

Well-educated macrophage membrane-camouflaged piezoelectric nanosystem reprograms immune microenvironment for bone regeneration

Xin Qian

Beijing Tsinghua Chang Gung Hospital

Xiongfa Ji

Guangdong Provincial People's Hospital

Yueyi Zhang

Beijing Tsinghua Chang Gung Hospital

Le Jiang

Tsinghua University

Liangyu Chen

Beijing Tsinghua Chang Gung Hospital

Yiqun Wang

Beijing Tsinghua Chang Gung Hospital

Wentao Li

Beijing Tsinghua Chang Gung Hospital

Chao Li

Beijing Tsinghua Chang Gung Hospital

Peng Yang

Guangdong Provincial People's Hospital

Yifan Song

Beijing Tsinghua Chang Gung Hospital

Lingan Huang

Beijing Tsinghua Chang Gung Hospital

Wei Rong

Beijing Tsinghua Chang Gung Hospital

Ronghui Deng

Beijing Tsinghua Chang Gung Hospital

Xiaodan Sun

Tsinghua University

Yu Zhang

Guangdong Provincial People's Hospital

Jia-kuo Yu

yujiakuo@126.com

Beijing Tsinghua Chang Gung Hospital

Research Article

Keywords: Piezocatalytic therapy, Macrophage membrane, Tumorous bone defect, SIRP α -CD47 axis, Bone repair.

Posted Date: October 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11096851/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Well-educated macrophage membrane-camouflaged piezoelectric nanosystem reprograms immune microenvironment for bone regeneration

Xin Qian ^{a,b,1}, Xiongfa Ji ^{c,1}, Yueyi Zhang ^{a,b,1}, Le Jiang ^d, Liangyu Chen ^{a,b}, Yiqun Wang ^{a,b}, Wentao Li ^{a,b}, Chao Li ^{a,b}, Peng Yang ^c, Yifan Song ^{a,b}, Lingan Huang ^{a,b}, Wei Rong ^{a,b}, Ronghui Deng ^{a,b,*}, Xiaodan Sun ^{d,*}, Yu Zhang ^{c,*}, Jia-kuo Yu ^{a,b,***}

a. Orthopaedics and Sports Medicine Center, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua Medicine, Tsinghua University, P.R. China.

b. Institute of Orthopaedics and Sports Medicine, Tsinghua Medicine, Tsinghua University, Beijing, P.R. China.

c. Department of Orthopedics, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Southern Medical University, Guangzhou 510080, P. R. China

d. State Key Laboratory of New Ceramics and Fine Processing School of Materials Science and Engineering Tsinghua University Beijing 100084, P. R. China.

E-mail addresses:

xinqian2020@163.com (X.Q.)

**ronghuideng@whu.edu.cn (Ronghui D.)*

**sunxiaodan@tsinghua.edu.cn (Xiaodan S.)*

**Zhangyu@gdph.org.cn (Yu Z.)*

****yujiakuo@126.com (Jia-kuo Y.)*

¹These authors contributed equally to this work.

Abstract

Tumorous bone defects caused by osteosarcoma require concurrent tumor eradication, immune microenvironment remodeling, and bone repair, yet integrating these functions into a single therapeutic platform remains challenging. Herein, we developed a “well-educated” macrophage membrane-camouflaged piezoelectric nanosystem, termed $M_{SIRP\alpha}$ -BT, comprising tetragonal barium titanate (BT) nanoparticles coated with membranes derived from $SIRP\alpha$ -overexpressing macrophages. The engineered membrane engages the CD47- $SIRP\alpha$ axis on tumor cells, thereby alleviating immunosuppression and remodeling the tumor immune microenvironment. Under ultrasound (US) irradiation, the piezoelectric BT core generated cytotoxic ROS, including hydroxyl radicals and superoxide anions, thereby inducing intracellular oxidative stress and apoptosis in osteosarcoma cells. Transcriptomic analysis indicated the involvement of DNA damage, cell-cycle arrest, apoptosis, and p53-associated signaling pathways. Under mild US stimulation, $M_{SIRP\alpha}$ -BT promoted osteogenic differentiation of BMSCs, as evidenced by increased expression of ALP, OPN, COL-1, and RUNX2, enhanced ALP staining, and increased calcium deposition. In a mouse femoral tumorous bone defect model, $M_{SIRP\alpha}$ -BT + US inhibited tumor growth, attenuated tumor-associated osteolysis, improved bone microarchitecture, and promoted local bone repair without apparent systemic toxicity. These findings indicate that $M_{SIRP\alpha}$ -BT integrates US-triggered piezocatalytic tumor therapy, immune microenvironment modulation, and osteogenic stimulation for osteosarcoma-associated tumorous bone defects.

Keywords

Piezocatalytic therapy; Macrophage membrane; Tumorous bone defect; $SIRP\alpha$ -CD47 axis; Bone repair.

1. Introduction

The reconstruction of complex bone defects remains a major challenge in orthopedic regenerative medicine[1]. Arising from severe trauma, infection, tumor resection, or revision surgery, these defects frequently exceed the intrinsic healing capacity of bone and are commonly accompanied by impaired vascularization, persistent inflammation, dysregulated cellular crosstalk, and disruption of the local regenerative niche[2]. Successful treatment therefore requires not only restoration of tissue volume and mechanical continuity, but also coordinated modulation of the pathological microenvironment to support cell recruitment, osteogenic differentiation, extracellular matrix deposition, and mineralized tissue formation[3]. Although tissue-engineered constructs have improved osteoconductivity and structural support, many remain inadequate when active pathological processes persist within the defect site, limiting their translational potential[4,5].

Tumor-associated bone defects represent a particularly challenging subset of complex skeletal injuries. Osteosarcoma, the most common primary malignant bone tumor in children and adolescents, is characterized by locally destructive growth and a high risk of pulmonary metastasis[6,7]. Surgical resection remains indispensable for local disease control, yet it often produces extensive defects in load-bearing bone. Furthermore, microscopic residual tumor cells at the resection margin may contribute to local recurrence. The postoperative site thus requires both effective elimination of residual malignant cells and establishment of a microenvironment permissive for bone repair. Conventional reconstructive materials generally lack intrinsic antitumor activity, whereas local or systemic anticancer therapies may compromise the viability and osteogenic function of cells involved in tissue regeneration[8,9]. Multifunctional platforms that integrate local tumor suppression, immune regulation, and skeletal regeneration are therefore urgently needed.

Macrophages are critical regulators of tumor progression and bone healing[10]. In osteosarcoma, tumor-associated macrophages commonly exhibit immunosuppressive and tumor-supportive properties, contributing to a microenvironment favorable for tumor persistence[11–13]. The CD47-signal regulatory protein alpha (SIRP α) axis

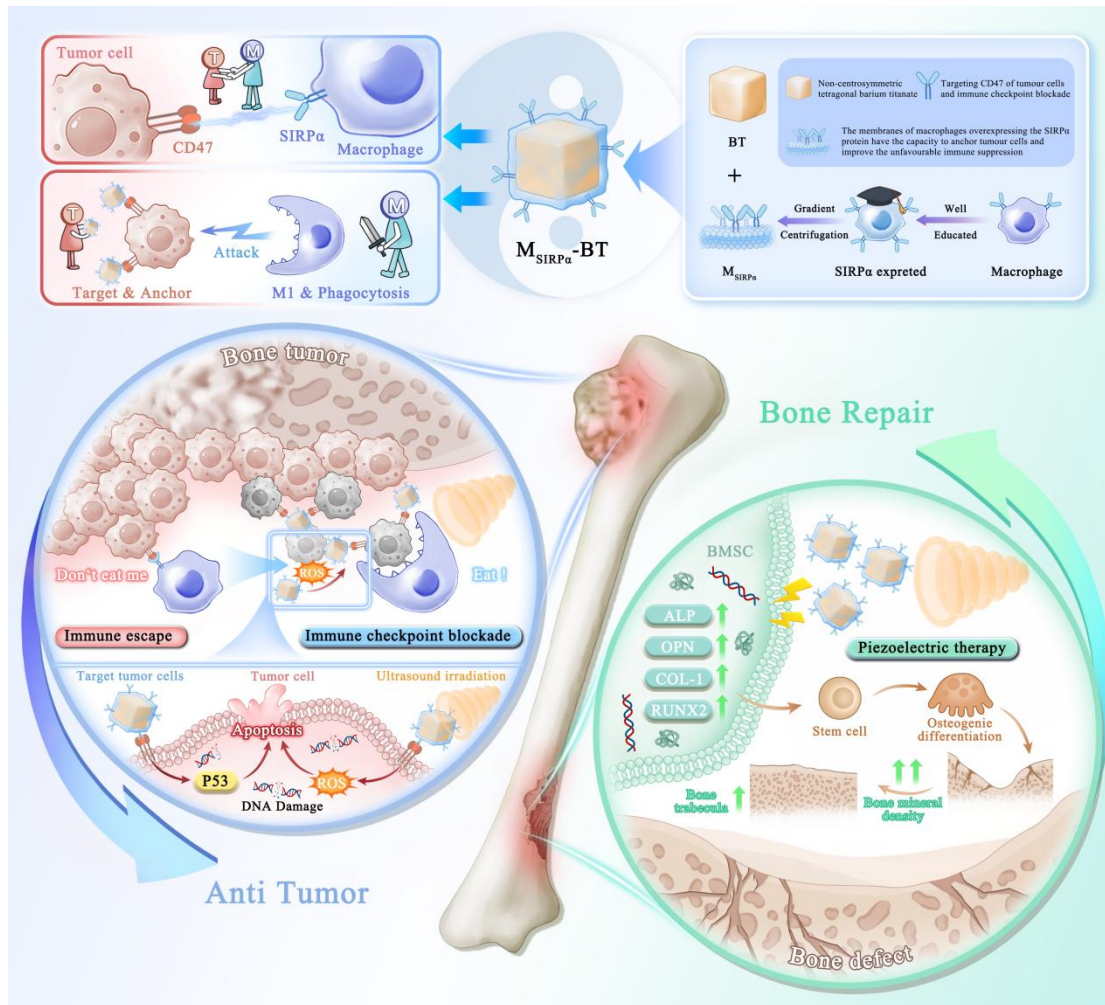
represents an important macrophage immune checkpoint in this setting. CD47 is frequently upregulated on tumor cells and interacts with SIRP α on macrophages to transmit a “don't eat me” signal, thereby suppressing phagocytic clearance[14,15]. Interference with CD47-SIRP α signaling has emerged as a promising approach to enhance macrophage-mediated phagocytosis and alleviate local immune suppression[16,17]. Nevertheless, checkpoint intervention alone is often insufficient for solid tumors, especially in the presence of residual tumor burden and an immunosuppressive tumor microenvironment (TME)[18–20]. Integration of macrophage-associated immune modulation with a localized tumoricidal modality may therefore offer a more effective strategy for postoperative tumor-associated bone defects.

Ultrasound-responsive piezoelectric nanomaterials provide a noninvasive approach for spatially controlled local therapy[21]. Under ultrasound stimulation, tetragonal barium titanate (BT) undergoes mechanical deformation and charge separation, generating a piezoelectric potential that drives interfacial redox reactions and reactive oxygen species (ROS) production[22]. This process can induce oxidative stress-mediated tumor-cell injury without the limited tissue penetration associated with light-based modalities or dependence on endogenous hydrogen peroxide. Piezoelectric stimulation has also been implicated in the regulation of bone-related cell behavior and may facilitate osteogenic differentiation and matrix mineralization under appropriate conditions[23]. However, bare inorganic piezocatalysts generally lack a bioactive interface for effective interaction with tumor and immune cells, thereby limiting their therapeutic performance *in vivo*[24].

Cell membrane coating technology provides a versatile strategy to improve the biological performance of nanomaterials. By preserving membrane-associated proteins and intercellular recognition motifs, cell membrane-camouflaged nanoparticles may acquire improved biocompatibility and disease-relevant biological interactions[25–27]. In particular, macrophage membrane coating introduces immune-associated functions into therapeutic nanosystems. Genetic engineering of source cells further enables stable enrichment of functional membrane proteins,

including SIRP α , while avoiding complex post-synthetic ligand modification[28,29]. Despite the individual advantages of macrophage membrane coating and piezocatalysis, their integration for the treatment of malignant tumor-associated complex bone defects remains insufficiently explored.

In this study, we developed an engineered macrophage membrane-camouflaged piezoelectric nanosystem, termed M_{SIRP α} -BT, by coating BT nanoparticles with membranes derived from SIRP α -overexpressing macrophages. As illustrated in Scheme 1, this bioinspired platform was designed to integrate antitumor activity, immune microenvironment modulation, and osteogenic stimulation for the management of osteosarcoma-associated bone defects. The SIRP α -enriched membrane was designed to function as a CD47-binding decoy interface. By competitively engaging CD47 on tumor cells, membrane-associated SIRP α may reduce the inhibitory interaction between tumor-cell CD47 and endogenous SIRP α on phagocytes, thereby facilitating macrophage-mediated phagocytic clearance. In parallel, ultrasound activation of the piezoelectric BT core generates ROS, which induce oxidative stress and apoptosis in tumor cells. The combined tumor-cell injury and enhanced phagocytic clearance were expected to alleviate local immunosuppression and contribute to remodeling of the tumor immune microenvironment. For bone regeneration, ultrasound-triggered piezoelectric stimulation provides local bioelectrical cues that support the osteogenic differentiation of BMSCs, as reflected by the increased expression of osteogenic markers, including ALP, OPN, COL-1, and RUNX2. Overall, M_{SIRP α} -BT was evaluated as a multifunctional strategy for local tumor suppression, immune microenvironment remodeling, and restoration of bone regenerative potential in osteosarcoma-associated bone defects.



Scheme 1. Schematic illustration of the engineered macrophage membrane-camouflaged piezoelectric nanosystem ($M_{SIRP\alpha}$ -BT) for coordinated tumor suppression, macrophage-associated immune modulation, and bone regeneration.

2. Materials and methods

2.1. Cell Culture

Murine RAW264.7 macrophages and K7M2 cells (both from ATCC, USA) were routinely cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (P/S, Gibco, USA), at 37 °C in a humidified 5% CO₂ incubator. K7M2-GFP cells, a stable cell line constitutively expressing green fluorescent protein (GFP) previously constructed in our laboratory, were cultured in the same medium supplemented with puromycin to maintain stable GFP expression. Primary bone marrow derived mesenchymal stem cells (BMSCs) were isolated from the femurs and tibiae of 4-week-old male Sprague-Dawley rats. The marrow cavity was flushed with DMEM, and the collected cells were triturated into a single-cell suspension. After erythrocyte lysis (Beyotime, China), adherent cells were cultured in the same complete medium, refreshed every 2-3 days to remove non-adherent hematopoietic elements. BMSCs at passages 3-5 were used for all experiments, and all cell lines were maintained in the logarithmic growth phase.

2.2. Construction of SIRPα-overexpressing RAW264.7 cell line

Recombinant lentiviral particles encoding SIRPα were obtained from OBiO (China). RAW264.7 cells were seeded into 12-well plates (Corning, USA) at 2×10^5 cells/well and incubated overnight. The next day, the medium was replaced with 1.5 mL of fresh complete medium containing lentivirus (MOI = 50) and $6 \mu\text{g mL}^{-1}$ polybrene. Cells were exposed to the lentiviral suspension for 4 h, after which the medium was replaced with fresh complete DMEM. At 24 h post-transduction, the medium was switched to fresh complete medium supplemented with $3 \mu\text{g mL}^{-1}$ puromycin (Beyotime, China) to select positively transduced cells. Cells were then transferred to 10-cm dishes (Corning, USA) and maintained under puromycin for an additional 48 h to eliminate untransduced cells. The surviving puromycin-resistant pools were expanded in selective medium and cryopreserved in liquid nitrogen for future use. The resulting cell lines were designated as RAW264.7-control

(untransduced), RAW264.7-blank (transduced with empty vector), and RAW264.7-SIRP α (transduced with SIRP α -overexpressing lentivirus).

