia, 25 mg/kg calcein (Coolaber, China) was delivered through intramuscular administration, which was prepared with PBS containing 2% sodium bicarbonate. Subsequent to micro-CT analysis, bone tissues containing implants were isolated and cut using a specialized hard tissue slicer. The harvested specimens underwent gradient ethanol dehydration using a total solvent volume approximately 50 times the sample volume. A series of ethanol concentrations (75%, 85%, 95%, and 100%) were applied for 10 days each, with the ethanol solution being renewed every 48 h. Following complete dehydration, non-decalcified hard tissue sections were fabricated through continuous grinding and polishing procedures. Ultimately, a fluorescence microscope was utilized to observe and capture images of the fluorochrome distribution, enabling comparative analysis of new bone growth across different experimental groups at various stages.

2.5.4. Van Gieson staining

Van Gieson histochemical staining was carried out on polished non-decalcified hard tissue slices. Specimens were incubated with Servicebio toluidine blue at 60 °C for more than 15 min and washed with pre-warmed distilled water. Afterwards, sections were submerged in acid fuchsin (Macklin, China) for 10 min at ambient temperature, with anhydrous ethanol applied to achieve proper color differentiation. Prepared slices were then imaged under a microscope.

2.5.5. Hematoxylin and eosin staining

Peri-implant bone tissues were trimmed into 1–2 mm fragments and decalcified in 10% ethylenediaminetetraacetic acid solution (Coolaber, China). The decalcifying liquid was prepared at a 10:1 liquid-to-tissue ratio and adjusted to pH 7.3 with sodium hydroxide (Macklin, China); the ethylenediaminetetraacetic acid solution

was renewed every two days throughout the two-month decalcification period. Specimens underwent stepwise ethanol dehydration (75%, 85%, 95%, and 100%; one day per gradient), xylene transparency (Damao AR, China; 7 h), overnight paraffin infiltration, and an additional 4 h immersion in fresh paraffin for embedding.

Obtained paraffin sections were subjected to drying, deparaffinization, and rehydration. After incubation in a humidified chamber, samples were stained with hematoxylin (Servicebio, China) for 10 s at ambient temperature and washed under running tap water for 20 min, followed by 15 s eosin counterstaining (Servicebio, China). Serial dehydration, clearing, and mounting were performed afterwards, and finished sections were observed and imaged using a light microscope.

2.5.6. Immunohistochemistry

The paraffin sections were deparaffinized and rehydrated, then processed with a universal two-step immunohistochemistry detection kit (mouse/rabbit enhanced polymer detection system; PV-9000, ZSGB-BIO, China) following the manufacturer's instructions. The sections were incubated with primary antibodies for osteocalcin (MA5-43930, Thermo Fisher Scientific, USA; 10 μ g/mL) and COL I (1:200; 14695-1-AP, Proteintech, USA). Subsequently, freshly prepared diaminobenzidine substrate solution (ZSGB-BIO, China) was added and incubated at room temperature for 5–8 min to visualize immunoreactivity. Hematoxylin was used for nuclear counterstaining for 20 s, then briefly differentiated in a 1% hydrochloric acid–ethanol solution, followed by a 2-min rinse under running tap water. Following dehydration, clearing, and mounting, the sections were prepared for microscopic observation. Images were subsequently obtained using an optical microscope.

2.5.7. Immunofluorescence staining of paraffin sections

Paraffin sections were treated with ultraviolet light for at least 4 h to remove residual autofluorescence. After deparaffinization and rehydration, they were blocked with goat serum and permeabilized with 0.1% Triton X-100 for 1 h. After application of primary antibodies against CCR7 and Arg-1 diluted 1:50 in PBS, the sections were incubated overnight at 4 °C in a humidified chamber. They were then washed in PBS on a shaker the following day. Following the washing step, sections incubated with the Arg-1 primary antibody were exposed to a goat anti-rabbit IgG H&L secondary antibody conjugated to Alexa Fluor 488 (1:50; ab150077, Abcam, United Kingdom). After incubation with the CCR7 primary antibody, sections were treated with a goat anti-rabbit IgG H&L secondary

antibody conjugated to Alexa Fluor 594 (1:50; ab150080, Abcam, United Kingdom) and kept in the dark at room temperature for 2 h. After washing with PBS, the sections were stained with DAPI for 10 min in the dark. Subsequent PBS washes were performed before dehydration and mounting, and images were obtained using a fluorescence microscope (TFM680, Nikon, Japan).

2.6. Statistical analysis

GraphPad Prism version 9 software (GraphPad Software, USA) was used for all statistical analyses, while ImageJ software (version 1.54p) was employed for image quantification. Quantitative data are presented as mean $\pm$ standard deviation. Differences between groups were analyzed using two-way ANOVA ($n = 3$) or one-way ANOVA ($n = 3$), followed by Tukey's post hoc test. A p -value of less than 0.05 was considered statistically significant.

3. Results

3.1. Material characterization

Figure 1A shows SA hydrogels containing different concentrations of MgO (0 wt%, 8 wt%, 12 wt%, and 16 wt%) and the corresponding composite scaffolds formed after infiltration of the hydrogels into the pores of pTa scaffolds. Figure 1B presents the compressive stress-strain curves of the pTa scaffold and pTa@SA-12MgO. The compressive yield strengths of the two scaffolds were 41.5 ± 1.6 MPa and 40.9 ± 2.0 MPa, respectively, with no significant difference between them. The infiltration of MgO-containing SA hydrogel hardly altered the scaffold's mechanical performance.

Figure 1C–F displays the microstructures of the composite scaffolds composed of pTa infiltrated with SA hydrogels containing different concentrations of nanosized MgO. The MgO-loaded SA hydrogels completely filled the interconnected pores of the pTa scaffold. EDS analysis (Figure 1G–J) revealed that the Mg content in the hydrogels increased markedly with increasing MgO loading. The Mg weight percentages in the pTa@SA-8MgO, pTa@SA-12MgO, and pTa@SA-16MgO groups were 21.74%, 26.50%, and 37.31%, respectively.

Elemental mapping results (Figure 1K) further demonstrated the homogeneous distribution of O, Mg, Ta, and Ca on the surface of the composite scaffolds, with no evident elemental aggregation.

Figure 2A displays the XRD spectra of pure pTa scaffolds and their surface-modified composite counterparts. Apart from the characteristic diffraction signals of Ta, newly emerged peaks assigned to SA were clearly observed in

the pTa@SA group, confirming the successful fabrication of the SA coating on the scaffold surface. For the pTa@SA-MgO group, new diffraction peaks appeared at approximately $2\theta = 43^\circ$ and 62° , which can be indexed to the (200) and (220) crystal planes of MgO (PDF#45-0946), confirming the successful incorporation of MgO. Overall, the formation of the composite coating did not alter the crystal structure of the tantalum substrate, indicating that the scaffold maintained good structural stability.

Figure 2B illustrates the Fourier transform infrared spectra of pTa@SA and pTa@SA-MgO composite scaffolds. Characteristic absorption peaks derived from O–H stretching and COO^- vibrational modes were detected in both groups, which verified the successful immobilization of SA on the scaffold substrate. Additional absorption peaks attributed to Mg–OH and Mg–O bonds were observed in the pTa@SA-MgO sample, further confirming the effective incorporation of MgO. During degradation, the pTa@SA-MgO scaffold interacted with the buffer solution, resulting in the sustained release of Mg^{2+} ions from the hydrogel for more than four weeks (Figure 2C).

