

Synergistic Sono-Chemodynamic Therapy of Renal Cell Carcinoma Using HKUST-1@TiO₂ Heterojunctions

Sinian Zheng¹, Cong Li², Jiayu Chen³, Kuo Zhang¹, Xiaobo Cui¹, Rubing Li¹, Zeming Weng¹

¹Department of Urology, Ningbo Medical Center Lihuili Hospital, Ningbo, Zhejiang, People's Republic of China; ²Department of Urology, Yuyao Hospital of Traditional Chinese Medicine, Ningbo, Zhejiang, People's Republic of China; ³Operating Room, Ningbo Medical Center Lihuili Hospital, Ningbo, Zhejiang, People's Republic of China

Correspondence: Rubing Li; Zeming Weng, Email nblirb@126.com; ZemingWeng1@126.com

Introduction: Renal cell carcinoma (RCC) is a common urinary malignancy with high postoperative recurrence, and current therapies are limited by toxicity or insufficient efficacy. Efficient sonodynamic therapy (SDT) strategies capable of generating reactive oxygen species (ROS) are urgently needed. This study aimed to develop a high-performance MOF-based heterojunction sonosensitizer to enhance ROS generation and achieve effective anti-tumor activity.

Methods: We synthesized HKUST-1@TiO₂ heterojunctions and characterized their morphology, electronic structure, and ROS generation capacity. In vitro, OSRC-2 cells were treated with HKUST-1@TiO₂ ± ultrasound; cell viability, proliferation, apoptosis, and intracellular ROS were assessed. In vivo, nude mice bearing OSRC-2 xenografts received HKUST-1@TiO₂ ± ultrasound; tumor growth, histopathology, and biosafety markers were analyzed.

Results: HKUST-1@TiO₂ exhibited efficient heterojunction formation, which enhanced charge separation and ROS production under ultrasound. In vitro, the combination treatment significantly reduced cell viability, decreased Ki67-positive area, and increased the number of TUNEL-positive cells. Intracellular ROS staining confirmed effective ROS accumulation in tumor cells. In vivo, tumor volume and weight were significantly reduced, with no detectable organ toxicity.

Conclusion: HKUST-1@TiO₂ heterojunctions effectively augment SDT through enhanced intracellular ROS generation, inducing tumor cell apoptosis and inhibiting proliferation. This study addresses the unmet need for efficient and safe SDT for RCC and provides a promising strategy with translational potential.

Keywords: renal cell carcinoma, sonodynamic therapy, metal-organic frameworks, heterojunction, reactive oxygen species, titanium dioxide

Introduction

Renal cell carcinoma (RCC) is a common urinary malignancy, ranking as the 14th most prevalent cancer globally, with over 400,000 new cases reported in 2020.¹⁻³ Despite its moderate incidence, postoperative recurrence and metastasis occur in approximately 20% of patients, posing significant challenges for clinical management.⁴⁻⁶ Current therapeutic strategies, including surgical resection, targeted therapy, and immunotherapy, are often limited by toxicity, insufficient efficacy, or drug resistance, highlighting an urgent need for novel, effective, and safe treatment modalities.

Nanomaterial-based therapies have emerged as promising alternatives, offering the potential for precise tumor targeting and enhanced therapeutic effects. Among these, sonodynamic therapy (SDT) utilizes ultrasound (US) to activate sonosensitizers, generating reactive oxygen species (ROS) to induce apoptosis in tumor cells.⁷⁻¹² SDT benefits from deep tissue penetration (up to ~10 cm), non-invasiveness, and spatially controlled activation, making it particularly attractive for treating deep-seated tumors such as RCC.^{11,12} However, the development of highly efficient sonosensitizers remains a critical challenge.

Metal-organic frameworks (MOFs) have gained increasing attention as SDT platforms due to their high surface area, tunable porosity, and abundant metal nodes capable of participating in redox reactions.^{13,14} HKUST-1, a copper-based MOF, exhibits excellent structural stability and can facilitate ROS generation through redox cycling of Cu^{2+} nodes.¹⁵ Nevertheless, native MOFs often suffer from low charge separation efficiency, as photo- or sonogenerated electron-hole (e^-/h^+) pairs recombine rapidly, limiting ROS production under ultrasound.¹⁶ To overcome these limitations, heterojunction engineering has been employed, coupling MOFs with semiconductors to promote directional charge migration, suppress carrier recombination, and enhance ROS generation.^{17,18} Recent studies have demonstrated that MOF-based heterojunctions, such as Z-scheme MOF-on-MOF systems or hypoxia-responsive Cu-MOF nanoparticles, can improve SDT efficacy and achieve synergistic tumor suppression.^{19,20}

Titanium dioxide (TiO_2) is widely used in photocatalysis and sonodynamic therapy due to its chemical stability, biocompatibility, and efficient electron transport capabilities, which facilitate effective charge separation and ROS production under external stimuli.^{21,22} Incorporating TiO_2 into MOF-based composites has been shown to enhance sonodynamic performance by providing an electron transport pathway and promoting interfacial charge coupling.^{23,24} Despite these advances, there remains a need for MOF- TiO_2 heterojunctions specifically designed to achieve efficient ROS generation and potent antitumor activity in RCC.