2.3. Fabrication of Tetragonal BT Nanoparticles

The synthetic procedure commenced with the preparation of a 60% ethanol aqueous solution (25 mL), into which 5.915 g of Ba(OH)₂·8H₂O (Aladdin, China) was charged. The mixture was heated in a water bath at 80 °C with magnetic stirring for 30 min to ensure complete dissolution. Sequential addition of tetrabutyl titanate (4.3 mL, Aladdin, China) and ammonia solution (25%, 2 mL, Aladdin, China) was then performed, and the reaction system was kept under stirring for a further 30 min. The reaction mixture was transferred into a 100 mL hydrothermal vessel and aged at 230 °C for 48 h. The resultant product was recovered, ball-milled, and sieved to produce the final tetragonal barium titanate (BT) nanoparticles.

2.4. Preparation of M_{SIRP α} -BT and M-BT

RAW264.7-SIRP α cells were cultured in 100-mm dishes at 5×10^6 cells per dish for 24 h. Cells were harvested using 0.25% trypsin-EDTA, washed thrice with PBS, and pelleted at $500 \times g$ for 5 min. The pellet was subjected to three freeze-thaw cycles at -80 °C, followed by ultrasonication (20% amplitude, 2 s on/3 s off) for 5 min. Cell membranes were isolated by sequential centrifugation at $500 \times g$ (10 min), $10,000 \times g$ (10 min), and $100,000 \times g$ (60 min) at 4 °C using an ultracentrifuge (Beckman Coulter, USA). Membrane protein content was quantified via a BCA assay (Thermo Scientific, USA). The isolated membranes (M_{SIRP α}) were mixed with BT nanoparticles at a 1:1 protein-to-NP weight ratio in PBS (0.5 mg mL⁻¹) and extruded 20 times through a 200-nm polycarbonate membrane using a mini extruder (Avanti, Australia). The resulting M_{SIRP α} -BT nanoparticles were collected by centrifugation at $10,000 \times g$ for 5 min to remove excess membranes and stored at 4 °C. For comparison, M-BT was identically prepared using membranes derived from RAW264.7-control cells under the same conditions.

2.5. Characterization of BT and $M_{SIRP\alpha}$ -BT

Surface morphology was examined by scanning electron microscopy (SEM, Zeiss, Germany), and internal nanostructures were visualized by transmission electron microscopy (TEM, Hitachi, Japan). Elemental mapping was acquired using energy-dispersive X-ray spectroscopy (EDS) integrated with the electron microscopes. Hydrodynamic diameter and zeta potential were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments, UK). Prior to all measurements, samples were dispersed in deionized water by ultrasonication to ensure homogeneous suspension.

2.6. Western blot analysis

Membrane proteins were extracted via differential centrifugation as described above and quantified using a BCA assay. Due to the close molecular weights of $SIRP\alpha$ (~90 kDa) and ATPase (~100 kDa), the target protein and internal control were resolved on separate parallel SDS-PAGE gels. After transfer onto PVDF membranes and blocking with 5% BSA, the blots were incubated overnight at 4 °C with primary antibodies against $SIRP\alpha$ (Abcam, UK) and ATPase (HuaAn Biotechnology, China), followed by HRP-conjugated secondary antibodies. Protein bands were visualized using an ECL kit and quantified with ImageJ software.

2.7. ROS staining

K7M2 cells were seeded into 6-well plates (Corning, USA) at 1×10^6 cells per well and cultured to approximately 80% confluence, followed by treatment according to the experimental grouping. For US-treated groups, the cells were irradiated with ultrasound (1.8 W cm^{-2} , 1.0 MHz, 50% duty cycle) for 5 min. After intervention, the culture medium was removed, and the cells were incubated with 10 μM DCFH-DA (ROS Assay Kit, Beyotime, China) diluted in serum-free medium for 20 min at 37 °C in the dark. The cells were then washed three times with serum-free medium to remove excess probe. Finally, intracellular ROS levels were visualized and imaged under an inverted fluorescence microscope (Nikon, Japan).

2.8. ESR spectroscopy for ROS detection

Electron spin resonance (ESR) spectra were recorded on a Bruker A300 spectrometer (Bruker, Germany) to identify reactive oxygen species (ROS) generated by piezoelectric catalysts under ultrasound (US) irradiation. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was employed as a spin-trapping agent for both hydroxyl radicals ($\cdot\text{OH}$) and superoxide anions ($\cdot\text{O}_2^-$). Four groups were examined at ambient temperature: DMPO alone (control), DMPO with US (US), DMPO with BT (BT), and DMPO with BT under US (BT + US).

2.9. Methylene blue degradation assay

The ROS-generating capacity of BT and $\text{M}_{\text{SIRP}\alpha}$ -BT was further evaluated by methylene blue (MB, Macklin, China) degradation. BT and $\text{M}_{\text{SIRP}\alpha}$ -BT (5 mg each) were dispersed in 5 mL of MB aqueous solution (10 mg L^{-1}) and stirred in the dark for 60 min to establish adsorption-desorption equilibrium. The suspensions were then exposed to US irradiation (1.8 W cm^{-2} , 1 MHz) for varying durations (0, 5, 10, and 15 min). At each time point, aliquots were collected and centrifuged at $10,000 \times g$ for 5 min to remove particulates, and the residual MB concentration in the supernatant was determined using a UV-Vis spectrophotometer (Thermo Scientific, USA).

2.10. ALP and ARS staining

BMSCs were seeded into 6-well plates and treated according to the experimental grouping, with designated groups receiving US irradiation (0.6 W cm^{-2} , 1.0 MHz, 50% duty cycle) for 1 min. The cells were then cultured in osteogenic induction medium. For alkaline phosphatase (ALP) staining, cells were fixed with 4% paraformaldehyde after 7 days of induction and incubated with BCIP/NBT substrate (Beyotime, China) at 37°C for 30 min. For alizarin red S (ARS) staining, cells were fixed after 14 days and stained with 2% ARS solution (Servicebio, China) for 10 min to visualize calcium deposits. All stained samples were imaged under a bright-field microscope, and semi-quantitative analysis was performed using ImageJ software.

2.11. Immunofluorescence staining

RAW264.7-control, RAW264.7-blank, and RAW264.7-SIRP α cells were seeded into confocal dishes (BioSharp, China) at 1×10^5 cells per dish. After 24 h, cells were rinsed thrice with PBS, fixed in 4% paraformaldehyde for 15 min, and blocked with 5% goat serum albumin (BOSTER, USA) in PBS for 1 h at 37 °C. The cells were then incubated with monoclonal antibody against SIRP α (abcam, UK) overnight at 4 °C, followed by goat anti-rabbit secondary antibody (abcam, UK) for 1 h at 37 °C. After three PBS washes, nuclei were counterstained with DAPI for 3 min. Stained cells were visualized under a Zeiss confocal microscope (Zeiss, Germany). Separately, BMSCs were subjected to the same immunofluorescence procedure following 7 days of culture under designated treatments, with ultrasound-exposed groups receiving irradiation at 0.6 W cm⁻² and 1 MHz. Runx2 expression was detected using a monoclonal antibody (HuaBio, China), and F-actin was stained with phalloidin (Solarbio, China), followed by DAPI counterstaining.

2.12. Real-time quantitative polymerase chain reaction (qRT-PCR)

RAW264.7-control, RAW264.7-blank, and RAW264.7-SIRP α cells were seeded into 6-well plates (Corning, USA) at 1×10^6 cells/well and cultured for 24 h. Total RNA was extracted using the Total RNA Kit I (Omega Bio-tek, USA), quantified on a Nanodrop 2000 (Thermo Scientific, USA), and reverse-transcribed into cDNA using the TransScript All-in-One First-Strand cDNA Synthesis SuperMix (Transgene, China). Real-time PCR was performed on a CFX Connect™ system (Bio-Rad, USA) with SYBR Green. Each sample was run in triplicate, and the entire experiment was repeated three times independently. Relative mRNA levels were calculated via the $2^{-\Delta\Delta C_t}$ method with GAPDH as the internal control. Primer sequences for SIRP α are listed in Table S1. Separately, BMSCs were subjected to the same qRT-PCR procedure following 72 h of culture under designated treatments, with ultrasound-exposed groups receiving irradiation at 0.6 W cm⁻² and 1 MHz. Osteogenic marker genes including ALP, OPN, COL-1, and RUNX2 were assessed,

and their corresponding primer sequences are provided in Table S2.

2.13. Flow cytometric analysis

RAW264.7-control, RAW264.7-blank, and RAW264.7-SIRP α cells were seeded into 6-well plates (1×10^6 cells/well) for 24 h. Cells were harvested, pelleted ($300 \times g$, 5 min, 4 °C), washed twice with ice-cold PBS, and stained with SIRP α monoclonal antibody (Abcam, UK) on ice for 15 min in the dark, followed by washing and centrifugation ($500 \times g$, 5 min, 4 °C). For the phagocytosis assay, K7M2-GFP cells were seeded (1×10^5 cells/well), cultured to 60% confluence, and co-cultured with RAW264.7 macrophages (1×10^5 cells/well) and different nanoparticles for 12 h. Cells were then harvested and stained as described above. For *in vivo* analysis, tumor tissues from K7M2-bearing mice were collected and dissociated using a Tumor Dissociation Kit (Miltenyi). Macrophage polarization was evaluated by staining with antibodies against F4/80, CD11b, CD86, and CD206 following the manufacturer's protocols. The gating strategy was as follows: macrophages were identified as F4/80⁺CD11b⁺, with M1 and M2 subsets defined as CD86⁺ and CD206⁺, respectively. All samples were resuspended in PBS, analyzed on a CytoFLEX flow cytometer (Beckman Coulter, USA), and processed with FlowJo software.

2.14. Time-lapse live-cell imaging

RAW264.7 macrophages and K7M2 osteosarcoma cells were cultured separately to appropriate confluence. K7M2 cells were seeded into Ibidi culture plates (Ibidi, Germany) at 1×10^5 cells per well and allowed to adhere for 12 h, followed by treatment according to the experimental grouping. Subsequently, RAW264.7 macrophages were added at a tumor-to-macrophage ratio of 1:10 and co-incubated for 2 h at 37 °C in 5% CO₂ to allow cell-cell interaction. Time-lapse imaging was performed using an Ibidi live-cell imaging system, with images captured every 30 min over a total observation period of 3 h under controlled environmental conditions (37 °C, 5% CO₂, humidified atmosphere). The acquired sequential images were processed and analyzed to monitor macrophage phagocytic activity toward tumor

cells.

2.15. Transcriptomic and proteomic analysis

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, USA) and subjected to paired-end 150-bp sequencing on an Illumina NovaSeq X Plus platform. Raw reads were quality-filtered using fastp and aligned to the mouse GRCm39 reference genome using HISAT2. Transcript assembly and expression quantification were performed using StringTie, and gene-level raw counts were used for differential expression analysis with DESeq2. Differentially expressed genes were identified using thresholds of $FDR < 0.05$ and $|\log_2FC| \geq 1$. GO and KEGG enrichment analyses were performed using the OmicStudio cloud platform.

For proteomic analysis, cells were lysed in RIPA buffer supplemented with a protease inhibitor cocktail, and protein concentrations were determined using a BCA assay. Equal amounts of protein were reduced, alkylated, and digested with trypsin at an enzyme-to-protein ratio of 1:50 overnight at 37 °C. The resulting peptides were labeled with TMT 10plex reagents, pooled, and fractionated by high-pH reversed-phase chromatography. NanoLC-MS/MS analysis was performed using a Vanquish Neo UHPLC system coupled with an Astral Orbitrap mass spectrometer (Thermo Fisher Scientific) in data-dependent acquisition mode. Raw MS data were processed using SpectroMine software with the Pulsar search engine against the UniProt Mus musculus database. False discovery rates at the peptide-spectrum match and peptide levels were controlled at $\leq 1\%$.

2.16. In vivo experiments

All animal procedures were approved by the Ethics Committee of Guangdong Province's General Hospital (approval no. KYD202140201). To establish the complex tumorous bone defect model, 4-6 week-old male BALB/c mice were anesthetized, and the lateral femoral condyle was exposed via blunt dissection. A 3 mm bone defect was created using a 12-gauge needle, after which pre-prepared tumor tissue was implanted into the defect and the wound was sutured. Tumor-bearing mice were randomly

divided into eight groups (n=5): Control, BT, M-BT, M_{SIRP α} -BT, US, BT + US, M-BT + US, and M_{SIRP α} -BT + US. For nanoparticle-treated groups, 100 μ L of BT, M-BT, or M_{SIRP α} -BT suspension (500 μ g mL⁻¹ in PBS) was administered via intratumoral injection. US-treated groups received pulsed US irradiation on days 1 and 7 post-injection at intensities of 1.8 and 0.6 W cm⁻², respectively, with irradiation durations of 5 and 1 min, respectively (1.0 MHz; 50% duty cycle). Tumor dimensions were measured every 7 days using a caliper, and tumor volume was calculated as $0.5 \times \text{length} \times \text{width}^2$. Because the lesion involved the femur and surrounding soft tissues, this measurement should be interpreted as the apparent tumor-bearing mass volume rather than the volume of tumor tissue alone. Body weight was recorded weekly and normalized to the initial measurement.

2.17. Histological examination

Tumor tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. The embedded tissues were sectioned at 4 μ m thickness, mounted onto glass slides, and dried overnight at 60 °C. For hematoxylin and eosin (H&E) staining, sections were deparaffinized, rehydrated, stained with hematoxylin and eosin, dehydrated, cleared, and mounted with coverslips. For immunofluorescence staining, antigen retrieval was performed by heating sections in citrate buffer using a microwave. After blocking with 10% serum, sections were incubated with primary antibodies overnight at 4 °C, followed by fluorophore-conjugated secondary antibodies for 1 h at room temperature. Nuclei were counterstained with DAPI, and sections were mounted for observation.

2.18. Statistical analysis

All quantitative data were expressed as mean $\pm$ standard deviation (SD). Intergroup differences were evaluated using Student's t-test for two-group comparisons, while one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for multiple group comparisons. Statistical significance was indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns denotes not significant.

Each experiment was performed with at least three independent replicates, with exact sample sizes (n) specified in the corresponding figure legends. All statistical computations were conducted using GraphPad Prism version 9.0 (GraphPad Software, USA).

Results and Discussion

3.1. Construction and physicochemical characterization of $M_{SIRP\alpha-BT}$

To fabricate the “well-educated” macrophage membrane-camouflaged piezoelectric nanosystem illustrated in Scheme 1, RAW264.7 macrophages were first engineered to stably overexpress SIRP α via lentiviral transduction followed by puromycin selection (Fig. 1a). Here, “well-educated” refers to the deliberate installation of a SIRP α -enriched, CD47-recognizing phenotype in source macrophages, thereby enabling their membrane to confer a programmed immunoregulatory interface to the nanoparticle surface[30,31]. RT-qPCR analysis revealed a marked increase in SIRP α mRNA expression in the engineered RAW264.7-SIRP α cells compared with untransduced controls (RAW264.7-Control) and empty-vector controls (RAW264.7-Blank) (Fig. 1b). Immunofluorescence staining consistently showed substantially enhanced SIRP α -associated fluorescence in the engineered macrophages (Fig. 1c). Flow cytometry further demonstrated that the proportion of SIRP α -positive cells was substantially increased in RAW264.7-SIRP α cells (94.5%) compared with RAW264.7-control cells (54.7%) and RAW264.7-blank cells (Fig. 1d). Western blotting of the membrane protein fractions isolated from RAW264.7-Control, RAW264.7-Blank, and RAW264.7-SIRP α cells further confirmed SIRP α enrichment, with ATPase used as a membrane-associated loading reference (Fig. 1e,f). These results demonstrated the successful generation of well-educated macrophages as the membrane source for $M_{SIRP\alpha-BT}$ fabrication.