Figure 2D shows the pH variations of composite scaffolds with different MgO contents during immersion in PBS for 14 days. The results demonstrated that the MgO-doped scaffold groups (8%, 12%, and 16%) exhibited a rapid increase in pH during the early stage of immersion, reaching a peak around day 3 and subsequently stabilizing, whereas the 0% group without MgO maintained a neutral pH throughout the entire period. These findings indicate that the incorporation of MgO effectively elevated the alkalinity of the system in a concentration-dependent manner.

To visually demonstrate the morphological evolution of the composite scaffolds during degradation, representative macroscopic photographs were taken after immersion for 1, 3, and 7 days (Figure 2E). After one day of immersion, the hydrogel phase in each group remained relatively intact and uniformly covered the surface of the pTa scaffold. With prolonged immersion, the hydrogel gradually degraded, resulting in progressive exposure of the underlying porous structure. Despite the gradual degradation of the hydrogel phase, the overall architecture of the pTa scaffolds remained stable without obvious structural collapse, indicating that metallic tantalum could provide sustained mechanical support throughout the degradation process. These observations were consistent with the sustained Mg^{2+} release behavior and pH variation trends shown in Figure 2C and 2D, suggesting that the gradual degradation of the hydrogel enabled prolonged Mg^{2+} release while preserving the structural integrity of the scaffolds.

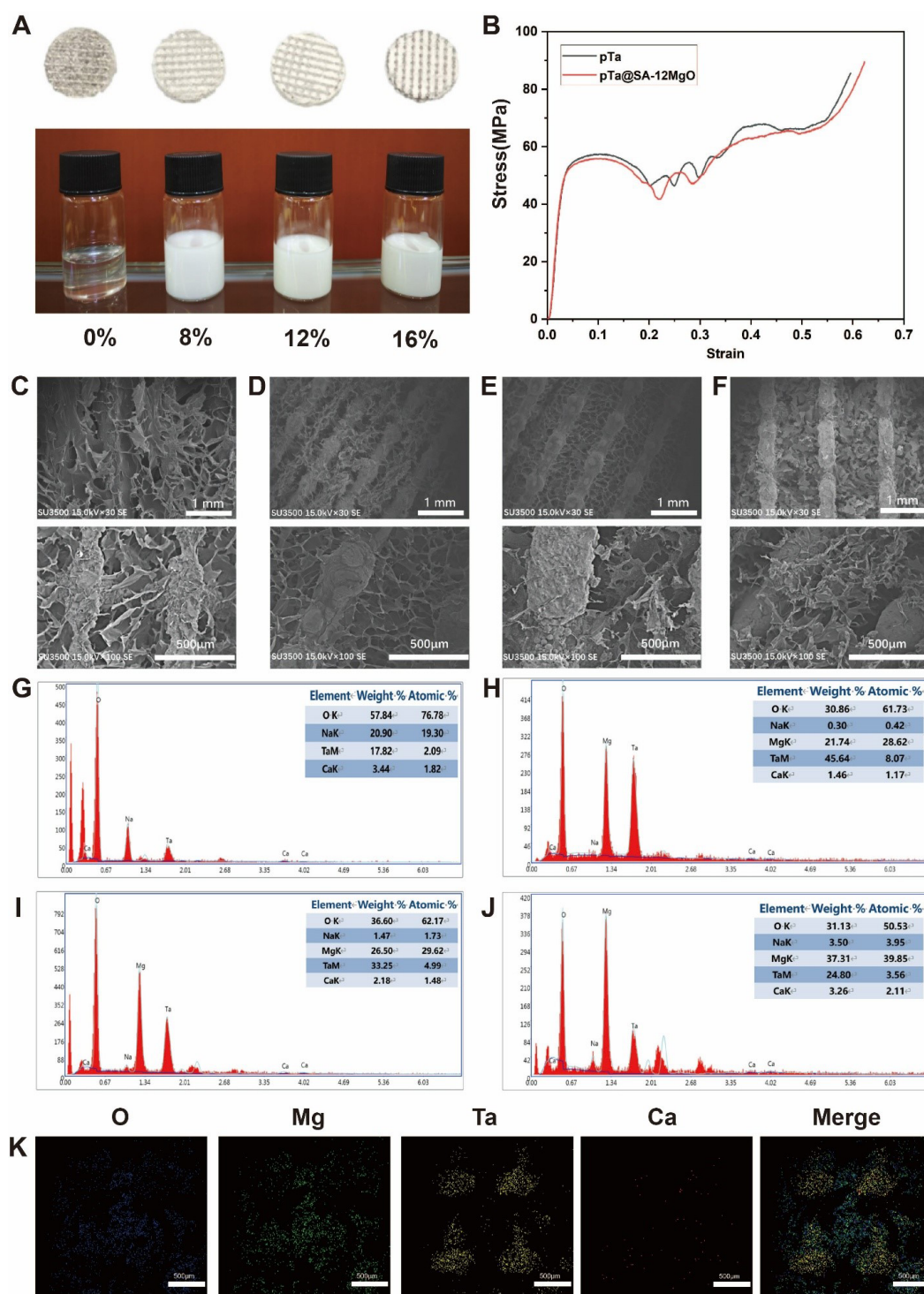


Figure 1. Microstructure and hydrogel property characterization of composite scaffolds. (A) Representative images of the pTa@SA-MgO hydrogel and the corresponding composite scaffold. (B) Compressive stress-strain curves of the pTa and pTa@SA-12MgO scaffolds. (C-F) Representative scanning electron microscopy images of the (C) pTa@SA, (D) pTa@SA-8MgO, (E) pTa@SA-12MgO, and (F) pTa@SA-16MgO scaffolds at low (scale bar = 1 mm, magnification = 30×) and high (scale bar = 500 μm, magnification = 100×) magnifications. (G-J) Corresponding energy-dispersive X-ray spectroscopy spectra and elemental compositions of the (G) pTa@SA, (H) pTa@SA-8MgO, (I) pTa@SA-12MgO, and (J) pTa@SA-16MgO scaffolds. (K) elemental mapping results of the composite scaffolds (scale bar = 500 μm, magnification = 50×). Abbreviations: MgO: Magnesium oxide; pTa: Porous tantalum; SA: Sodium alginate.