Based on this background, we rationally designed and fabricated HKUST-1@ TiO_2 heterojunction composites to address the limitations of native MOFs in SDT. The porous structure of HKUST-1 enhances ultrasound scattering and cavitation effects, promoting local energy enrichment, while TiO_2 serves as an efficient electron transport medium to facilitate spatial charge separation. The interfacial coupling between HKUST-1 and TiO_2 effectively suppresses electron-hole recombination and sustains ROS generation. Furthermore, HKUST-1 serves as a Cu^{2+} reservoir that depletes intracellular GSH to generate Cu^+ , which subsequently triggers a high-efficiency Fenton-like reaction to amplify $\cdot\text{OH}$ production, thereby synergistically enhancing the therapeutic efficacy of SDT. Collectively, this rational design is expected to enhance SDT efficacy, providing a promising strategy for the treatment of RCC with potential clinical translation.

Materials and Methods

Reagents and Materials

HKUST-1 was purchased from Karger Advanced Carbon Materials Co., Ltd.; anhydrous ethanol was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd.; tetrabutyl titanate and hydrofluoric acid (HF, 40 wt%, analytical grade) were obtained from Sigma-Aldrich; the TUNEL assay kit was purchased from YaMei Biochemical Technology Co., Ltd.; and the Ki67 antibody was sourced from Affinity Biosciences.

Synthesis of HKUST-1@ TiO_2

HKUST-1 crystals (25 mg) were ultrasonically dispersed in 50 mL of ethanol to obtain a homogeneous suspension. Then, 250 μL of tetrabutyl titanate (Sigma-Aldrich) was added to the suspension under constant stirring at room temperature and stirred for 20 minutes. Subsequently, 3.5 mL of deionized water and 105 μL of hydrofluoric acid (HF) were slowly added to the mixture, and stirring was continued for 20 minutes. The resulting suspension was transferred to a 100 mL stainless steel autoclave lined with polytetrafluoroethylene (PTFE) and heated at 180 °C for 12 hours. After cooling to room temperature, the product was collected by centrifugation and washed with alternating ethanol and deionized water for at least three cycles until the supernatant reached a neutral pH (≈ 7.0), with additional washes performed if necessary. Finally, the powder was vacuum-dried at 80 °C for 24 h to ensure the complete removal of any residual HF and volatile acids.

The synthesis of pure TiO_2 followed the same procedure, but without the addition of HKUST-1.

Material Characterization

DLS and zeta potential measurements were carried out with a Zetasizer Nano from Malvern Panalytical Limited (ZEN3600). The textural properties and specific surface area of the samples (HKUST-1) were characterized by nitrogen adsorption-desorption isotherms at 77.3 K using a BSD-660M A3S|B3M surface area and pore size analyzer (BSD Instrument) via the static volumetric method. UV-visible (UV-vis) absorption spectra was recorded using a UV-visible

spectrophotometer. Transmission electron microscopy (TEM) images were acquired using a JEOL JEM-F200 instrument (Japan) at an accelerating voltage of 200 keV to provide detailed information on particle morphology and microstructure. Scanning electron microscopy (SEM) observations were conducted using a ZEISS GeminiSEM 300 (Germany) to examine surface morphology and sample uniformity. X-ray photoelectron spectroscopy (XPS) measurements were performed on an ESCALAB 250Xi system (Thermo Fisher Scientific) equipped with an Mg X-ray source. X-ray diffraction (XRD) patterns were collected using a Rigaku D/MAX-2550 V diffractometer with a Cu K α X-ray source.