The SIRP α -enriched membrane was designed to recognize CD47, an antiphagocytic checkpoint signal broadly expressed by tumor cells[32]. Rather than chemically conjugating recombinant SIRP α to the nanoparticle surface, the use of membranes from engineered macrophages preserves SIRP α within its native

lipid-bilayer context[33]. Thus, the resulting membrane shell serves not merely as a physical camouflage layer, but as an immunologically programmed interface capable of mediating CD47-associated tumor-cell recognition. The functional consequences of this well-educated membrane interface for tumor-cell interaction, macrophage polarization, and phagocytic activity were investigated in subsequent experiments.

Tetragonal barium titanate (BT) nanoparticles were synthesized as the ultrasound-responsive piezoelectric core. SEM images showed that BT nanoparticles possessed a relatively uniform cuboidal morphology with smooth surfaces (Fig. 1g). The selected-area electron diffraction pattern and lattice fringes observed in the inset indicated good crystallinity of the synthesized particles (Fig. 1h). XRD analysis revealed the characteristic peak splitting near $2\theta \approx 45^\circ$, corresponding to the tetragonal (002) and (200) planes of BT (Fig. 1i). This tetragonal phase provides the structural basis for piezoelectric polarization under ultrasound-induced mechanical deformation[23]. TEM and EDS elemental mapping further confirmed the morphology of bare BT and the homogeneous distribution of Ba, Ti, and O throughout the nanoparticles (Fig. 1j,k and Fig. S1).

Membranes isolated from RAW264.7-SIRP α cells were fused with BT nanoparticles by extrusion to obtain M_{SIRP α} -BT[34]. TEM revealed a distinct shell surrounding the electron-dense BT core, consistent with successful membrane deposition on the nanoparticle surface (Fig. 1l). EDS elemental mapping provided additional evidence of membrane coating: a peripheral phosphorus signal was observed in M_{SIRP α} -BT but was absent from bare BT nanoparticles (Fig. 1m), consistent with the phospholipid-rich nature of the macrophage membrane. These results support the successful integration of an immunologically programmed macrophage membrane shell with the piezoelectric BT core.

Membrane coating was further supported by changes in colloidal properties. The hydrodynamic diameter increased from 192.1 nm for bare BT to 252.8 nm after membrane coating, whereas the zeta potential shifted from -11.2 to -32.4 mV (Fig. 1n). These changes were consistent with the formation of a membrane-derived outer interface. Flow-cytometric analysis detected SIRP α -positive signals in 72.4% of the

M_{SIRP α} -BT population, whereas bare BT exhibited only background signals (Fig. S3a,b), indicating that the engineered SIRP α signature was retained after membrane isolation and extrusion[35]. Moreover, TEM analysis after storage at 4 °C for 4 weeks showed preservation of the core-shell morphology (Fig. S3c), supporting the short-term structural stability of M_{SIRP α} -BT.

The baseline cytocompatibility of BT and M_{SIRP α} -BT was assessed in C3H10T1/2 cells (hereafter termed C3H10 cells) in the absence of ultrasound stimulation. CCK-8 analysis showed that both formulations maintained cell viability above 80% after 24, 48, and 72 h of incubation (Fig. S2a). Notably, M_{SIRP α} -BT exhibited higher cell viability than bare BT, particularly at 48 h, suggesting that membrane camouflage partially attenuated the basal cytotoxicity of the inorganic core[36]. This finding was corroborated by live/dead staining, in which C3H10 cells treated with M_{SIRP α} -BT displayed predominantly Calcein-AM-positive signals and fewer PI-positive cells than those exposed to bare BT (Fig. S2b).

Taken together, these results confirm the successful construction of M_{SIRP α} -BT, in which a tetragonal piezoelectric BT core is integrated with a stable, SIRP α -enriched “well-educated” macrophage membrane shell. This design establishes a multifunctional platform in which the membrane component is programmed to modulate the local immune microenvironment, while the BT core provides ultrasound-activated piezoelectric functionality for subsequent antitumor and osteogenic applications.

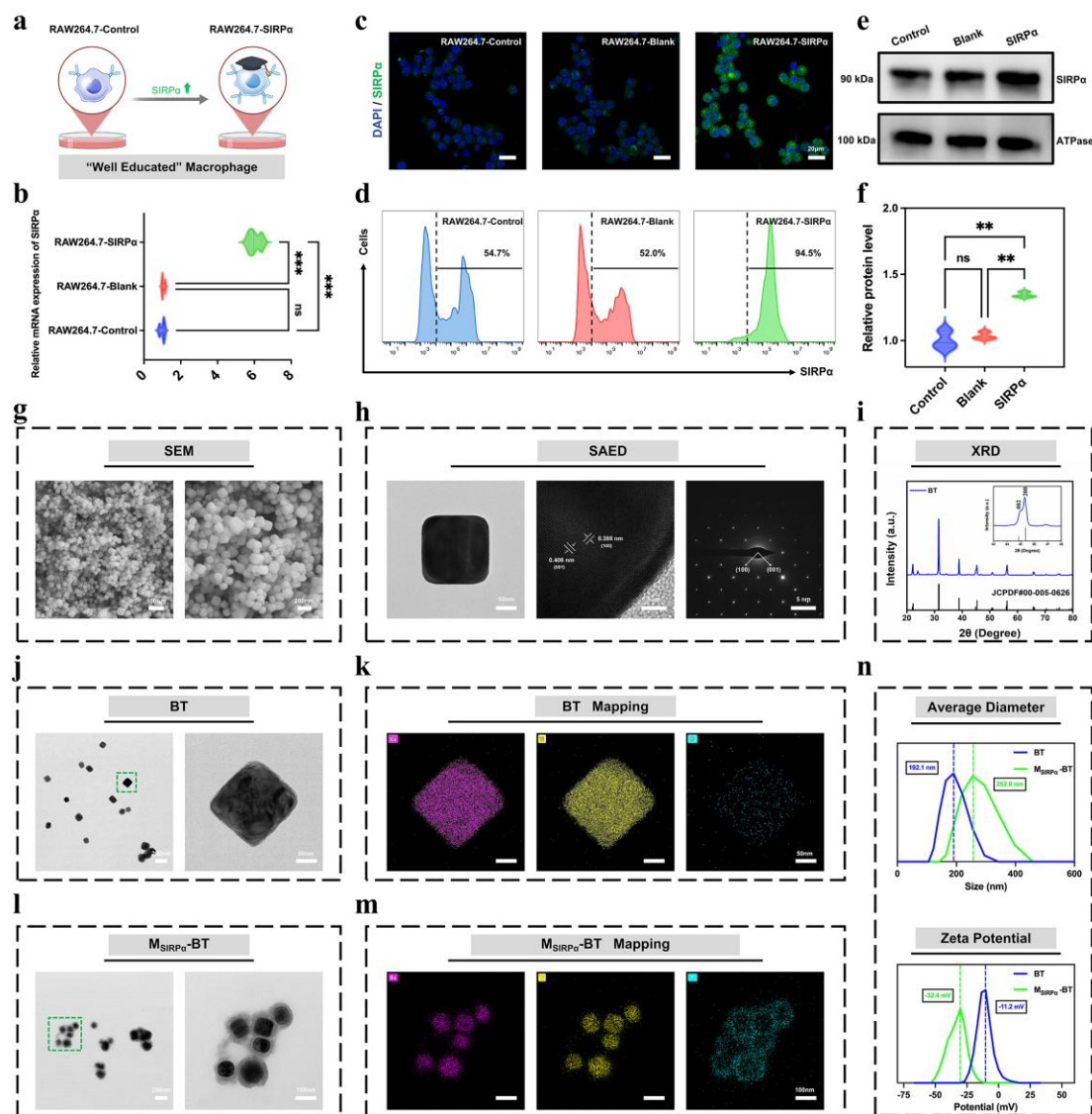


Figure 1. Construction and characterization of SIRP α -engineered macrophages and MSIRP α -BT nanoparticles. (a) Schematic illustration of the preparation of SIRP α -overexpressing RAW264.7 macrophages via lentiviral transduction. (b) RT-qPCR analysis of SIRP α mRNA levels in Control, Blank, and SIRP α groups. (c, d) Immunofluorescence staining and flow cytometry analysis confirming the successful overexpression of SIRP α protein on the engineered RAW264.7 macrophages (Blue: DAPI; Green: SIRP α . Scale bars = 20 μ m). (e, f) Western blot analysis of SIRP α in the membrane proteins isolated from the Control, Blank, and SIRP α groups, and the corresponding densitometric quantification (ATPase used as the internal control for membrane proteins). (g) SEM images of the synthesized BT nanoparticles. Scale bars = 500 nm and 200 nm. (h, i) SAED pattern with lattice fringes and XRD pattern of BT

nanoparticles matching the standard tetragonal phase. Scale bars = 50 nm and 5 nm. (j, k) TEM images and EDS elemental mapping of bare BT nanoparticles. Scale bars = 200 nm and 50 nm. (l, m) TEM images and EDS elemental mapping of macrophage membrane-coated $M_{SIRP\alpha}$ -BT. Scale bars = 200 nm and 100 nm. (n) Hydrodynamic size distributions and zeta potentials of BT and $M_{SIRP\alpha}$ -BT nanoparticles. Data are presented as mean $\pm$ SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. Ultrasound-triggered piezoelectric ROS generation of $M_{SIRP\alpha}$ -BT

After confirming the structural integrity of the tetragonal BT core, we investigated its piezoelectric properties, which underpin the US-responsive catalytic activity of $M_{SIRP\alpha}$ -BT. Piezoresponse force microscopy (PFM) revealed a characteristic displacement-voltage butterfly loop and an approximately 180° phase reversal over an applied bias range of -10 V to +10 V (Fig. 2a), confirming the piezoelectric behavior of the BT nanoparticles. Under US irradiation, acoustic pressure induces cyclic deformation of the non-centrosymmetric BT lattice, leading to polarization charge separation and the formation of an interfacial electric field. The resulting charge carriers can participate in redox reactions with the surrounding medium, thereby generating ROS[37] (Fig. 2b).

Electron spin resonance (ESR) spectroscopy with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin-trapping agent was used to identify the ROS species generated during US activation. In aqueous solution, the BT + US group exhibited the characteristic 1:2:2:1 quartet signal of the DMPO- $\cdot$ OH adduct, whereas in methanol solution, signals attributable to the DMPO- $\cdot$ O₂⁻ adduct were detected[38] (Fig. 2e). In contrast, radical signals were negligible in the US-only and BT-only groups. These findings indicate that ROS production requires the combined application of BT and US, supporting a piezocatalytic mechanism.

The catalytic activity of the membrane-coated nanosystem was further assessed using methylene blue (MB) degradation. US irradiation alone caused negligible MB degradation over 15 min (Fig. 2f). In comparison, the absorbance of MB at 664 nm progressively decreased in the presence of BT under US irradiation, indicating

ROS-mediated dye degradation[39]. Importantly, $M_{SIRP\alpha}$ -BT showed degradation behavior comparable to that of bare BT, suggesting that macrophage membrane camouflage did not substantially impair the piezocatalytic activity of the BT core. This preservation of catalytic function is essential because the membrane shell is intended to provide biological functionality without compromising US-triggered ROS production.

Intracellular ROS production was assessed in K7M2 cells using DCFH-DA staining. Minimal fluorescence was observed in the control, US-only, and material-only groups (Fig. 2c). By contrast, US exposure markedly increased intracellular ROS signals in both the BT + US and $M_{SIRP\alpha}$ -BT + US groups, with the latter showing the strongest fluorescence intensity (Fig. 2c,d). Since the MB degradation assay indicated comparable cell-free catalytic activity between BT and $M_{SIRP\alpha}$ -BT, the greater intracellular ROS accumulation observed for $M_{SIRP\alpha}$ -BT under US may reflect enhanced nanoparticle-cell association mediated by the $SIRP\alpha$ -enriched membrane. Such close contact could increase the local availability of short-lived ROS at the tumor-cell surface and facilitate intracellular oxidative stress.

The PFM, ESR, MB degradation, and intracellular DCFH-DA results indicate that $M_{SIRP\alpha}$ -BT retains the piezoelectric and piezocatalytic properties of the BT core after membrane coating. Under US activation, the nanosystem generates $\cdot OH$ and $\cdot O_2^-$, enabling localized oxidative stress in osteosarcoma cells. This ROS-generating capability provides a mechanistic basis for the subsequent evaluation of $M_{SIRP\alpha}$ -BT mediated tumor-cell injury, macrophage associated tumor-cell clearance, and immune microenvironment modulation.

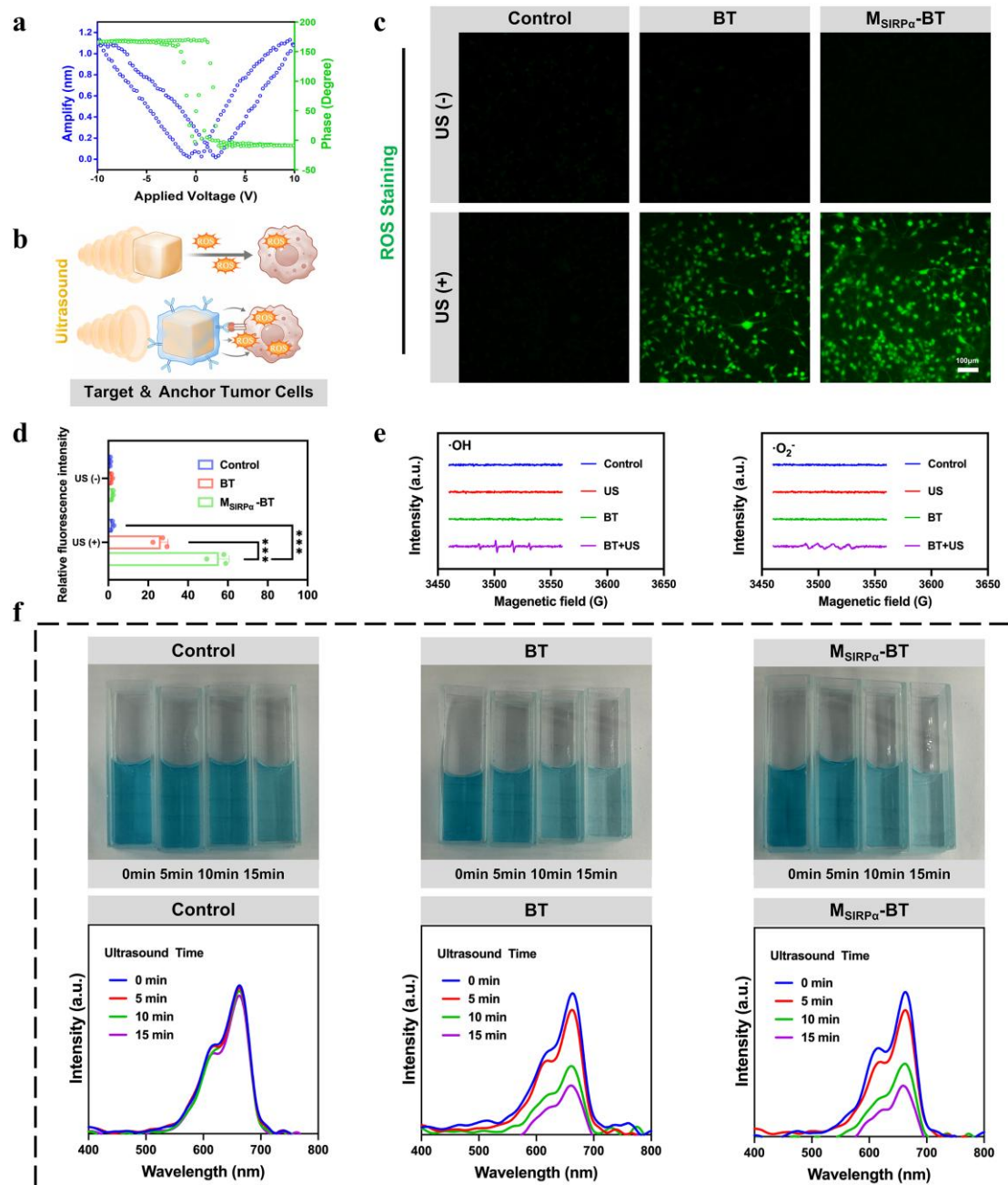


Figure 2. Piezoelectric effect and *in vitro* ROS generation of BT and $M_{SIRP\alpha}$ -BT nanoplateforms. (a) PFM amplitude (blue) and phase (green) curves of BT nanoparticles under applied voltage (-10 V to +10 V). (b) Schematic illustration of ultrasound-triggered piezocatalytic effect for targeted ROS generation. (c) Intracellular ROS staining of K7M2 cells treated with Control, BT, and $M_{SIRP\alpha}$ -BT with or without US irradiation. Scale bars = 100 μ m. (d) Quantitative analysis of relative fluorescence intensity from (c). (e) ESR spectra of hydroxyl radicals ($\cdot$ OH, left) and superoxide anions ($\cdot$ O₂⁻, right) trapped by DMPO under different treatments.