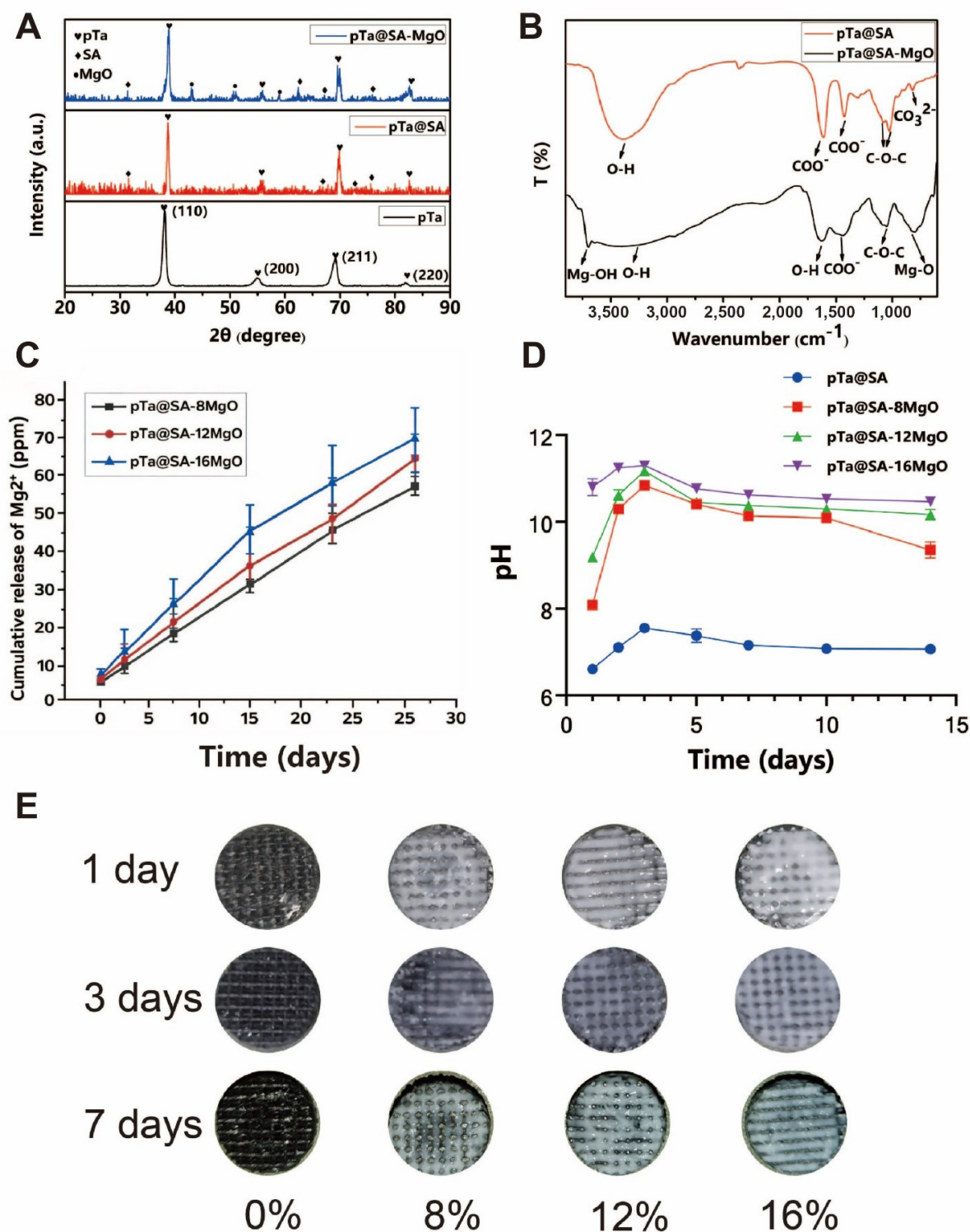


Figure 2. Material characterization of the fabricated composite scaffolds. (A) X-ray diffraction patterns of different composite scaffold samples. (B) Fourier transform infrared spectra of the pTa@SA and pTa@SA-MgO composite scaffolds. (C) *In vitro* Mg²⁺ release behavior of the pTa@SA-MgO scaffold. (D) pH changes of the surrounding environment triggered by composite scaffolds during immersion. (E) Representative macroscopic images of the composite scaffolds during the degradation process.

Abbreviations: MgO: Magnesium oxide; pTa: Porous tantalum; SA: Sodium alginate.

3.2. *In vitro* experimental results

As a biomaterial scaffold, favorable biocompatibility is a prerequisite for clinical application. Cell proliferation on pTa@SA, pTa@SA-8MgO, pTa@SA-12MgO, and pTa@SA-16MgO composite scaffolds was evaluated, as shown in Figure 3A, with the pTa@SA group serving as the control.

After one day of incubation, the pTa@SA-8MgO, pTa@SA-12MgO, and pTa@SA-16MgO groups displayed significantly enhanced cell viability compared with the control group ($p < 0.05$). With the culture duration extended to day 3, cellular viability was elevated across all experimental groups. Notably, the pTa@SA-12MgO group yielded the maximum OD value, which was statistically higher than that of the control group ($p < 0.01$). Moreover, both the pTa@SA-8MgO and pTa@SA-12MgO groups showed markedly enhanced proliferation compared with the control ($p < 0.0001$). On day 7, cell proliferation was further enhanced in all MgO-containing groups, which exhibited significantly higher viability than the control group ($p < 0.0001$). Among them, the pTa@SA-12MgO group demonstrated the most pronounced proliferative effect and was significantly superior to both the pTa@SA-8MgO and pTa@SA-16MgO groups ($p < 0.0001$). Collectively, these results indicate that the incorporation of

MgO effectively promotes cell proliferation, with the pTa@SA-12MgO scaffold showing the optimal performance; therefore, this formulation was selected for subsequent experiments.

Live/dead staining (Figure 3B) confirmed the high viability of MC3T3-E1 cells treated with various scaffold extracts, with negligible dead cells observed in all groups, thereby confirming good biocompatibility of the materials. The pTa@SA-12MgO group exhibited distinctly greater cell proliferation at days 4 and 7 than the pTa and pTa@SA groups.

Figure 3C illustrates the cell spreading morphology and cytoskeletal fluorescence intensity, while Figure 3F presents the corresponding quantitative analysis. At 2 h, no significant differences in cell spreading were observed among the groups. In contrast, at 24 h, the cytoskeletal fluorescence intensity of the pTa@SA-12MgO group was approximately 1.5-fold higher than that of the pTa group, indicating a superior ability to promote cell spreading.

Figure 3D depicts osteoblast migration in response to different scaffold extracts. Cells steadily filled the scratched area during culture, with the pTa@SA-MgO group showing the best wound closure. No significant differences