(f) Degradation photographs and corresponding UV-vis absorption spectra of methylene blue solutions in different groups under US irradiation for different durations (0-15 min). Data are presented as mean $\pm$ SD (n = 3). *** $p < 0.001$.

3.3. *In vitro synergistic anti-tumor efficacy and macrophage phagocytosis restoration*

The antitumor activity of M_{SIRP α} -BT was evaluated in K7M2 cells after confirmation of its US-triggered intracellular ROS-generating activity. Dose-response analysis showed that, under the antitumor US parameters (1.8 W cm⁻², 1.0 MHz, 50% duty cycle, 5 min), M_{SIRP α} -BT reduced K7M2 cell viability in a concentration-dependent manner, with an IC₅₀ below 300 μ g mL⁻¹ (Fig. S4). A concentration of 500 μ g mL⁻¹ was therefore selected for subsequent *in vitro* experiments to ensure a robust therapeutic response.

The effects of BT, M-BT, and M_{SIRP α} -BT were then compared in the presence or absence of US (Fig. 3a). In the absence of US, BT, M-BT, and M_{SIRP α} -BT showed limited cytotoxicity, indicating limited cytotoxicity under the tested conditions. Under US irradiation, cell viability decreased substantially in the BT, M-BT, and M_{SIRP α} -BT treated groups, confirming that the antitumor activity was dependent on piezoelectric activation of the BT core. Among all groups, M_{SIRP α} -BT + US resulted in a >90% reduction in cell viability relative to the untreated control. Live/dead staining showed predominantly Calcein-AM-positive viable cells in the US(-) groups, whereas abundant PI-positive dead cells were observed following M_{SIRP α} -BT + US treatment (Fig. 3c). Annexin V-FITC/PI staining further demonstrated that M_{SIRP α} -BT + US induced the highest proportion of apoptotic K7M2 cells (Fig. 3d,e). The apoptotic fraction increased from 4.99% in the Control + US group to 23.8% in the M_{SIRP α} -BT + US group. Together with the intracellular ROS data shown in Fig. 2, these findings support that US-activated M_{SIRP α} -BT induces oxidative stress and apoptosis in osteosarcoma cells.

BT and M_{SIRP α} -BT showed comparable ROS-associated MB degradation in the cell-free system (Fig. 2f), indicating that membrane coating did not markedly alter the intrinsic piezocatalytic activity of the BT core. Therefore, the superior cellular

response of the $M_{SIRP\alpha}$ -BT + US group may be attributable to enhanced nanoparticle-tumor cell association conferred by the $SIRP\alpha$ -enriched membrane. By interacting with CD47 expressed on tumor cells, membrane-associated $SIRP\alpha$ may retain the nanosystem near the tumor-cell surface, increasing the local availability of short-lived ROS generated during US activation.

Beyond direct tumor-cell injury, we investigated whether $M_{SIRP\alpha}$ -BT could facilitate macrophage-mediated clearance of K7M2-GFP cells. Flow cytometric analysis showed that $M_{SIRP\alpha}$ -BT + US produced the highest phagocytosis rate, reaching 30.2%, compared with 9.62% in the Control + US group and 18.6% in the M-BT + US group (Fig. 3f,g). Time-lapse live-cell imaging further visualized the dynamic interactions between macrophages and tumor cells. In the $M_{SIRP\alpha}$ -BT + US group, macrophage engulfment of K7M2-GFP cells was observed within 2 h, whereas apparent engulfment was not detected in the control or $M_{SIRP\alpha}$ -BT US(-) groups during the 3-h observation period (Fig. 3h).

The enhanced phagocytic activity likely results from the combined effects of membrane-associated $SIRP\alpha$ and US-induced tumor-cell injury. The $SIRP\alpha$ -enriched membrane was designed to competitively engage tumor-cell CD47, potentially reducing inhibitory CD47 signaling to endogenous $SIRP\alpha$ on macrophages[40]. Simultaneously, oxidative stress and apoptosis induced by US-activated piezocatalysis may render tumor cells more susceptible to macrophage recognition and engulfment[41]. Direct mechanistic validation using CD47/ $SIRP\alpha$ blockade or rescue experiments, together with assays of relevant pro-phagocytic signals, will be important in future studies. Overall, these results show that $M_{SIRP\alpha}$ -BT integrates US-triggered piezocatalytic tumor-cell injury with enhanced macrophage-associated phagocytic clearance. This dual mode of action provides the basis for subsequent analyses of treatment-associated molecular changes and *in vivo* antitumor immune modulation.

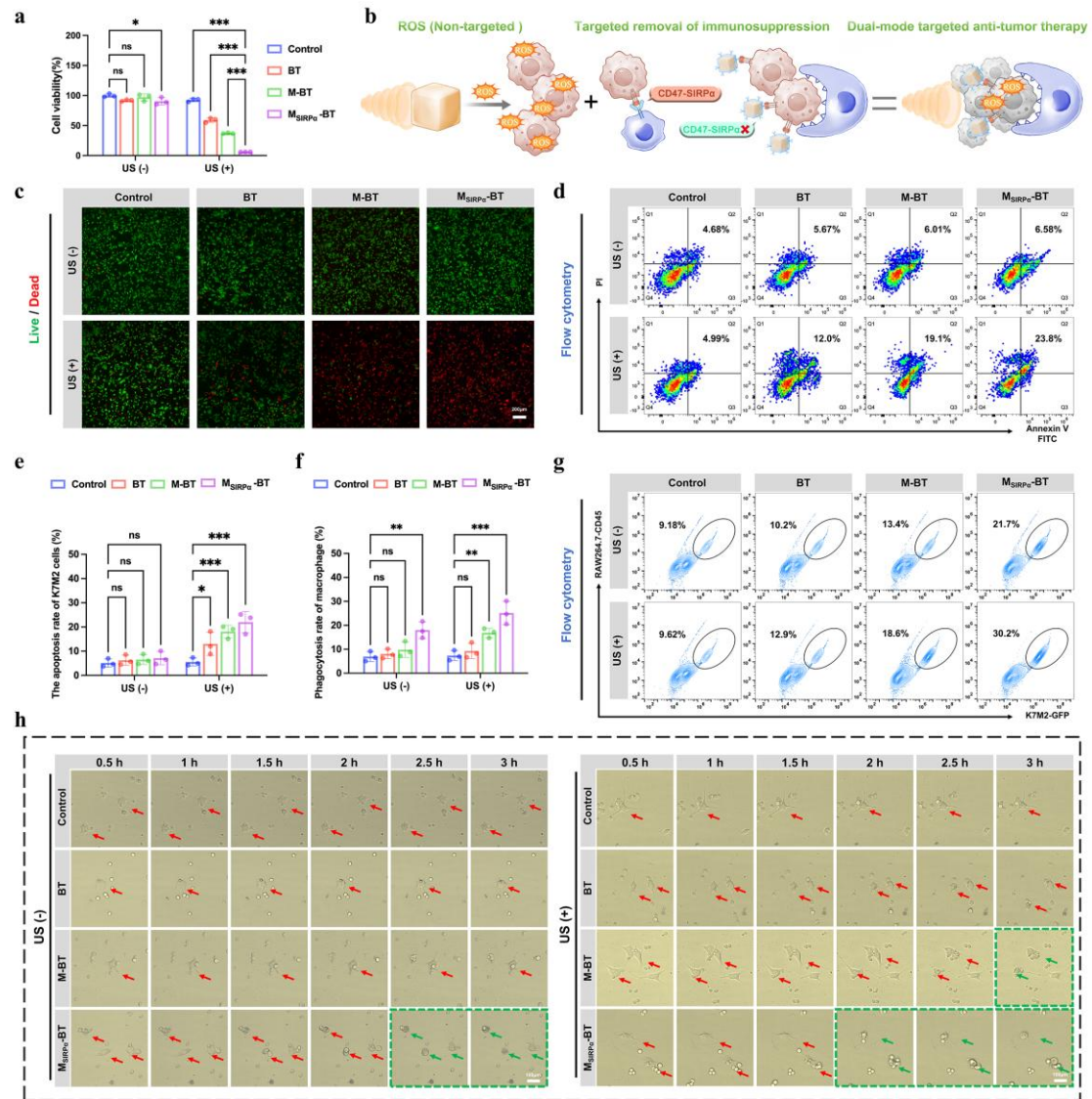


Figure 3. *In vitro* synergistic anti-tumor efficacy and immune activation of US-triggered $M_{SIRP\alpha}$ -BT. (a) Cell viability of K7M2 cells treated with different groups with or without US irradiation (1.8 W cm^{-2} , 1.0 MHz, 50% duty cycle, 5 min). (b) Schematic illustration of the dual-mode targeted anti-tumor therapy combining ROS-induced damage and targeted relief of CD47-SIRP α immunosuppression. (c) Representative live/dead staining images of K7M2 cells after different treatments (Calcein-AM, green; PI, red. Scale bar = 100 μ m). (d) Representative flow-cytometric plots of Annexin V-FITC/PI staining in K7M2 cells after the indicated treatments. (e, f) Quantitative analysis of the cell apoptosis rates (from d) and macrophage phagocytosis rates (from g). (g) Representative flow-cytometric analysis of macrophage phagocytosis of K7M2-GFP cells. (h) Time-lapse live-cell imaging

capturing the dynamic phagocytic process of macrophages on K7M2 tumor cells within 3 h. Red arrows indicate tumor cells; green arrows indicate phagocytosed cells. Scale bars = 100 μ m. Data are presented as mean $\pm$ SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4. Multi-omics analysis reveals molecular mechanisms of $M_{SIRP\alpha}$ -BT mediated tumor suppression

Transcriptomic RNA sequencing and tandem mass tag (TMT)-based proteomic analyses were performed in K7M2 cells to characterize molecular changes induced by $M_{SIRP\alpha}$ -BT + US. Untreated K7M2 cells served as the control. Transcriptomic profiling revealed extensive treatment-associated gene-expression changes, with 1,833 genes upregulated and 2,351 genes downregulated following $M_{SIRP\alpha}$ -BT + US exposure (Fig. 4a,b). KEGG enrichment analysis showed that upregulated genes were significantly enriched in pathways related to the cell cycle, DNA replication, and p53 signaling, whereas downregulated genes were mainly associated with ribosomal function and cellular senescence (Fig. 4c). GO analysis further identified enrichment of biological processes involved in DNA damage responses, DNA repair, and cell-cycle regulation (Fig. 4d). Together, these transcriptomic changes indicate that $M_{SIRP\alpha}$ -BT + US induces extensive cellular stress and activates programs associated with genome surveillance and cell-cycle control.

The p53 signaling pathway provides a plausible framework linking US-triggered piezocatalytic ROS generation to the apoptotic phenotype observed in K7M2 cells. As illustrated in the KEGG p53 signaling pathway map (Fig. 4e), p53 functions as a central stress-responsive regulator that can coordinate cell-cycle arrest, DNA repair, senescence, and apoptosis in response to genotoxic stress. In the present system, the substantial ROS generation induced by $M_{SIRP\alpha}$ -BT under US activation may disrupt intracellular redox homeostasis and induce oxidative damage, including DNA damage[42,43]. The resulting stress response could activate p53-associated transcriptional programs and promote the expression of downstream effectors involved in cell-cycle arrest and apoptosis, such as Cdkn1a (p21), Bax, and Puma[44].

This interpretation is consistent with the elevated apoptotic fraction observed by Annexin V-FITC/PI staining (Fig. 3d,e).

To determine whether the transcriptional alterations were reflected at the protein level, TMT-based proteomic analysis was performed using the same treatment comparison. The volcano plot and correlation scatter plot demonstrated substantial remodeling of the K7M2 proteome after M_{SIRP α} -BT + US treatment (Fig. 4f,g). KEGG and GO enrichment analyses of differentially expressed proteins similarly identified the p53 signaling pathway, cell-cycle regulation, and apoptosis-associated processes among the significantly altered functional categories (Fig. 4h-k). Integrated transcriptomic-proteomic analysis further demonstrated concordant enrichment of the p53 signaling pathway, DNA replication, and mismatch repair at both the mRNA and protein levels (Fig. S5a,b), strengthening the association between M_{SIRP α} -BT + US treatment and activation of stress-related molecular networks.

These multi-omics data complement the preceding functional experiments. US-activated M_{SIRP α} -BT generated intracellular ROS (Fig. 2), induced substantial tumor-cell injury and apoptosis (Fig. 3), and enhanced macrophage-associated phagocytosis. The observed enrichment of p53- and DNA damage-related pathways offers a molecular explanation for how piezocatalytic oxidative stress may be converted into cell-cycle dysregulation and apoptotic cell death[45]. In turn, oxidatively damaged or apoptotic tumor cells may be more susceptible to macrophage recognition and clearance[46]. The transcriptomic and proteomic datasets indicate that M_{SIRP α} -BT + US elicits a broad oxidative-stress-associated cellular response in K7M2 cells, with p53 signaling emerging as a prominent candidate pathway.