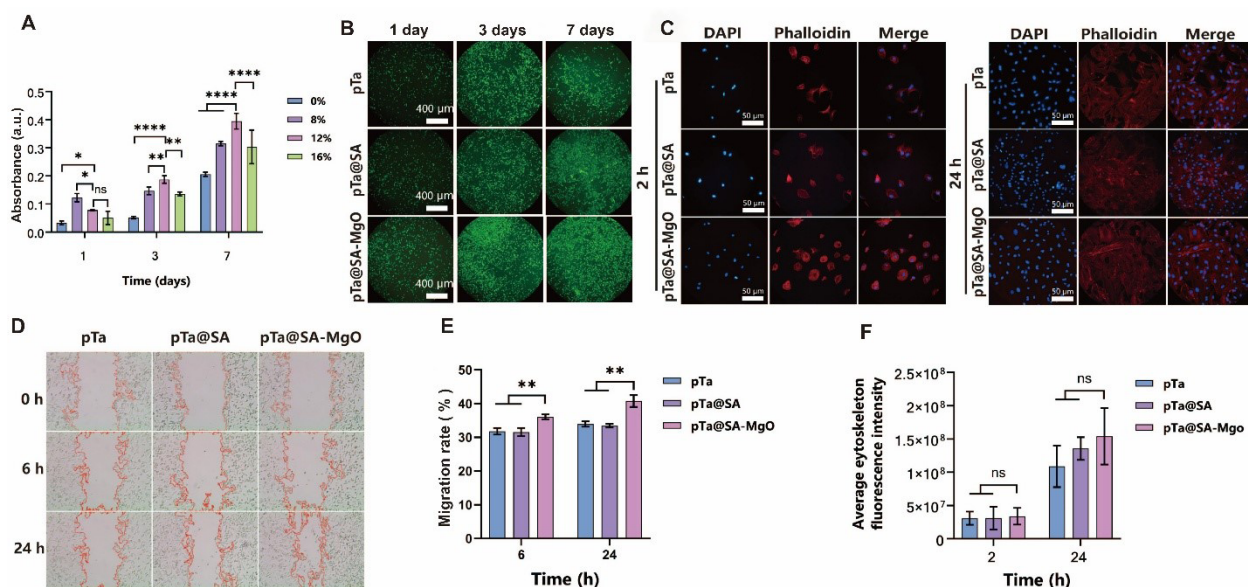


Figure 3. Cell adhesion, proliferation, and migration upon treatment with different scaffolds. (A) Cell Counting Kit-8 assay of MC3T3-E1 cells cultured with extracts from MgO-containing alginate/tantalum scaffolds for 1, 4, and 7 days. (B) Live/dead fluorescence imaging of MC3T3-E1 cells at different culture times (scale bar = 400 μ m, magnification = 10 $\times$). (C) Phalloidin and DAPI staining of cells treated with three types of scaffold extracts for 2 h and 24 h (scale bar = 50 μ m, magnification = 40 $\times$). (D) Scratch wound assay images of MC3T3-E1 cells at 0, 6, and 24 h (scale bar = 200 μ m, magnification = 10 $\times$). (E) Statistical results of cell migration rates. (F) Mean fluorescence intensity of the cytoskeleton. * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$ indicate statistical significance, whereas “ns” indicates no statistical significance.

Abbreviations: DAPI: 4',6-diamidino-2-phenylindole; MgO: Magnesium oxide; pTa: Porous tantalum; SA: Sodium alginate.

were observed among groups at 6 h (Figure 3E). The pTa@SA-MgO group exhibited the fastest migration at 24 h, verifying the promotive effect of MgO on osteoblast migration.

Alkaline phosphatase expression in the early stage and extracellular matrix mineralization are typical indicators of osteogenic differentiation of cells cultured on material surfaces. Immunofluorescence staining was performed to detect ALP distribution. ALP expression was observed in all groups at day 7 (Figure 4A), and the pTa@SA-12MgO group exhibited the highest ALP level. Quantitative analysis (Figure 4C) showed highly significant differences between the pTa@SA-12MgO and the other two groups (p

< 0.0001).

Based on the screening results, the selected pTa@SA-12MgO group was further investigated for its osteogenic potential. ALP staining and ARS staining were separately applied to assess the early and late stages of osteogenic differentiation. As shown in Figure 4E and the corresponding quantitative analysis performed using ImageJ (Figure 4F), the pTa@SA-12MgO group exhibited a 2.1-fold increase in early-stage osteogenesis and a 1.1-fold enhancement in late-stage osteogenesis compared with the pTa group. Meanwhile, the pTa@SA group displayed comparable results to the pTa group. ALP activity was used to assess early osteogenic differentiation,

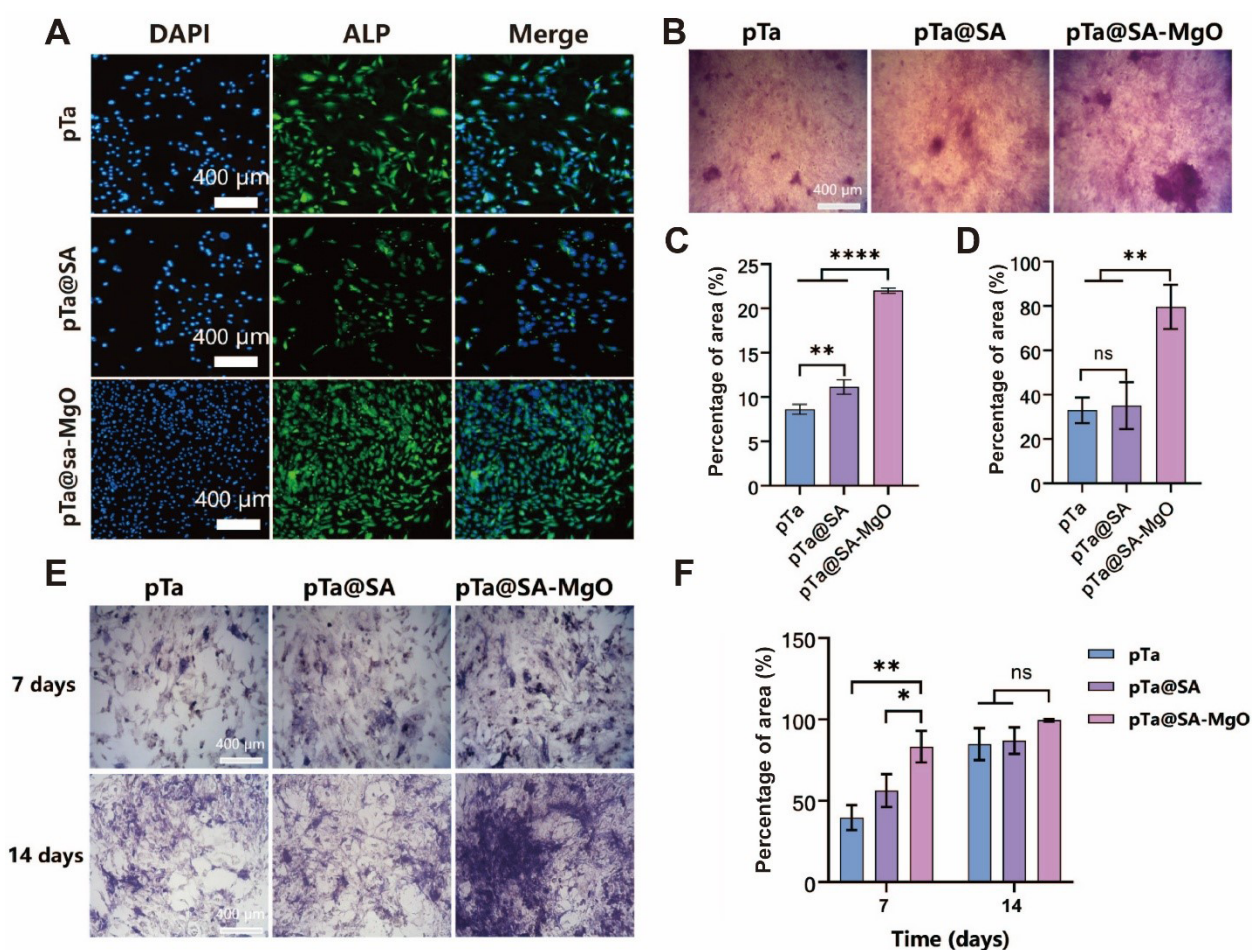


Figure 4. Osteogenic differentiation performance of MC3T3-E1 cells cultured with scaffold extracts. (A) Immunofluorescent staining of ALP in MC3T3-E1 cells after seven days of incubation with different extraction media (scale bar = 400 μ m, magnification = 40 $\times$). (B) Alizarin red S staining results of MC3T3-E1 cells after 21 days of osteogenic induction using pTa, pTa@SA and pTa@SA-MgO scaffold extracts (scale bar = 400 μ m, magnification = 40 $\times$). (C) Quantitative analysis of ALP-positive areas corresponding to the immunofluorescence images. (D) Quantitative analysis of mineralized matrix area based on alizarin red S staining results at day 21. (E) ALP staining of MC3T3-E1 cells after seven and 14 days of osteogenic culture with different scaffold extracts (scale bar = 400 μ m, magnification = 40 $\times$). (F) Quantitative comparison of ALP-positive areas at days 7 and 14. * $p < 0.05$, * $p < 0.01$, and **** $p < 0.0001$ indicate statistical significance, whereas “ns” indicates no statistical significance.

Abbreviations: ALP: Alkaline phosphatase; DAPI: 4',6-diamidino-2-phenylindole; MgO: Magnesium oxide; pTa: Porous tantalum; SA: Sodium alginate.

while ARS staining was employed to evaluate late-stage mineralization. As illustrated in Figure 4B, the pTa@SA-12MgO group demonstrated a pronounced promotive effect on both early and late osteogenic differentiation.

Gene expression in MC3T3-E1 cells at days 7 and 14 is shown in Figure 5A and 5B. The pTa@SA-12MgO group significantly upregulated all osteogenic genes. The pTa group moderately enhanced gene expression on day 7, while significant differences from the pTa@SA-12MgO group persisted on day 14, which is consistent with prior studies about pure tantalum. The pTa@SA group showed limited osteogenic activity at both time points. All these data are consistent with ALP and ARS staining results.

Figure 5D includes immunofluorescence images and quantitative analysis of M2 marker Arg-1 and M1 marker CCR7. The pTa group showed weak anti-inflammatory activity, as documented in previous studies. In the pTa@SA-12MgO group, Arg-1 fluorescence intensity was approximately 1.4-fold that of the pTa group, and CCR7 intensity decreased to approximately 0.3-fold, indicating effective inhibition of M1 polarization and enhanced anti-inflammatory effects. The pTa@SA group inhibited both

macrophage phenotypes, with stronger suppression on M1 cells. Since CCR7 expression was higher than Arg-1, this group still exhibited a slight pro-inflammatory tendency. Quantitative data are presented in Figure 5E.

Analysis of macrophage-related gene expression (Figure 5C) revealed that the pTa@SA-12MgO group exhibited partial suppression of pro-inflammatory gene expression while more strongly promoting the expression of anti-inflammatory genes in macrophages. The other two groups showed only slight increases or decreases in relative gene expression levels, and overall, the pTa group demonstrated a relatively weak inhibitory effect on inflammation. Although the pTa@SA group showed a certain reduction in pro-inflammatory gene expression, its overall gene expression profile still suggested a relatively pronounced pro-inflammatory tendency. By comparing the effects of different experimental groups on macrophage differentiation, it is evident that the incorporation of Mg²⁺ ions markedly promoted polarization toward the anti-inflammatory phenotype. Previous studies have also reported the anti-inflammatory effects of magnesium ions; however, the underlying mechanisms remain to be further elucidated.

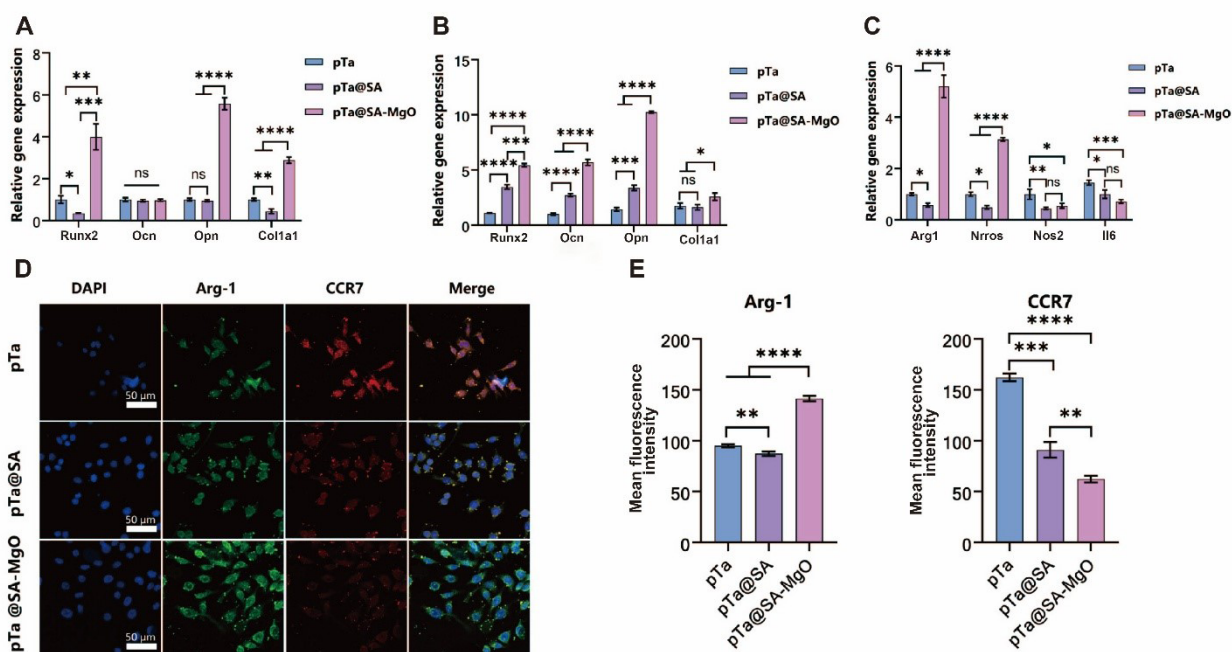


Figure 5. Cell responses to different scaffolds. (A, B) Relative expression of osteogenic genes in MC3T3-E1 cells at days (A) 7 and (B) 14. (C) Expression of macrophage polarization-related genes in RAW264.7 cells after seven days of treatment. (D) Immunofluorescence staining of RAW264.7 cells cultured with scaffold extracts for 48 h (scale bar = 50 μ m, magnification = 40 $\times$). (E) Quantitative analysis of fluorescence intensity of red and green channels. * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001 indicate statistical significance, whereas “ns” indicates no statistical significance.

Abbreviations: Arg-1: Arginine 1; CCR7: C-C chemokine receptor type 7; DAPI: 4',6-diamidino-2-phenylindole; MgO: Magnesium oxide; pTa: Porous tantalum; SA: Sodium alginate.

3.3. *In vivo* experimental results

A rabbit femoral bone defect model was constructed in this study, and no postoperative infection or mortality occurred during the six- and 12-week observation periods. All experimental rabbits survived the entire study process, and all implanted scaffolds remained stable without loosening throughout the implantation cycle. All specimen samples were successfully collected at the scheduled time points. Micro-CT reconstructed images of femoral samples at six and 12 weeks after implantation are displayed in Figure 6A and 6B.