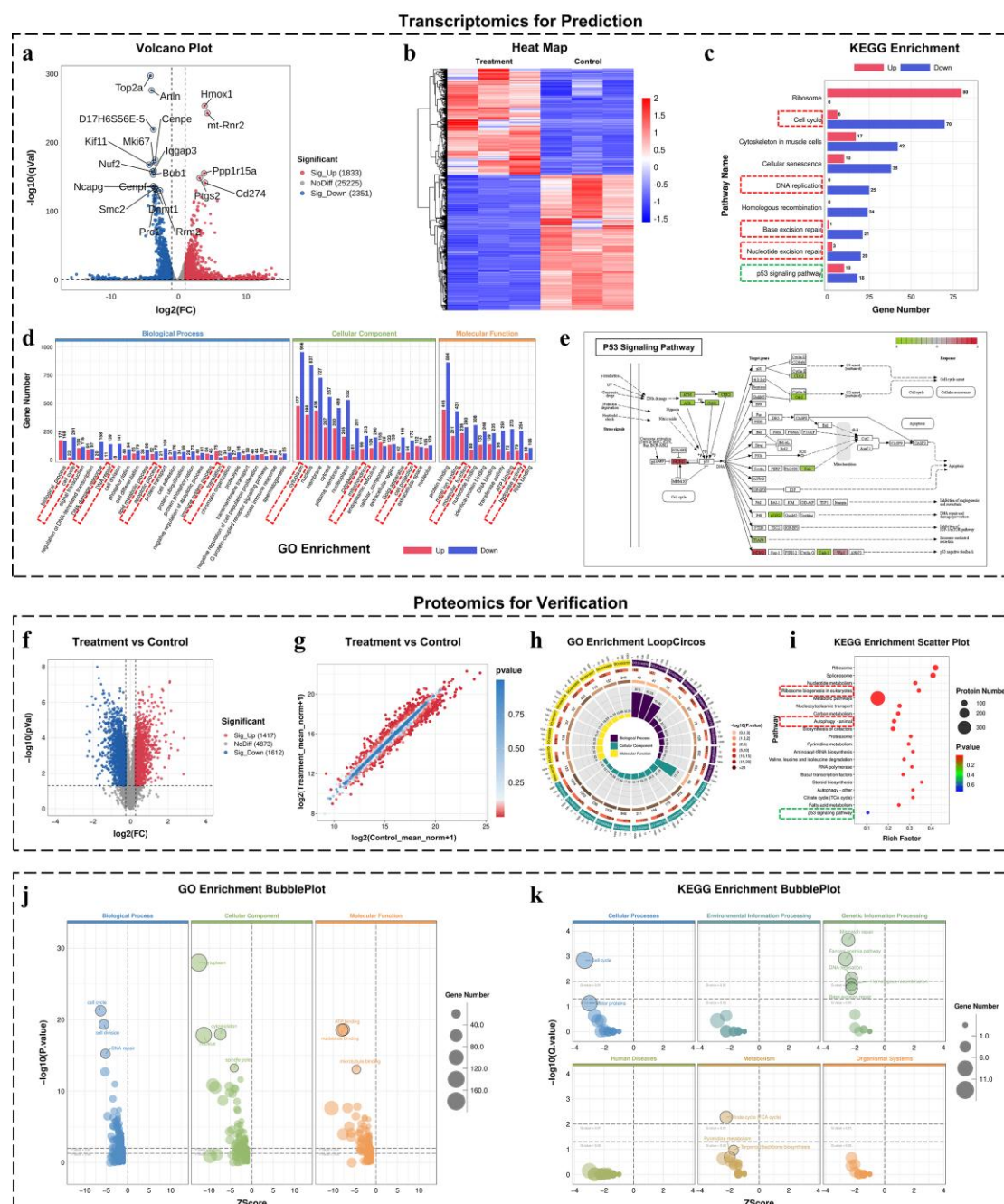


Figure 4. Transcriptomic prediction and proteomic verification of the anti-tumor mechanism mediated by $M_{SIRP\alpha}$ -BT. (a, b) Volcano plot and heat map illustrating the differentially expressed genes (DEGs) in the Treatment group compared to the Control group from transcriptomic analysis. (c, d) KEGG enrichment analysis of the up- and down-regulated DEGs, alongside GO enrichment analysis categorized by biological processes, cellular components, and molecular functions. (e) Schematic diagram of the canonical p53 signaling pathway[47]. (f, g) Volcano plot and correlation scatter plot of the significantly changed proteins in the proteomic

verification. (h, i) GO enrichment LoopCircos map and KEGG enrichment scatter plot of the proteomic data. (j, k) GO and KEGG enrichment bubble plots illustrating the distribution of enriched terms based on Z-scores and biological functional categories.

3.5. *M_{SIRP α} -BT promotes ultrasound-assisted osteogenic differentiation of BMSCs*

The osteogenic effects of M_{SIRP α} -BT were evaluated in bone marrow derived mesenchymal stem cells (BMSCs) under low-intensity US. BMSCs were treated with BT, M-BT, or M_{SIRP α} -BT in the presence or absence of US (0.6 W cm⁻², 1.0 MHz, 50% duty cycle, 1 min), followed by osteogenic induction[5].

After 3 days, RT-qPCR analysis showed that ultrasound-activated M_{SIRP α} -BT significantly increased the expression of osteogenic genes, including ALP, OPN, COL-1, and RUNX2, compared with the corresponding non-ultrasound group and control treatment (Fig. 5a). Although nanoparticle treatment alone elicited limited changes in gene expression without US, US irradiation markedly upregulated the osteogenic genes in all nanoparticle-treated groups. Notably, no significant differences were detected among the BT + US, M-BT + US and M_{SIRP α} -BT + US groups, indicating that the osteogenic response was driven primarily by US-triggered piezoelectric stimulation of the BT core rather than by the membrane coating. This suggests that the SIRP α -enriched membrane shell improves biocompatibility while preserving the transmission of piezoelectric microcurrents to the BMSC membrane, a process essential for osteogenic signal transduction. ALP staining performed on day 7 revealed significantly enhanced ALP staining in the M_{SIRP α} -BT + US group (Fig. 5b and Fig. S6a). At day 14, Alizarin Red S staining demonstrated marked calcium deposition following M_{SIRP α} -BT + US treatment (Fig. 5c and Fig. S6b). Immunofluorescence staining further confirmed RUNX2 expression in BMSCs treated with M_{SIRP α} -BT + US (Fig. 5d and Fig. S6c), indicating enhanced osteogenic commitment at the protein level[48].

These findings suggest that M_{SIRP α} -BT can provide a dual-function therapeutic effect in the setting of osteosarcoma-associated bone defects. Under the relatively mild US conditions used for the BMSC experiments, the BT core generates localized

piezoelectric stimulation without causing the extensive oxidative damage observed under the antitumor US parameters. This bioelectrical stimulation may promote osteogenic gene expression and matrix mineralization, with the membrane coating contributing to biocompatibility and local piezoelectric signal conduction[49]. Notably, the US parameters used for osteogenic induction are substantially milder than those required for tumor ablation, indicating that the biological response of this nanosystem can be tuned according to the intended therapeutic application.

Under the tested conditions, $M_{SIRP\alpha}$ -BT + US enhanced osteogenic markers and mineralized matrix formation in BMSCs in vitro to a degree comparable with uncoated BT + US, indicating that membrane camouflage does not compromise the intrinsic osteoinductive capacity of tetragonal BT. Together with its US-triggered antitumor activity and increased macrophage-associated tumor-cell uptake, these findings support further evaluation of $M_{SIRP\alpha}$ -BT in osteosarcoma-related bone defect models.

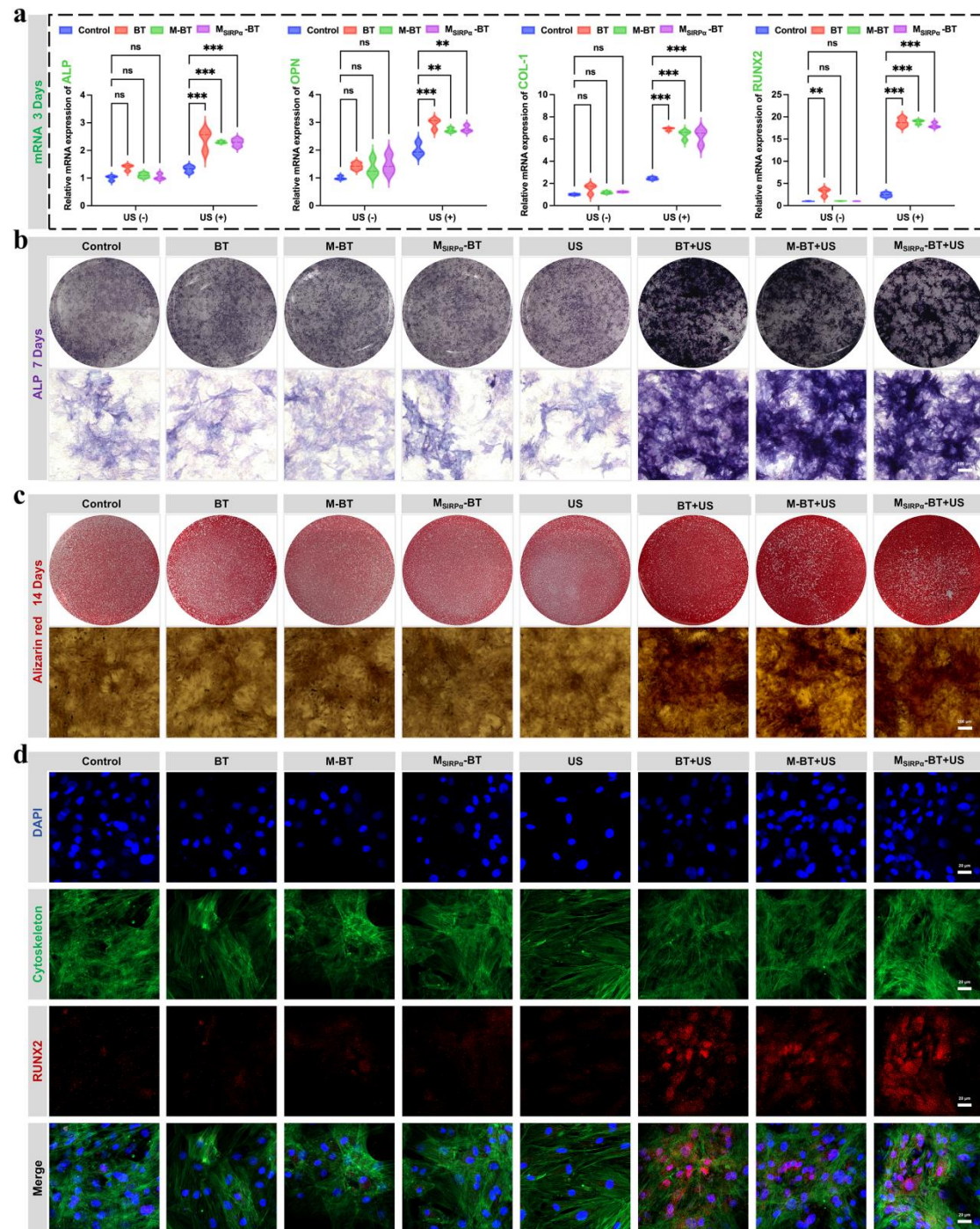


Figure 5. *In vitro* osteogenic differentiation of BMSCs mediated by $M_{SIRP\alpha}$ -BT under US stimulation (0.6 W cm^{-2} , 1.0 MHz, 50% duty cycle, 1 min). (a) RT-qPCR analysis of osteogenic-related genes (ALP, OPN, COL-1, and RUNX2) in BMSCs after 3 days of culture. (b, c) Representative macroscopic and microscopic images of ALP staining at day 7 and Alizarin Red S (ARS) staining at day 14. Scale bars = 100 μ m and 200 μ m, respectively. (d) Immunofluorescence images showing RUNX2 (red) and cytoskeleton (green) expression in BMSCs (nuclei stained with DAPI, blue). Scale

bars = 20 μm . Data are presented as mean $\pm$ SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.6. *In vivo* antitumor efficacy and bone repair in a tumor-bearing bone defect model

The *in vivo* activity of $\text{M}_{\text{SIRP}\alpha}\text{-BT}$ was evaluated in a mouse femoral tumor-bearing bone defect model. As shown in Fig. 6a, tumor-bearing mice received intratumoral injections of PBS, BT, M-BT, or $\text{M}_{\text{SIRP}\alpha}\text{-BT}$, followed by pulsed US irradiation on days 1 and 7 post-injection, at US intensities of 1.8 W cm^{-2} and 0.6 W cm^{-2} , respectively (1.0 MHz, 50% duty cycle, 5 min per session). Relative tumor volume and body weight were monitored for 5 weeks.

Representative photographs showed progressive enlargement of tumors in the untreated and non-US treated groups during the 5-week observation period (Fig. 6b). BT-containing formulations combined with US reduced tumor growth, and the $\text{M}_{\text{SIRP}\alpha}\text{-BT} + \text{US}$ group showed the greatest reduction among the treatment groups. Resected femur specimens containing the tumor lesions were also smaller in the $\text{M}_{\text{SIRP}\alpha}\text{-BT} + \text{US}$ group than in the control and comparator groups (Fig. 6c). The tumor volume was measured based on the total mass of the tumor-bearing femur, which includes the tumor as well as the affected surrounding bone and soft tissues. Longitudinal relative tumor volume measurements showed sustained suppression of tumor growth in the $\text{M}_{\text{SIRP}\alpha}\text{-BT} + \text{US}$ group (Fig. 6f).

The enhanced antitumor activity of $\text{M}_{\text{SIRP}\alpha}\text{-BT} + \text{US}$ can be attributed to the coordinated functions of the piezoelectric core and engineered membrane shell. Under US stimulation, the BT core undergoes piezoelectric polarization, promoting charge separation and interfacial redox reactions that generate cytotoxic ROS[50]. The resulting oxidative stress induces tumor-cell damage and apoptosis, consistent with the *in vitro* cytotoxicity results and the enrichment of DNA damage-, cell cycle-, and p53-related signaling pathways. Moreover, the $\text{SIRP}\alpha$ -enriched macrophage membrane provides a CD47-recognizing interface that facilitates the interaction of nanoparticles with CD47-expressing tumor cells[51,52]. The superior efficacy of $\text{M}_{\text{SIRP}\alpha}\text{-BT} + \text{US}$ over BT + US and M-BT + US further demonstrates that the

engineered membrane contributes therapeutic functionality beyond nanoparticle camouflage.

Tumor progression in osteosarcoma is commonly accompanied by severe osteolysis and destruction of local bone architecture[53]. Therefore, micro-computed tomography (micro-CT) was performed to assess bone repair at the tumor-bearing femur. The control group exhibited pronounced cortical destruction, trabecular erosion, and progressive tumor-associated osteolysis (Fig. 6d). In contrast, M_{SIRP α} -BT + US treatment substantially preserved structural continuity and improved trabecular organization. Quantitative analysis demonstrated significantly increased bone mineral density (BMD), trabecular thickness (Tb.Th), bone volume fraction (BV/TV), and trabecular number (Tb.N) in the M_{SIRP α} -BT + US group compared with the other treatment groups (Fig. 6g,h, and Fig. S7a,b). H&E staining revealed extensive tissue disruption in the control groups, whereas M_{SIRP α} -BT + US treatment resulted in improved local architecture and evident osteoid-like regenerated tissue within the tumor-bearing bone region (Fig. S8).

The active bone repair effect likely results from the integration of tumor ablation and piezoelectric osteogenic stimulation. Effective elimination of tumor cells limits continuous osteolysis and reduces the adverse influence of the tumor microenvironment on bone homeostasis[54]. Meanwhile, the piezoelectric signals generated by the BT core under US stimulation provide local bioelectrical cues that promote osteogenic differentiation and matrix deposition. Although the high-intensity US condition was primarily applied for tumor ablation *in vivo*, the improved bone microstructure and increased osteogenic activity indicate that the M_{SIRP α} -BT platform retains a bone-repairing function within the tumorous bone niche. These findings demonstrate that M_{SIRP α} -BT + US achieves efficient tumor inhibition while actively promoting restoration of the local bone structure. Notably, although a single intratumoral injection was administered on day 0, two separate US interventions were performed on days 1 and 7. This protocol is supported by the chemically stable nature of BaTiO₃, which is resistant to rapid *in vivo* degradation[22]. Previous studies have confirmed that intratumorally delivered inorganic nanoparticles, especially

piezoelectric nanomaterials, can maintain high concentrations and stable structures at the lesion site for several weeks through cellular uptake and matrix adsorption[25]. Even upon phagocytosis, the BT core retains its piezoelectric activity; lysosomal degradation of the membrane shell may further expose the active surface. Under local US irradiation, the internalized BT can still generate piezoelectric microcurrents or intracellular ROS, which justifies the repeated ultrasound stimulations.