Quantitative micro-CT analysis demonstrated that the pTa@SA-12MgO group exhibited a higher BV/TV than the pure pTa group at both detection time points. At 12 weeks post-surgery, the BV/TV of the pTa@SA-12MgO group was approximately 1.2-fold higher than that of the pTa group (Figure 6C and 6D). Van Gieson staining of hard tissue sections further validated the bone regeneration performance of different scaffolds (Figure 6E). The pTa@SA-12MgO group exhibited significantly enhanced bone ingrowth, with the newly formed bone area reaching approximately 1.4-fold that of the pTa group. Particularly,

at 12 weeks after implantation, new bone tissues grew inward across the scaffold structure and formed partial interconnected bone networks (Figure 6F).

The osteogenic mineralization capacity of different scaffolds was evaluated by analyzing the spacing between labeled lines at two different time points. Calcium nodule deposition was observed in all three experimental groups (Figure 7A), and the results confirmed that the pTa@SA-12MgO group exhibited a faster bone formation rate than the pTa group at identical culture stages.

Immunohistochemical staining was further performed to detect the expression of osteogenic markers (Figure 7C). At both postoperative time points, the pTa@SA-MgO group displayed remarkably stronger positive staining of OCN and COL I compared with the pTa group. Specifically, the positive expression areas of OCN and COL I in the pTa@SA-MgO group were 1.4-fold and 1.8-fold higher than those in the pTa group, respectively (Figure 7D). Collectively, these findings demonstrate that the pTa@SA-MgO scaffold exhibits enhanced *in vivo* osteogenic performance and can effectively promote bone regeneration.

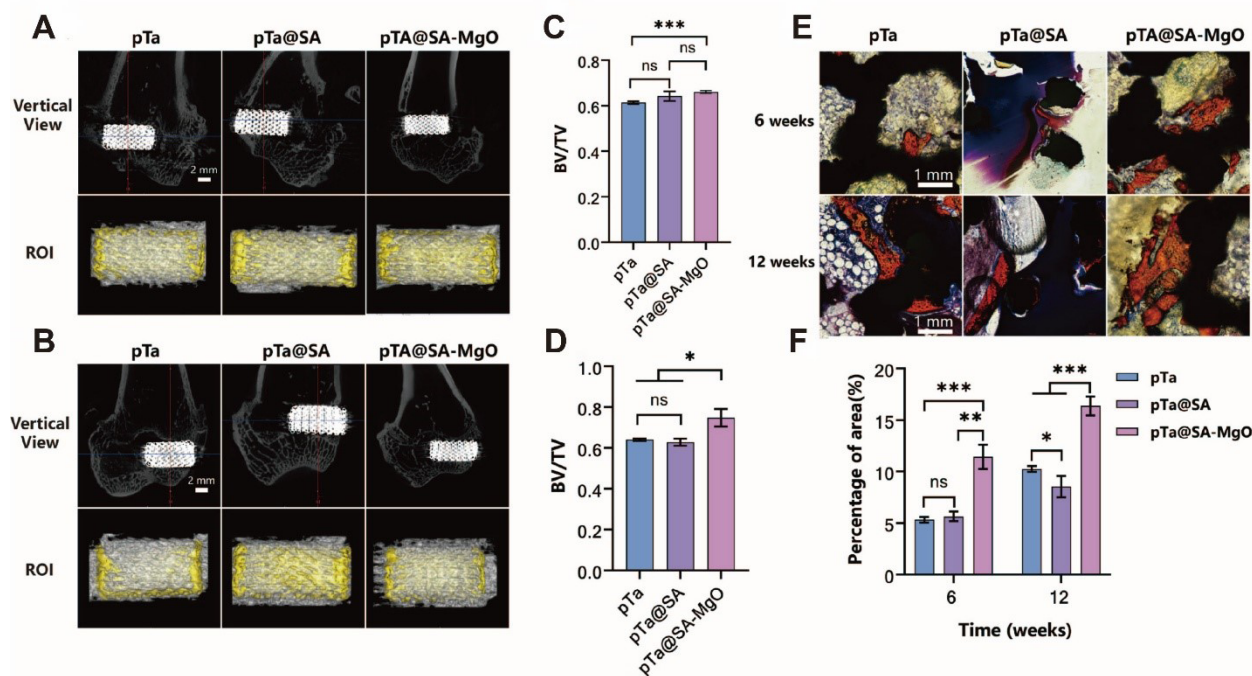


Figure 6. *In vivo* bone regeneration assessment of different scaffolds using a rabbit femoral implantation model at six and 12 weeks postoperatively. (A, B) Typical micro-computed tomography reconstruction images of scaffold implantation regions at (A) week 6 and (B) week 12 (scale bar = 2 mm). (C, D) Quantitative analysis of trabecular number per unit length at (C) week 6 and (D) week 12. (E) Van Gieson staining of hard tissue sections harvested from the implantation sites (scale bar = 1 mm, magnification = 10 $\times$). (F) Corresponding morphometric analysis of the proportion of newly formed bone area (red-stained areas) corresponding to the staining images. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, indicate statistical significance, whereas "ns" indicates no statistical significance. Abbreviations: BV/TV: Bone volume fraction; MgO: Magnesium oxide; pTa: Porous tantalum; ROI: Region of interest; SA: Sodium alginate.

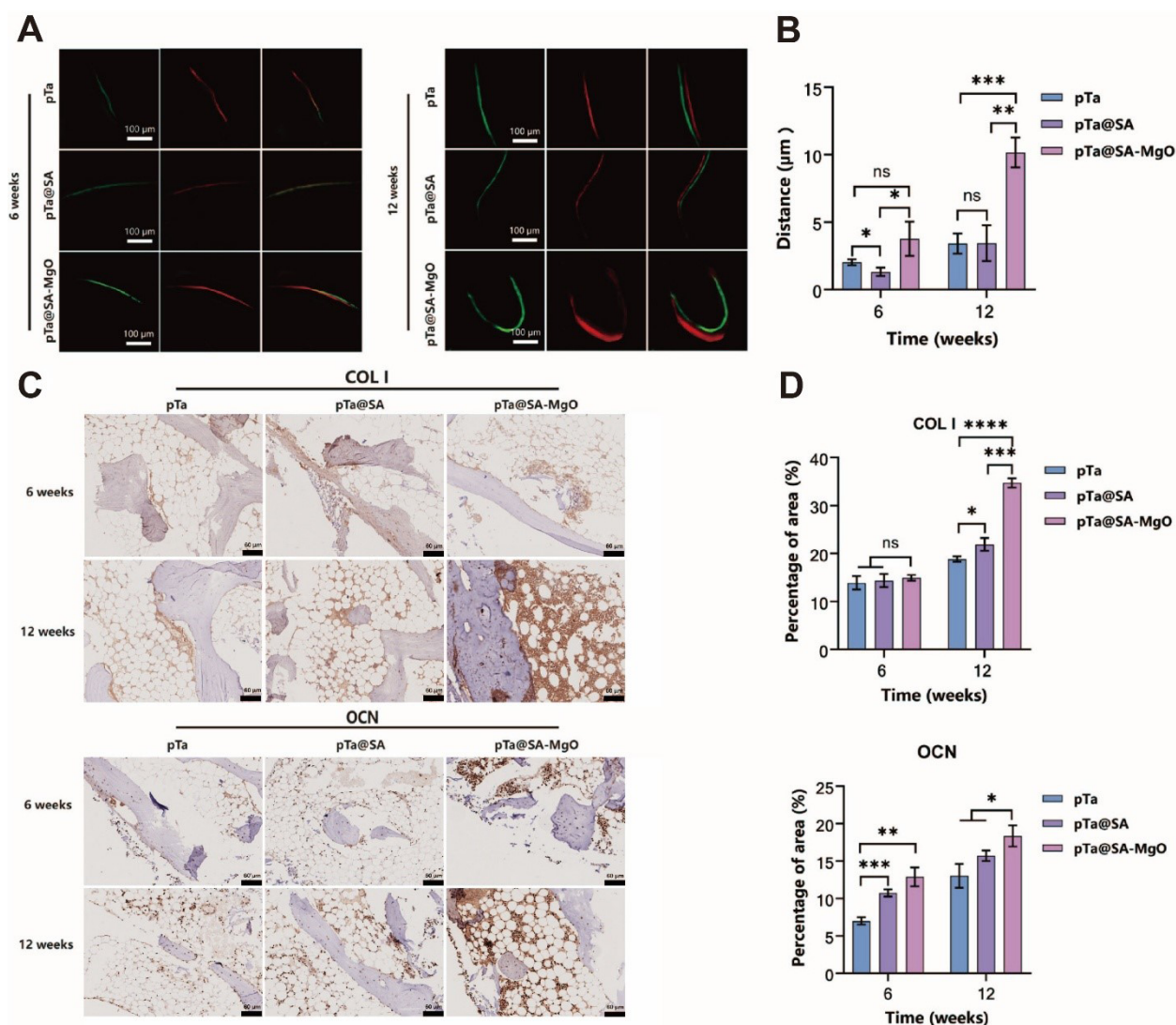
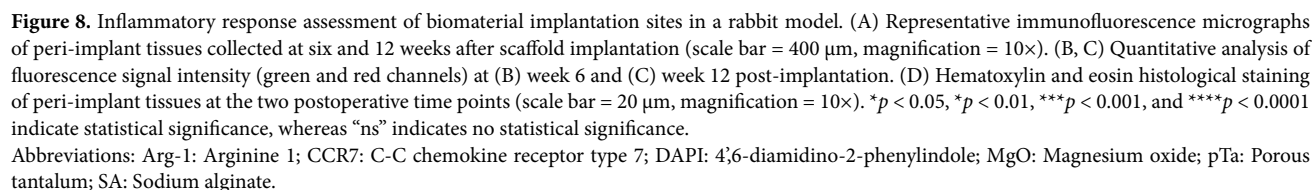


Figure 7. *In vivo* bone regeneration evaluation of different scaffolds in rabbit implantation models after six and 12 weeks of healing. (A) Fluorescent labeling imaging for visualization of newly regenerated bone tissues (scale bar = 100 μm, magnification = 10×). (B) Quantitative analysis of bone formation rates in the pTa, pTa@SA, and pTa@SA-MgO groups. (C) Immunohistochemical staining of tissue sections collected at two postoperative time points (scale bar = 60 μm, magnification = 5×). (D) Semi-quantitative quantification of the positive brown-stained areas. * $p < 0.05$, * $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ indicate statistical significance, whereas “ns” indicates no statistical significance.

Abbreviations: COL I: Collagen type I; MgO: Magnesium oxide; OCN: Osteocalcin; pTa: Porous tantalum; SA: Sodium alginate.

Figure 8A presents the immunofluorescence staining results of key inflammatory biomarkers. At the two detected time points, the fluorescence intensity of the M2 macrophage marker Arg-1 was significantly upregulated in the pTa@SA-MgO group, reaching 2.2-fold of that in the pTa group. In contrast, the fluorescence level of the M1 macrophage marker CCR7 was downregulated, declining to half of the value obtained from the pTa group (Figure 8B and 8C). These findings suggest that the pTa@SA-MgO scaffold possesses promising anti-inflammatory properties.

Additionally, hematoxylin and eosin staining was conducted for histological observation of tissues surrounding the implants (Figure 8D). The morphological characteristics of periprosthetic soft tissue and bone tissue can effectively reflect the local microenvironment during bone regenerative repair. Compared with the pTa and pTa@SA groups, the pTa@SA-MgO group exhibited reduced inflammatory cell infiltration and a lower degree of inflammatory response. In conjunction with the immunohistochemical and immunofluorescence



findings, these results suggest that the composite scaffold can, to a certain extent, modulate the local immune microenvironment and suppress excessive inflammatory responses, thereby providing more favorable conditions for bone regeneration.

Specifically, as the implantation time increased, the osteogenic effect of the pTa@SA-MgO group continued to enhance, indicating that this material has potential for promoting bone tissue regeneration and repair.

4. Discussion

In this study, we successfully developed a composite scaffold (pTa@SA-MgO) consisting of MgO-loaded SA hydrogel and 3D-printed pTa, and systematically evaluated its overall performance in large bone defect repair. The results show that this composite scaffold not only inherits the excellent mechanical properties and biocompatibility of pTa but also achieves controlled release of Mg^{2+} via the hydrogel carrier, demonstrating significant advantages in promoting osteoblast proliferation, migration, differentiation, as well as modulating the immune microenvironment.^{32,33}

The pTa scaffold was prepared using SLM technology. Its diamond-shaped pore structure (porosity 75%, pore size 500 μm) not only provides sufficient specific surface area for cell adhesion and tissue ingrowth but also, more importantly, its compressive yield strength (37.1 ± 2.5 MPa) and elastic modulus (2.98 ± 0.14 GPa) match those of natural bone tissue, effectively avoiding the common “stress shielding” effect often observed with traditional metal implants.^{12,41,42} Scanning electron microscopy and EDS analyses revealed that the SA hydrogel can completely fill the pores of the pTa scaffold, and the magnesium content significantly increases with the addition of MgO. XRD and Fourier transform infrared results further confirmed the successful incorporation of MgO, laying the foundation for the subsequent realization of biological functions.

In vitro release experiments demonstrated that the pTa@SA-MgO composite scaffold was capable of continuously releasing Mg^{2+} over a period of 28 days, and that the release kinetics could be precisely regulated by adjusting the MgO content. Notably, the pTa@SA-12MgO group maintained the Mg^{2+} concentration within the optimal range for biological activity (approximately 50 ppm),⁴³ which is markedly superior to conventional surface-coating strategies, where limitations in coating thickness and ion loading often prevent the attainment of therapeutically effective concentrations. For example, magnesium-containing coatings typically release only a few ppm of Mg^{2+} per day, which is far below the ideal concentration range required for biological efficacy.^{44,45} Meanwhile, pH monitoring results indicated that the incorporation of

MgO moderately increased the alkalinity of the system (pH 10–11), and such a mildly alkaline microenvironment has been demonstrated to be beneficial for osteoblast activity and bone matrix mineralization.^{46,47}

The cellular experimental results demonstrated that the pTa@SA-MgO composite scaffold exhibited significant advantages in promoting osteoblast activity. Cell counting kit-8 and live/dead staining assays revealed that all MgO-containing groups significantly enhanced the proliferation of MC3T3-E1 cells, with the pTa@SA-12MgO group showing the most pronounced effect, indicating that an appropriate concentration of Mg^{2+} exerts a positive regulatory effect on cell growth, which is consistent with previous reports.^{48,49}