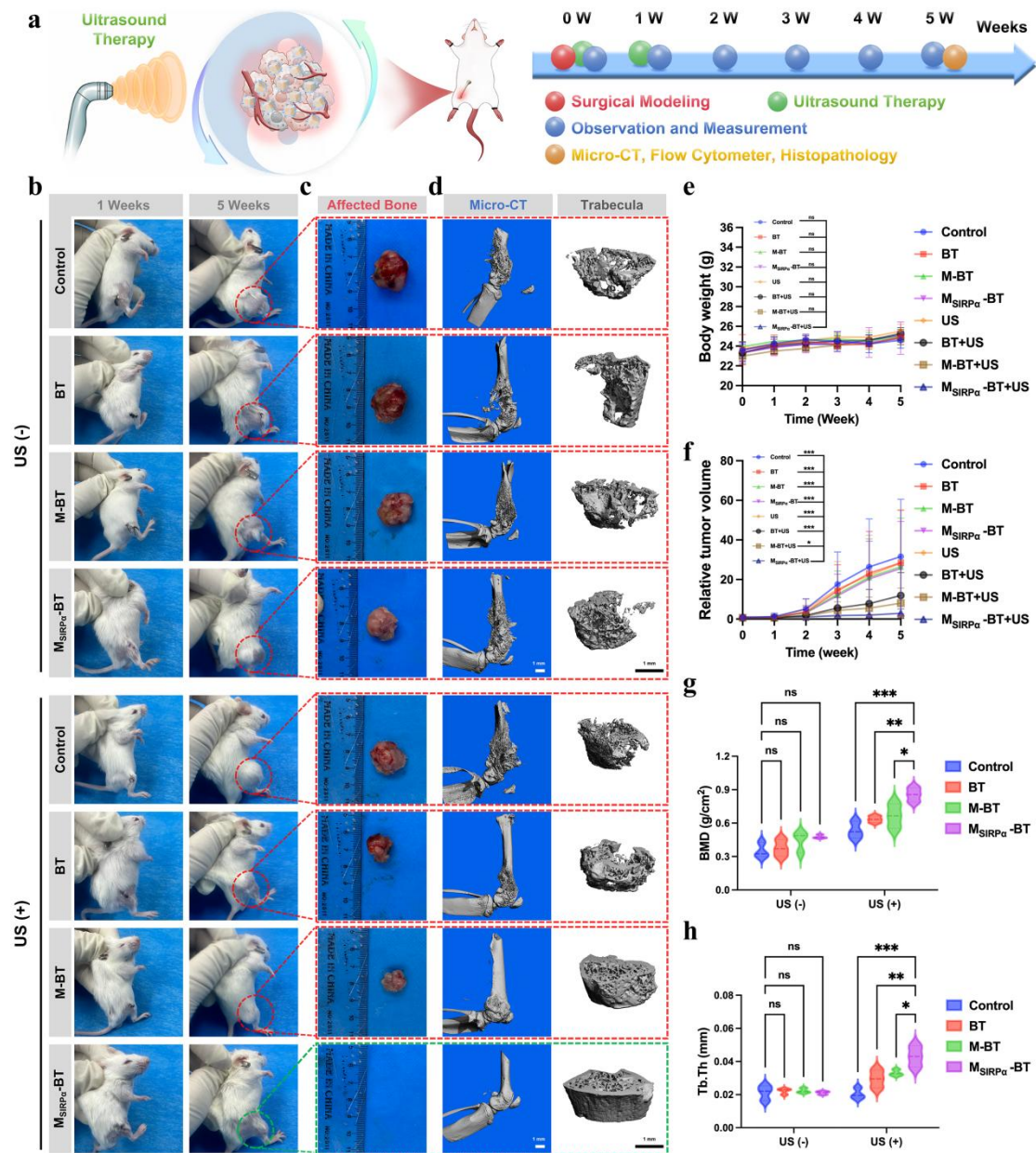


Figure 6. *In vivo* anti-tumor and bone regeneration effects of $M_{SIRP\alpha}$ -BT combined with US in a tumor-bearing bone defect model. (a) Schematic illustration of the

experimental timeline and ultrasound therapy schedule. (b) Representative gross photographs of mice in different groups at week 1 and week 5. Dashed circles indicate the tumor sites. (c) Representative photographs of the resected femur specimens containing the tumor lesions alongside a ruler. (d) Micro-CT 3D reconstructed images of the tumor-bearing femur and trabecular bone. Scale bars = 1 mm. (e, f) Body weight and relative tumor volume curves of mice in different groups over 5 weeks. (g, h) Quantitative analysis of bone mineral density (BMD) and trabecular thickness (Tb.Th) as evaluated by micro-CT. Data are presented as mean $\pm$ SD (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001.

3.7. *In vivo* remodeling of the tumor immune microenvironment and biosafety evaluation

Beyond direct tumor killing and bone protection, a key objective was to remodel the immunosuppressive tumor microenvironment *in vivo*. We thus analyzed the polarization state of tumor-associated macrophages (TAMs) within the resected tumor tissues via flow cytometry. Macrophages were identified as F4/80⁺CD11b⁺ cells, with the CD86⁺ and CD206⁺ subsets characterizing M1-like and M2-like populations, respectively[55,56]. As shown in Fig. 7a, the ratio of M1-type (CD86⁺) to M2-type (CD206⁺) macrophages was substantially increased in the M_{SIRP α} -BT + US group compared with the control and other US-treated groups (also quantified in Fig. S9). This shift indicates that the SIRP α -enriched membrane shell, in conjunction with ROS-induced tumor-cell injury, promotes the polarization of TAMs toward a pro-inflammatory, antitumorigenic M1 phenotype. This immunomodulatory effect is critical for reversing the immunosuppressive TME that typically fosters osteosarcoma recurrence and metastasis.

To further investigate the mechanisms underlying the enhanced antitumor immune response, we examined tumor-cell apoptosis and macrophage-related immune activation in tumor tissues. TUNEL staining revealed a marked increase in apoptotic tumor cells in the M_{SIRP α} -BT + US group (Fig. 7b). Immunofluorescence staining further showed increased co-localization of CD11b-positive macrophages

with CD47-positive tumor cells after M_{SIRP α} -BT + US treatment (Fig. 7c), suggesting enhanced macrophage-tumor cell interactions. In addition, iNOS expression, a marker of M1-like macrophage polarization, was substantially elevated in this group (Fig. 7d)[57]. These findings indicate that the local ROS burst induced by M_{SIRP α} -BT + US directly promotes tumor-cell apoptosis while remodeling the tumor immune microenvironment toward a pro-inflammatory, antitumor state. This response is consistent with the p53-associated stress signaling identified by transcriptomic analysis (Fig. 4), linking piezocatalytic oxidative injury to downstream macrophage activation and antitumor immunity.

In parallel, the osteogenic and regenerative effects observed *in vivo* were further confirmed by histological examination of the bone tissue surrounding the defect. H&E and Masson's trichrome staining showed that the M_{SIRP α} -BT + US group exhibited significantly more new bone formation and collagen deposition compared to other groups (Fig. 7e, f). Immunohistochemical staining revealed that the expression of ALP and RUNX2, key markers of osteogenesis, was markedly upregulated in the M_{SIRP α} -BT + US group (Fig. 7g, h)[58]. These results strongly suggest that the locally activated piezoelectric signals, combined with the improved immune microenvironment, create a favorable niche for the osteogenic differentiation of resident BMSCs, thereby promoting new bone formation.

The *in vivo* biosafety of M_{SIRP α} -BT was assessed by histological analysis of major organs (heart, liver, spleen, lung, and kidney). H&E staining revealed no significant pathological abnormalities or signs of inflammatory lesions in the M_{SIRP α} -BT + US group compared with the untreated control (Fig. S10), confirming the favorable biocompatibility of the nanosystem. In summary, these results demonstrate that M_{SIRP α} -BT achieves a multifaceted therapeutic outcome: suppressing primary tumor growth, remodeling the TME to activate anti-tumor immunity, and promoting local bone regeneration, all while maintaining a high safety profile. This positions the engineered nanosystem as a promising candidate for the comprehensive management of osteosarcoma-associated complex bone defects.

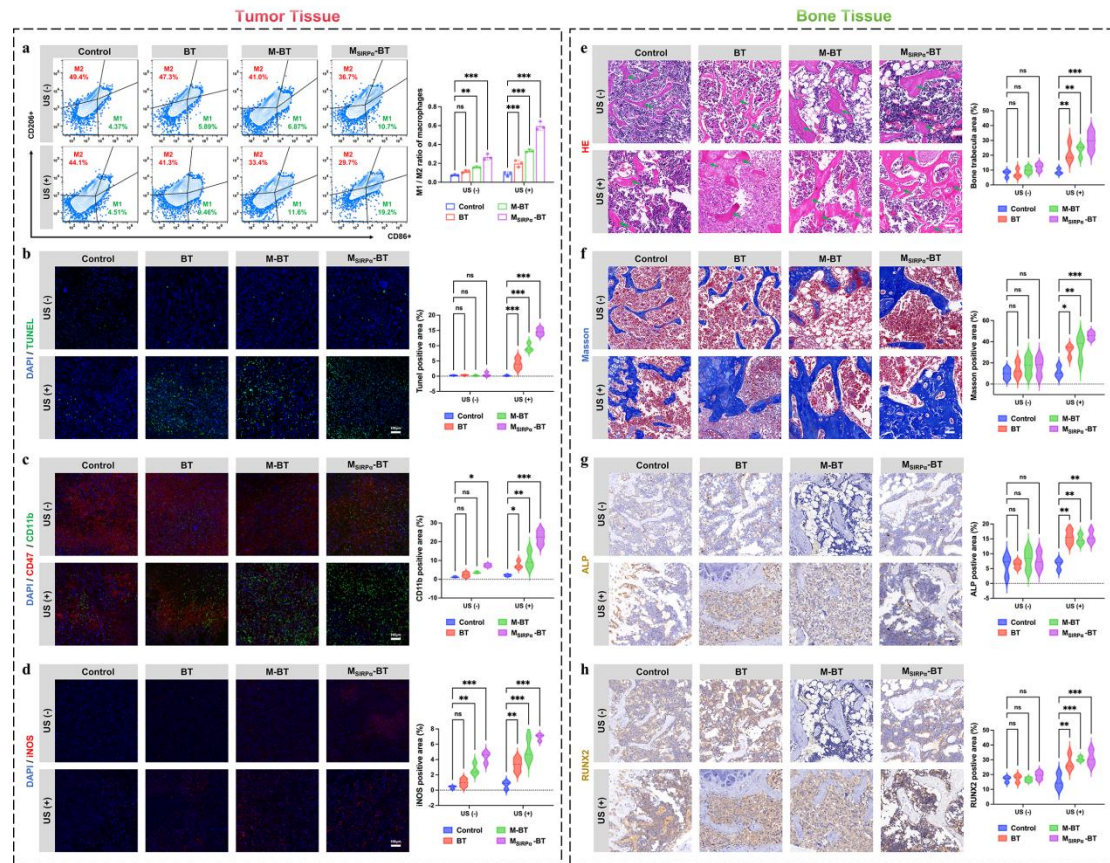


Figure 7. *In vivo* evaluation of tumor immune microenvironment remodeling and bone regeneration. (a) Flow cytometry analysis of M1/M2 macrophage polarization in tumor tissues and the corresponding quantitative M1/M2 ratios. (b-d) Representative immunofluorescence images showing TUNEL (green), CD11b/CD47 (green/red), and iNOS (red) expression in tumor tissues; nuclei were counterstained with DAPI (blue). Scale bars = 100 μ m. (e, f) H&E and Masson's trichrome staining of bone tissues, with quantitative analysis of bone formation area and collagen deposition. Scale bars = 50 μ m. (g, h) Immunohistochemical (IHC) staining and quantitative analysis of ALP and RUNX2 expression in bone tissues. Scale bars = 50 μ m. Data are presented as mean $\pm$ SD (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001.

Conclusion

This study presents a “well-educated” engineered macrophage membrane-camouflaged piezoelectric nanosystem, $M_{SIRP\alpha}$ -BT, comprising tetragonal barium titanate nanoparticles coated with membranes derived from SIRP α -overexpressing RAW264.7 macrophages. The membrane coating preserved the SIRP α surface signature while retaining the piezoelectric and ROS-generating properties of the BT core under ultrasound stimulation. Integrating a SIRP α -enriched membrane interface with an ultrasound-responsive tetragonal barium titanate core enables the platform to simultaneously address the intertwined challenges of residual tumor cell eradication, immunosuppressive microenvironment reversal, and bone tissue regeneration. The engineered membrane shell competes with endogenous macrophages for CD47 on tumor cells, effectively blocking the “don’t eat me” signal and restoring phagocytic clearance. Under ultrasound activation, the piezoelectric core generates reactive oxygen species to induce DNA damage and p53-mediated apoptosis, a mechanism corroborated by transcriptomic and proteomic analyses. By switching to a lower ultrasound intensity, the same platform provides localized bioelectrical cues that promote osteogenic differentiation of bone marrow mesenchymal stem cells and matrix mineralization. In a mouse femoral tumorous bone defect model, $M_{SIRP\alpha}$ -BT combined with ultrasound suppressed primary tumor growth, remodeled the tumor immune microenvironment toward a pro-inflammatory M1 phenotype, and attenuated osteolysis while promoting new bone formation, all without detectable systemic toxicity. Thus, this integrated piezoelectric-immuno synergistic platform provides a promising paradigm for local tumor control and functional bone restoration, with significant potential for the comprehensive management of tumorous bone defects.

Acknowledgements

This work was supported by no specific funding.

Author Contributions

X.Q., X.J., and Y.Z. (Yueyi Zhang) contributed equally to this work. X.Q. and Y.Z. (Yueyi Zhang) performed the methodology and software; X.J. contributed to conceptualization. L.J. synthesized the BT nanomaterials; L.C. conducted the bioinformatics analysis; Y.W. and W.L. carried out the data curation and formal analysis. C.L. and P.Y. performed the in vitro experiments and validation; Y.S. and L.H. contributed to visualization and investigation. W.R. provided resources and assisted with the animal model establishment. R.D. supervised the project and reviewed the manuscript. X.S., Y.Z. (Yu Zhang), and J.-K.Y. conceived the study, designed the experiments, provided overall supervision, and revised the manuscript for critical intellectual content. All authors read and approved the final manuscript.

Data availability

Data is provided within the manuscript or supplementary information files.

Conflict of Interest

The authors declare no conflict of interest.

Supporting information

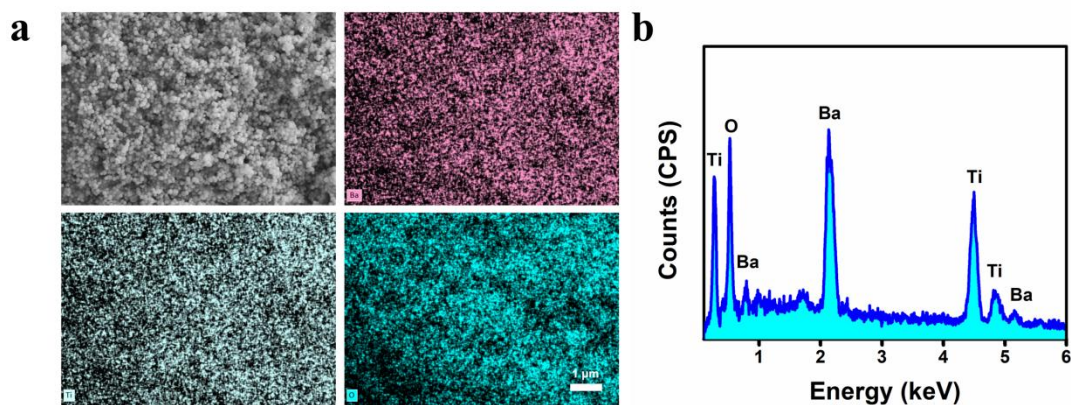


Figure S1. EDS elemental mapping and spectrum analysis of BT nanoparticles. (a) SEM morphology image and corresponding EDS elemental mapping of Ba, Ti, and O elements. Scale bar = 1 μm . (b) EDS spectrum confirming the elemental composition of BT nanoparticles.

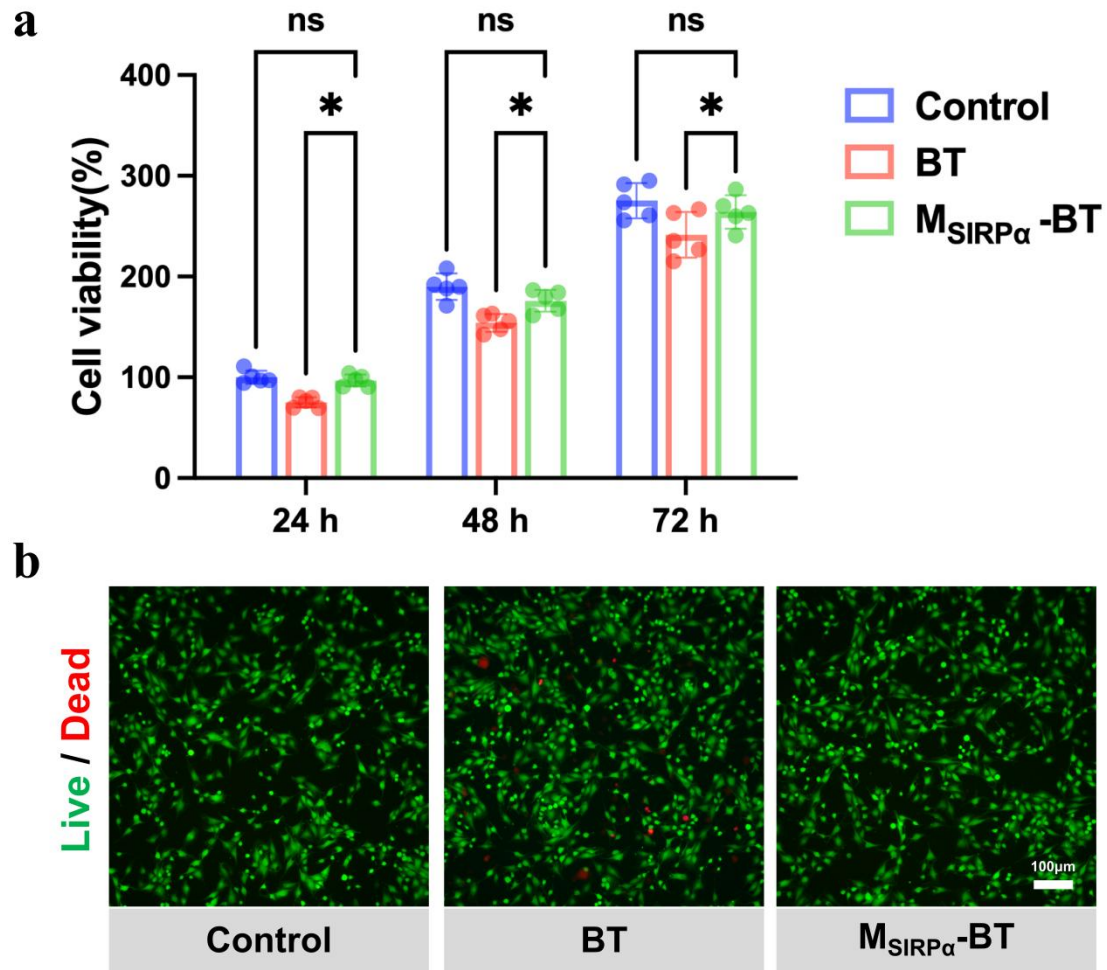


Figure S2. *In vitro* cytocompatibility of BT and M_{SIRPα}-BT nanoparticles in C3H10 cells in the absence of ultrasound stimulation. (a) Cell viability of C3H10 cells incubated with Control, BT, or M_{SIRPα}-BT for 24, 48, and 72 h, as determined by the CCK-8 assay. (b) Representative live/dead staining images of C3H10 cells after incubation with the indicated formulations (live cells, Calcein-AM, green; dead cells, PI, red). Scale bar = 100 μm. Data are presented as mean ± SD (n = 3). **p* < 0.05.

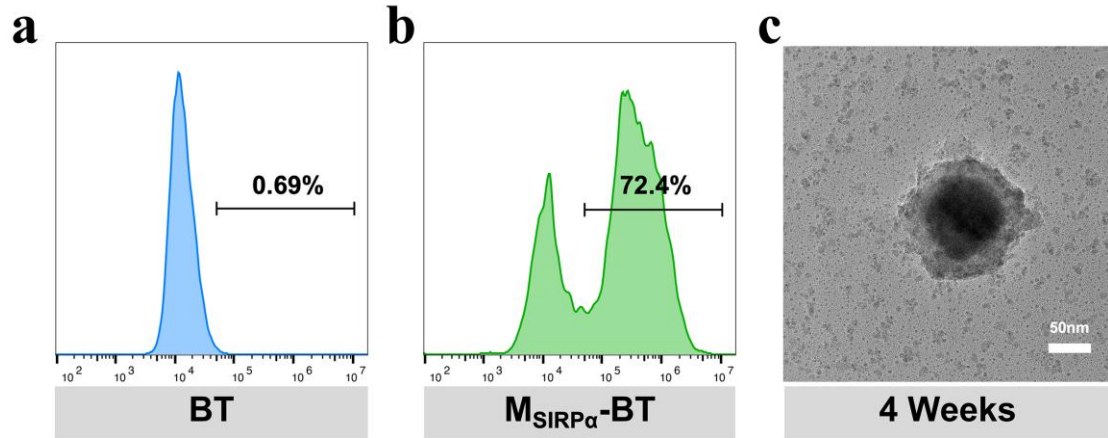


Figure S3. Validation of membrane coating, SIRP α modification, and stability of $M_{SIRP\alpha}$ -BT nanoparticles. (a, b) Flow cytometry analysis detecting SIRP α protein signals on bare BT (a) and $M_{SIRP\alpha}$ -BT nanoparticles (b). (c) Representative TEM image of $M_{SIRP\alpha}$ -BT nanoparticles stored at 4 °C for 4 weeks, demonstrating a stable core-shell structure. Scale bar = 50 nm.

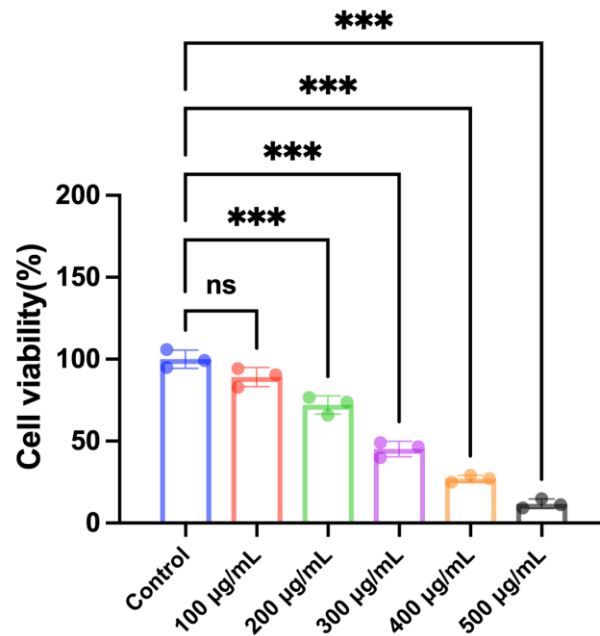


Figure S4. Concentration-dependent cytotoxicity of $M_{SIRP\alpha}$ -BT against K7M2 cells under the antitumor US conditions. CCK-8 assay of K7M2 cells treated with varying $M_{SIRP\alpha}$ -BT concentrations under US (1.8 W cm⁻², 1.0 MHz, 50% duty cycle, 5 min). Data are mean $\pm$ SD (n=3). *** $p < 0.001$.

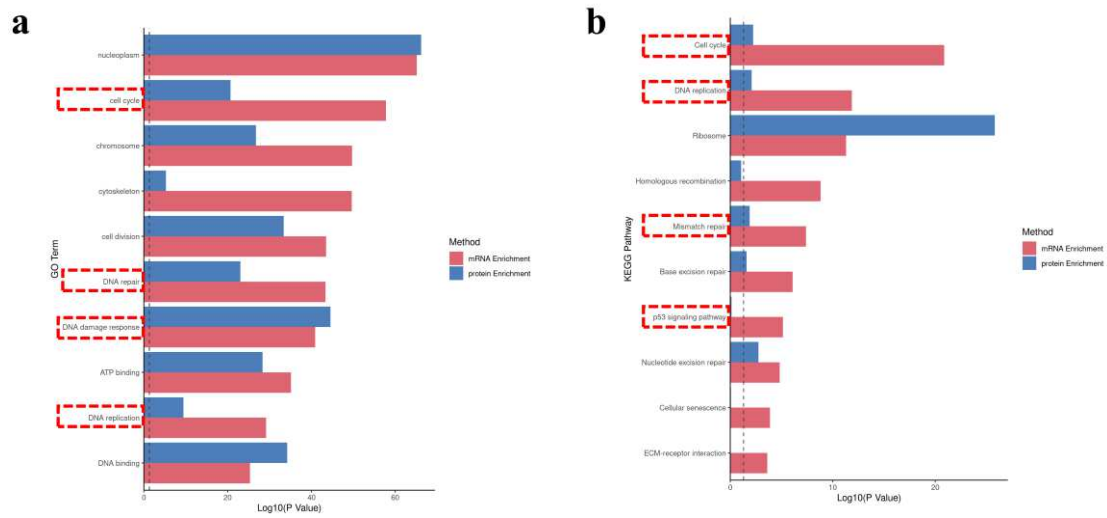


Figure S5. Integrated transcriptomic and proteomic enrichment analysis of M_{SIRPα}-BT treatment. (a) GO enrichment analysis comparing differentially expressed mRNAs and proteins. (b) KEGG pathway enrichment analysis comparing differentially expressed mRNAs and proteins.

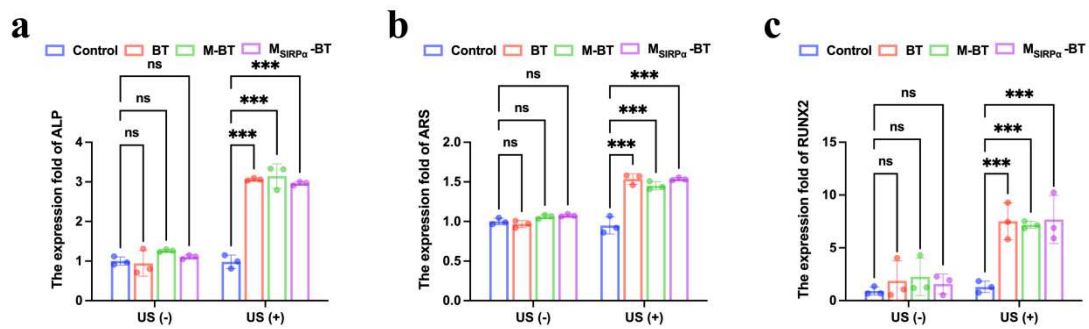


Figure S6. Quantification of ALP staining, ARS staining, and RUNX2 immunofluorescence in BMSCs. (a-c) Quantification of ALP-positive, ARS-positive mineralized area or calcium deposition, and RUNX2 fluorescence intensity, respectively, in the indicated groups with or without US stimulation. Data are presented as mean $\pm$ SD ($n = 3$). *** $p < 0.001$.

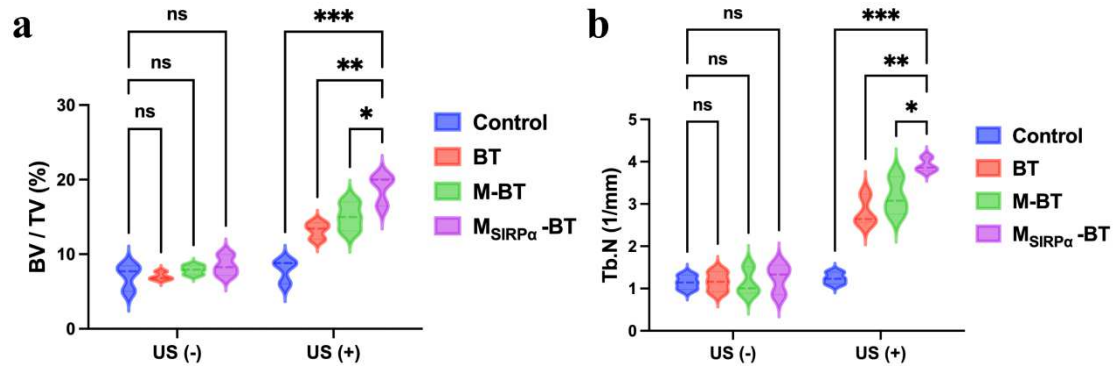


Figure S7. Micro-CT quantitative analysis of bone regeneration. (a, b) Quantitative analysis of bone volume fraction (BV/TV) and trabecular number (Tb.N) in different groups with or without US irradiation. Data are presented as mean $\pm$ SD (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001.

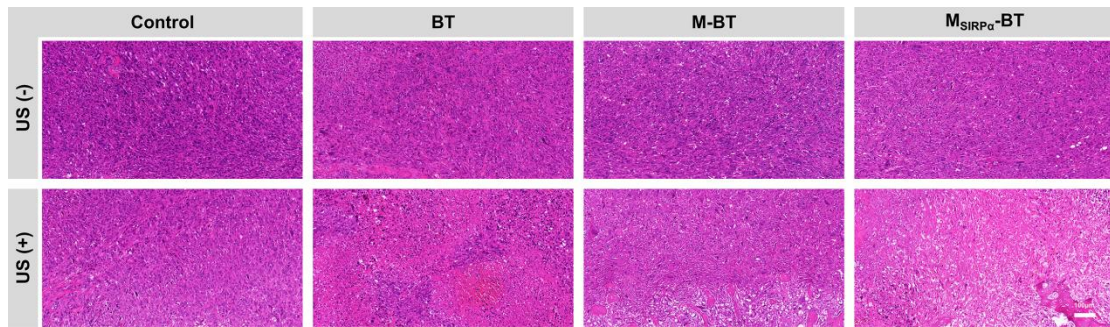


Figure S8. H&E staining of bone tumor tissues in different groups with or without US irradiation. Scale bar = 100 μ m.

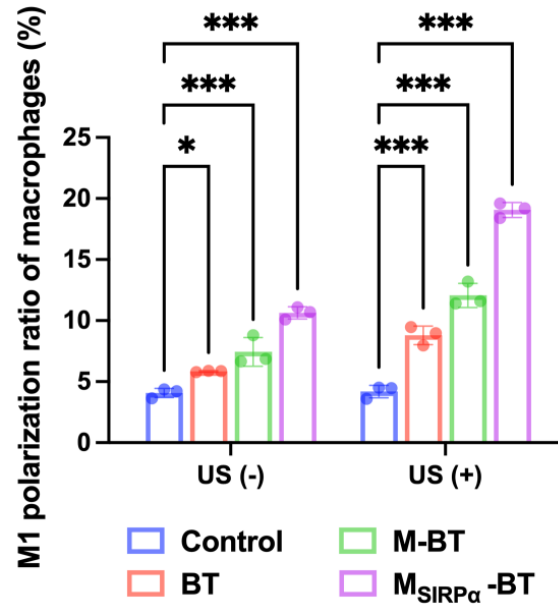


Figure S9. M1 polarization ratio of macrophages in bone tumor samples from different experimental groups. Quantitative analysis of the M1 polarization ratio of macrophages in bone tumor tissues in different groups with or without US irradiation. Data are presented as mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

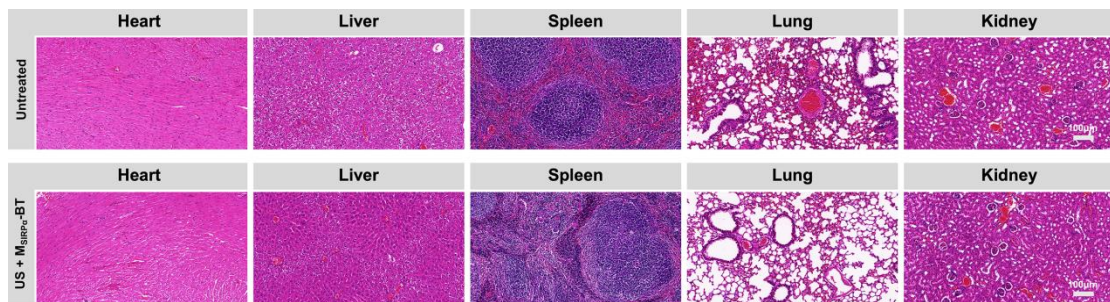


Figure S10. H&E staining of major organs. Representative H&E-stained sections of vital organs (heart, liver, spleen, lung, and kidney) in the Untreated and US + MSIRP α -BT groups. Scale bars = 100 μ m.