Cytoskeletal staining further demonstrated that the pTa@SA-12MgO group markedly promoted cell spreading, with fluorescence intensity approximately 1.5-fold higher than that of the pTa group. Scratch wound assays further confirmed that the incorporation of MgO effectively enhanced osteoblast migration. Bone repair and regeneration are complex biological processes that largely depend on the migration of cells, particularly osteoblasts and mesenchymal stem cells, toward the defect site. Effective cell migration is critical for successful bone defect repair, as it facilitates the recruitment of key cells involved in bone formation and remodeling to the injury site.⁵⁰ Numerous studies have emphasized the importance of cell migration in promoting bone regeneration and have provided insights into potential therapeutic strategies.^{51,52}

Both ALP immunofluorescence staining and ALP staining results demonstrated that the pTa@SA-12MgO group exhibited a significantly enhanced early osteogenic differentiation, with the ALP-positive area reaching approximately 2.1-fold that of the pTa group. qPCR analysis further confirmed that the expression of key osteogenic genes, including *Runx2*, *Ocn*, *Opn*, and *Col1a1*, was significantly upregulated in this group, indicating that Mg^{2+} promotes osteogenic differentiation at the transcriptional level.

Meanwhile, ARS staining revealed that the pTa@SA-MgO composite scaffold also exhibited superior capability in promoting extracellular matrix mineralization, providing strong support for bone formation and maturation *in vivo*. Mg^{2+} synergistically promotes osteogenesis through multiple pathways and plays a crucial role in maintaining skeletal health.⁵³ As an essential cofactor for key enzymes such as ALP, Mg^{2+} enhances enzymatic activity and directly participates in hydroxyapatite formation, which is the core component of bone mineralization.⁵⁴ At the cellular level, Mg^{2+} stimulates osteoblast proliferation and differentiation, promotes collagen secretion to provide structural support

for the bone matrix, and simultaneously suppresses excessive osteoclast activation, thereby maintaining the dynamic balance of bone remodeling.⁵⁵ At the molecular level, Mg^{2+} enhances osteogenesis by regulating signaling pathways such as transient receptor potential melastatin 7/phosphoinositide 3-kinase/protein kinase B pathway, thereby promoting the expression of osteogenesis-related genes.⁵⁶ Clinical studies have demonstrated that adequate magnesium intake significantly increases bone mineral density and reduces fracture risk.⁵⁷ Collectively, these mechanisms highlight the multifunctional role of Mg^{2+} in bone development and maintenance, underscoring its considerable potential in bone defect repair and regenerative therapies.

Macrophages act as the primary regulators of the early inflammatory response surrounding implanted biomaterials, and their polarization states directly influence the bone regeneration process.^{58,59} Immunofluorescence staining and qPCR analyses revealed that the pTa@SA-MgO group significantly promoted macrophage polarization toward the anti-inflammatory M2 phenotype, with Arg-1 fluorescence intensity reaching 1.4-fold that of the pTa group, while simultaneously suppressing polarization toward the pro-inflammatory M1 phenotype, as evidenced by a reduction of CCR7 fluorescence intensity to 0.3-fold of the pTa group. These findings are consistent with previous reports demonstrating the anti-inflammatory properties of Mg^{2+} , indicating that the composite scaffold effectively improves the local immune microenvironment at the implantation site through controlled Mg^{2+} release, thereby creating favorable conditions for subsequent bone regeneration.^{32,33,48,60,61}

The *in vivo* experimental results demonstrated that the pTa@SA-MgO composite scaffold exhibited excellent bone repair capacity in a rabbit bone defect model. Micro-CT analysis revealed that after 12 weeks of implantation, the pTa@SA-MgO group showed significantly higher BV/TV and Tb.N compared with the control group. Van Gieson staining further confirmed a markedly increased extent of bone ingrowth in the pTa@SA-MgO group, with the bone area reaching approximately 1.4-fold that of the pTa group. Fluorochrome labeling analysis showed a pronounced increase in the new bone formation rate in the pTa@SA-MgO group, indicating that the composite scaffold effectively promotes the dynamic process of bone tissue formation.

The novelty of this study is mainly reflected in the following aspects. First, this study addresses the functional limitations of conventional single-component materials by adopting a composite scaffold design strategy that integrates structural mechanical stability with tunable

biological functionality. The pTa scaffold provides robust mechanical support, while the SA hydrogel serves as an ideal carrier for magnesium ions, enabling their sustained and controllable release. Second, this study addresses a critical limitation of existing technologies, namely the insufficient release concentration of bioactive metal ions. Conventional surface coating approaches are constrained by physical limitations and therefore struggle to maintain therapeutically effective ion concentrations. In contrast, by dispersing nano-sized MgO particles within the hydrogel network, this study markedly enhances ion loading capacity and enables precise regulation of ion release kinetics through adjustment of hydrogel degradation rates and MgO content. Third, the multifaceted biological effects of magnesium ions are comprehensively demonstrated in this study. Beyond its well-established osteogenic effects, magnesium ions were found to exert significant immunomodulatory functions, and this dual-action mechanism is of great importance for the repair of bone defects.

Although this study has yielded encouraging results, several limitations persist and should be addressed in future investigations. First, although pTa@SA-12MgO was identified as the optimal composition in this study, bone defects of different sizes and anatomical locations may require tailored optimization of MgO content and hydrogel formulations to meet personalized therapeutic demands. Second, the precise molecular mechanisms by which Mg^{2+} regulates macrophage polarization have yet to be fully elucidated. Future studies should focus on clarifying how Mg^{2+} modulates inflammatory responses through key signaling pathways, such as nuclear factor kappa-light-chain-enhancer of activated B cells and mitogen-activated protein kinase pathways, which would provide a deeper theoretical basis for the rational optimization of composite scaffold design. Third, the maximum observation period in this study was limited to 12 weeks, whereas complete regeneration of large-segment bone defects typically requires a longer time course. Therefore, long-term animal studies are necessary to comprehensively evaluate the long-term safety and stability of the proposed biomaterial system.

In summary, the successfully developed pTa@SA-MgO composite scaffold in this study exhibited excellent overall performance, including robust mechanical support, enhanced osteogenic cellular activity, and effective modulation of the local immune microenvironment. This novel bone repair material not only provides a promising therapeutic strategy for the clinical treatment of large-segment bone defects but also offers new insights into the functional design of bone tissue engineering scaffolds.

Future studies should focus on personalized material customization, deeper elucidation of the underlying biological mechanisms, and translational research toward clinical applications, thereby facilitating the progression of this innovative technology from bench to bedside.

5. Conclusion

In this study, a composite scaffold integrating nano-MgO-loaded SA hydrogel with 3D-printed pTa was successfully developed. The scaffold exhibited favorable structural stability and mechanical support while enabling sustained Mg^{2+} release, which enhanced osteogenic cellular responses and modulated the local biological microenvironment. These findings demonstrate that the pTa@SA-MgO scaffold provides a functional platform combining mechanical reliability with bioactivity for bone regeneration. Although further *in vivo* validation and long-term evaluation are required, this strategy offers a promising direction for the design of multifunctional metallic scaffolds for large bone defect repair.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

All *in vivo* animal operations were ethically reviewed and approved by the Animal Welfare and Ethics Committee of Zhongshan Hospital, Dalian University (No. DW2025-008-01).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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