Table S1. Mouse SIRP α qPCR primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
SIRP α	GCTACCCACAACCTGGAATGTC	GGTTATTTCCCTGGCGTTCTT
GAPDH	TGTGATGGGTGTGAACCACG	CAGTGAGCTTCCCGTTCAGC

Table S2. Rat repair-related qRT-PCR primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ALP	CAACCTGACTGACCCTTCC	CGATGGCCTCATCCATCTCC
OPN	CCAGCCAAGGACCAACTACA	AGTGTTTGCTGTAATGCGCC
COL-1	TGGCAACCTCAAGAAGTCCC	ACAAGCGTGCTGTAGGTGAA
RUNX2	CAGCAGCTTTGCAGGAAGTG	CCCTCCCTAGTGAGAGCCTT
GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA

References

1. Henkel J, Woodruff MA, Epari DR, Steck R, Glatt V, Dickinson IC, et al. Bone Regeneration Based on Tissue Engineering Conceptions — A 21st Century Perspective. *Bone Res.* 2013;1:216–48. <https://doi.org/10.4248/BR201303002>
2. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. *Nat Rev Rheumatol.* 2012;8:133–43. <https://doi.org/10.1038/nrrheum.2012.1>
3. Zhang Y, Xu J, Ruan YC, Yu MK, O’Laughlin M, Wise H, et al. Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats. *Nat Med.* 2016;22:1160–9. <https://doi.org/10.1038/nm.4162>
4. Xue X, Hu Y, Wang S, Chen X, Jiang Y, Su J. Fabrication of physical and chemical crosslinked hydrogels for bone tissue engineering. *Bioact Mater.* 2022;12:327–39. <https://doi.org/10.1016/j.bioactmat.2021.10.029>
5. Li C, Li W, Deng R, Huang L, Song Y, Wei X, et al. Programming Ordered Tissue Reconstruction via Artificial Bionic Tendon Sheath for Tendinopathy Treatment. *Adv Fiber Mater.* 2026;8:629–54. <https://doi.org/10.1007/s42765-025-00637-3>
6. Beird HC, Bielack SS, Flanagan AM, Gill J, Heymann D, Janeway KA, et al. Osteosarcoma. *Nat Rev Dis Primer.* 2022;8:77. <https://doi.org/10.1038/s41572-022-00409-y>
7. Smrke A, Anderson PM, Gulia A, Gennatas S, Huang PH, Jones RL. Future Directions in the Treatment of Osteosarcoma. *Cells.* 2021;10:172. <https://doi.org/10.3390/cells10010172>
8. Picci P. Osteosarcoma (Osteogenic sarcoma). *Orphanet J Rare Dis.* 2007;2:6. <https://doi.org/10.1186/1750-1172-2-6>
9. Quadros M, Momin M, Verma G. Design strategies and evolving role of biomaterial assisted treatment of osteosarcoma. *Mater Sci Eng C Mater Biol Appl.* 2021;121:111875. <https://doi.org/10.1016/j.msec.2021.111875>
10. Zhu T, Han J, Yang L, Cai Z, Sun W, Hua Y, et al. Immune Microenvironment in Osteosarcoma: Components, Therapeutic Strategies and Clinical Applications. *Front Immunol.* 2022;13:907550. <https://doi.org/10.3389/fimmu.2022.907550>
11. Xu J, Ding L, Mei J, Hu Y, Kong X, Dai S, et al. Dual roles and therapeutic targeting of tumor-associated macrophages in tumor microenvironments. *Signal Transduct Target Ther.* 2025;10:268. <https://doi.org/10.1038/s41392-025-02325-5>
12. Buddingh EP, Kuijjer ML, Duim RAJ, Bürger H, Agelopoulos K, Myklebost O, et al. Tumor-Infiltrating Macrophages Are Associated with Metastasis Suppression in High-Grade Osteosarcoma: A Rationale for Treatment with Macrophage Activating Agents. *Clin Cancer Res.* 2011;17:2110–9. <https://doi.org/10.1158/1078-0432.CCR-10-2047>

13. Heymann M-F, L     F, Heymann D. The contribution of immune infiltrates and the local microenvironment in the pathogenesis of osteosarcoma. *Cell Immunol.* 2019;343:103711. <https://doi.org/10.1016/j.cellimm.2017.10.011>
14. Willingham SB, Volkmer J-P, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, et al. The CD47-signal regulatory protein alpha (SIRP  ) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad Sci.* 2012;109:6662  7. <https://doi.org/10.1073/pnas.1121623109>
15. Jaiswal S, Jamieson CHM, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 Is Upregulated on Circulating Hematopoietic Stem Cells and Leukemia Cells to Avoid Phagocytosis. *Cell.* 2009;138:271  85. <https://doi.org/10.1016/j.cell.2009.05.046>
16. Sheng S, Jin L, Zhang Y, Sun W, Mei L, Zhu D, et al. A Twindrive Precise Delivery System of Platelet-Neutrophil Hybrid Membrane Regulates Macrophage Combined with CD47 Blocking for Postoperative Immunotherapy. *ACS Nano.* 2024;18:4981  92. <https://doi.org/10.1021/acsnano.3c10862>
17. Weiskopf K. Cancer immunotherapy targeting the CD47/SIRP   axis. *Eur J Cancer.* 2017;76:100  9. <https://doi.org/10.1016/j.ejca.2017.02.013>
18. Logtenberg MEW, Scheeren FA, Schumacher TN. The CD47-SIRP   Immune Checkpoint. *Immunity.* 2020;52:742  52. <https://doi.org/10.1016/j.immuni.2020.04.011>
19. Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD, et al. CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells. *Cell.* 2009;138:286  99. <https://doi.org/10.1016/j.cell.2009.05.045>
20. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol.* 2019;16:563  80. <https://doi.org/10.1038/s41571-019-0218-0>
21. Chen S, Zhu P, Mao L, Wu W, Lin H, Xu D, et al. Piezocatalytic Medicine: An Emerging Frontier using Piezoelectric Materials for Biomedical Applications. *Adv Mater.* Deerfield Beach, Fla.; 2023;35:e2208256. <https://doi.org/10.1002/adma.202208256>
22. Zhu P, Chen Y, Shi J. Piezocatalytic Tumor Therapy by Ultrasound-Trigged and BaTiO  3-Mediated Piezoelectricity. *Adv Mater.* Deerfield Beach, Fla.; 2020;32:e2001976. <https://doi.org/10.1002/adma.202001976>
23. Liu X, Wan X, Sui B, Hu Q, Liu Z, Ding T, et al. Piezoelectric hydrogel for treatment of periodontitis through bioenergetic activation. *Bioact Mater.* 2024;35:346  61. <https://doi.org/10.1016/j.bioactmat.2024.02.011>
24. Nel AE, M    ler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater.* 2009;8:543  57. <https://doi.org/10.1038/nmat2442>

25. Fang RH, Gao W, Zhang L. Targeting drugs to tumours using cell membrane-coated nanoparticles. *Nat Rev Clin Oncol*. 2023;20:33–48. <https://doi.org/10.1038/s41571-022-00699-x>
26. Fang RH, Kroll AV, Gao W, Zhang L. Cell Membrane Coating Nanotechnology. *Adv Mater*. 2018;30:1706759. <https://doi.org/10.1002/adma.201706759>
27. Qian X, Lu T, Huang C, Zheng D, Gong G, Chu X, et al. Bioinspired Sonodynamic Nano Spray Accelerates Infected Wound Healing via Targeting and Disturbing Bacterial Metabolism. *Adv Funct Mater*. 2024;34:2315576. <https://doi.org/10.1002/adfm.202315576>
28. Fang RH, Hu C-MJ, Luk BT, Gao W, Copp JA, Tai Y, et al. Cancer Cell Membrane-Coated Nanoparticles for Anticancer Vaccination and Drug Delivery. *Nano Lett*. 2014;14:2181–8. <https://doi.org/10.1021/nl500618u>
29. Yin T, Fan Q, Hu F, Ma X, Yin Y, Wang B, et al. Engineered Macrophage-Membrane-Coated Nanoparticles with Enhanced PD-1 Expression Induce Immunomodulation for a Synergistic and Targeted Antiglioblastoma Activity. *Nano Lett*. 2022;22:6606–14. <https://doi.org/10.1021/acs.nanolett.2c01863>
30. Logtenberg MEW, Scheeren FA, Schumacher TN. The CD47-SIRP α Immune Checkpoint. *Immunity*. 2020;52:742–52. <https://doi.org/10.1016/j.immuni.2020.04.011>
31. Matlung HL, Szilagyi K, Barclay NA, Van Den Berg TK. The CD47-SIRP α signaling axis as an innate immune checkpoint in cancer. *Immunol Rev*. 2017;276:145–64. <https://doi.org/10.1111/imr.12527>
32. Chao MP, Weissman IL, Majeti R. The CD47–SIRP α pathway in cancer immune evasion and potential therapeutic implications. *Curr Opin Immunol*. 2012;24:225–32. <https://doi.org/10.1016/j.coi.2012.01.010>
33. Parodi A, Quattrocchi N, Van De Ven AL, Chiappini C, Evangelopoulos M, Martinez JO, et al. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. *Nat Nanotechnol*. 2013;8:61–8. <https://doi.org/10.1038/nnano.2012.212>
34. Ji X, Qian X, Luo G, Yang W, Huang W, Lei Z, et al. Engineered macrophage nanoparticles enhance microwave ablation efficacy in osteosarcoma via targeting the CD47-SIRP α Axis: A novel Biomimetic immunotherapeutic approach. *Bioact Mater*. 2025;47:248–65. <https://doi.org/10.1016/j.bioactmat.2025.01.012>
35. Kroll AV, Jiang Y, Zhou J, Holay M, Fang RH, Zhang L. Biomimetic Nanoparticle Vaccines for Cancer Therapy. *Adv Biosyst*. 2019;3:1800219. <https://doi.org/10.1002/adbi.201800219>
36. Fong D, Grégoire-Gélinas P, Cheng AP, Mezheritsky T, Lavertu M, Sato S, et al. Lysosomal rupture induced by structurally distinct chitosans either promotes a type 1 IFN response or activates the inflammasome in macrophages. *Biomaterials*. 2017;129:127–38. <https://doi.org/10.1016/j.biomaterials.2017.03.022>

37. Shen J, Wu S, Wang Y, Yan Z, Liu T, Sun X, et al. Mechano-bioactive hydrogel bioelectronics for mechanical-electrical-bioenergetic conversion and glia-modulating neural regeneration. *Nat Commun.* 2025;16:11582. <https://doi.org/10.1038/s41467-025-66779-5>
38. Buettner GR. Spin Trapping: ESR parameters of spin adducts 1474 1528V. *Free Radic Biol Med.* 1987;3:259–303. [https://doi.org/10.1016/S0891-5849\(87\)80033-3](https://doi.org/10.1016/S0891-5849(87)80033-3)
39. Zhang W, Yang Z, Zhang Y, Wang L, Zhang X, Mao J, et al. Multi-Enzyme Mimetic Molybdenum Nitride Nanozymes Reshape Subgingival Microenvironment for Synergistic Periodontitis Therapy via ROS Regulation and Microbiome Remodeling. *Adv Sci.* 2026;13:e17770. <https://doi.org/10.1002/advs.202517770>
40. Chao MP, Alizadeh AA, Tang C, Myklebust JH, Varghese B, Gill S, et al. Anti-CD47 Antibody Synergizes with Rituximab to Promote Phagocytosis and Eradicate Non-Hodgkin Lymphoma. *Cell.* 2010;142:699–713. <https://doi.org/10.1016/j.cell.2010.07.044>
41. Ravichandran KS. Beginnings of a Good Apoptotic Meal: The Find-Me and Eat-Me Signaling Pathways. *Immunity.* 2011;35:445–55. <https://doi.org/10.1016/j.immuni.2011.09.004>
42. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol.* 2020;21:363–83. <https://doi.org/10.1038/s41580-020-0230-3>
43. Barzilai A, Yamamoto K-I. DNA damage responses to oxidative stress. *DNA Repair.* 2004;3:1109–15. <https://doi.org/10.1016/j.dnarep.2004.03.002>
44. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol.* 2008;9:402–12. <https://doi.org/10.1038/nrm2395>
45. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature.* 2009;461:1071–8. <https://doi.org/10.1038/nature08467>
46. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. *Nature.* 2002;417:182–7. <https://doi.org/10.1038/417182a>
47. Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res.* 2021;49:D545–51. <https://doi.org/10.1093/nar/gkaa970>
48. Langenbach F, Handschel J. Effects of dexamethasone, ascorbic acid and β -glycerophosphate on the osteogenic differentiation of stem cells in vitro. *Stem Cell Res Ther.* 2013;4:117. <https://doi.org/10.1186/scrt328>
49. Jacob J, More N, Kalia K, Kapusetti G. Piezoelectric smart biomaterials for bone and cartilage tissue engineering. *Inflamm Regen.* 2018;38:2. <https://doi.org/10.1186/s41232-018-0059-8>
50. Song Y, Xu S, Zhang J, Zhang T, Wu R, Feng G, et al. Ultrasound-Mediated Piezocatalysis

Triggers NO Release to Augment Targeted Immunotherapy of Pancreatic Cancer. *ACS Nano*. 2025;19:32654–73. <https://doi.org/10.1021/acsnano.5c10301>

51. Oldenborg P-A. CD47: A Cell Surface Glycoprotein Which Regulates Multiple Functions of Hematopoietic Cells in Health and Disease. *ISRN Hematol*. 2013;2013:1–19. <https://doi.org/10.1155/2013/614619>

52. Murata Y, Kotani T, Ohnishi H, Matozaki T. The CD47-SIRP signalling system: its physiological roles and therapeutic application. *J Biochem (Tokyo)*. 2014;155:335–44. <https://doi.org/10.1093/jb/mvu017>

53. Kansara M, Teng MW, Smyth MJ, Thomas DM. Translational biology of osteosarcoma. *Nat Rev Cancer*. 2014;14:722–35. <https://doi.org/10.1038/nrc3838>

54. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer*. 2002;2:584–93. <https://doi.org/10.1038/nrc867>

55. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14:399–416. <https://doi.org/10.1038/nrclinonc.2016.217>

56. Locati M, Curtale G, Mantovani A. Diversity, Mechanisms, and Significance of Macrophage Plasticity. *Annu Rev Pathol Mech Dis*. 2020;15:123–47. <https://doi.org/10.1146/annurev-pathmechdis-012418-012718>

57. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity*. 2014;41:14–20. <https://doi.org/10.1016/j.immuni.2014.06.008>

58. Ducy P, Starbuck M, Priemel M, Shen J, Pinero G, Geoffroy V, et al. A Cbfa1-dependent genetic pathway controls bone formation beyond embryonic development. *Genes Dev*. 1999;13:1025–36. <https://doi.org/10.1101/gad.13.8.1025>