Ultrasound-Enhanced Peroxidase-Like Activity of HKUST-1@TiO₂

The glutathione (GSH) depletion capability of different systems (TiO₂, HKUST-1, and HKUST-1@TiO₂) was evaluated using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) assay. Briefly, the samples (at a final concentration of 100 μ g/mL) were incubated with GSH solution (1.0 mM) in phosphate-buffered saline (PBS, pH 7.4) containing H₂O₂ (100 μ M) at 37 °C for 1 h. After incubation, the mixtures were centrifuged at 10,000 rpm for 10 min to remove the solid materials. Subsequently, 100 μ L of the supernatant was mixed with 10 μ L of DTNB reagent (10 mM). The mixture was allowed to react in the dark for 15 min. The concentration of residual GSH was quantified by measuring the absorbance of the yellow TNB product at 412 nm using UV-Vis spectrophotometer.

The peroxidase-like (POD) activity of HKUST-1@TiO₂ was assessed using TMB as a substrate in the presence of H₂O₂. In a typical experiment, 0.8 mM TMB, 1 mM H₂O₂, and 200 μ g mL⁻¹ of HKUST-1@TiO₂ were mixed in phosphate-buffered saline (PBS). After the reaction proceeded for the predetermined time, the absorbance of the solution was recorded using a UV-visible spectrophotometer to monitor the oxidation of TMB.

All experiments involving ultrasound irradiation in this study were conducted using consistent parameters: a frequency of 1.0 MHz, a power density of 1.0 W cm⁻², and a 50% duty cycle, at set time 5 minutes.

Cell Culture and Treatment

Human renal cell carcinoma cell line (OSRC-2, obtained from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China) was cultured in 1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated in a 37°C, 5% CO₂ humidified incubator for routine cultivation. When the cells reached 80–90% confluence, they were passaged after digestion with trypsin. OSRC-2 cells were seeded and incubated overnight for adhesion. For ultrasound treatment, cells were exposed to 1.0 MHz ultrasound at 1.0 W/cm² with a 50% duty cycle for 5 minutes, using a probe positioned 1 cm above the cell monolayer.

The experiments were divided into six groups: Control, Control+US, TiO₂, TiO₂+US, HKUST-1@TiO₂, and HKUST-1@TiO₂+US. Cells were seeded at the same density into 96-well plates or culture dishes. After overnight adhesion, the corresponding treatments were performed.

Live/Dead Cell Staining Assay

The Live/Dead Cell Staining Kit was used to distinguish live and dead cells in each group. After washing the treated cells with PBS, they were incubated with the dye working solution containing Calcein-AM and propidium iodide (PI) at room temperature in the dark for 15–20 minutes. Green fluorescence indicated live cells, while red fluorescence indicated dead cells. The cells were observed and photographed under an inverted fluorescence microscope.

CCK-8 Assay for Cell Viability

For concentration-dependent cytotoxicity analysis, OSRC-2 cells were treated with HKUST-1@TiO₂ or TiO₂ at a series of concentrations (0, 25, 50, 100, 200, and 400 mg/mL) and evaluated by CCK-8 assay after 24 h. Based on the dose-response results and consideration of maintaining measurable therapeutic differences among groups, 200 mg/mL was selected as the working concentration for subsequent comparative in vitro experiments, including the group-wise evaluation with or without ultrasound. At the specified time points after treatment, 10 μ L of CCK-8 solution was added to each well, followed by incubation for 2 hours. The absorbance at 450 nm was measured using a microplate reader. Cell viability was expressed as the absorbance value (OD), reflecting cell proliferative capacity.

Intracellular ROS Detection

To directly assess intracellular ROS generation, OSRC-2 cells were incubated with 10 μ M DCFH-DA for 30 min at 37°C following respective treatments. Cells were washed three times with PBS and observed under an inverted fluorescence microscope. The fluorescence intensity was quantified using ImageJ software to evaluate intracellular ROS levels. This experiment provides direct evidence linking HKUST-1@TiO₂ catalytic activity to intracellular ROS accumulation and tumor cell death.²⁵

Ki67 Immunofluorescence Staining

Treated cells were fixed and permeabilized with 4% paraformaldehyde for 15 minutes and 0.1% Triton X-100 for 10 minutes. After blocking with 5% goat serum for 30 minutes, the Ki67 primary antibody was added, and the cells were incubated overnight at 4°C. The next day, a fluorescence-labeled secondary antibody was added.

Experimental Animals and Ethical Approval

Female BALB/c nude mice (4 weeks old, weighing 18–22 g) were obtained and acclimatized for one week prior to experimentation. All animal experiments were performed at the accredited animal facility of Wuhan Myhalic Biotechnology Co., Ltd. (Wuhan, China) and were conducted in strict accordance with the Guidelines for the Management and Use of Laboratory Animals and the Chinese national standard GB/T 35892-2018 (Laboratory Animal—Guideline for Ethical Review of Animal Welfare).

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Wuhan Myhalic Biotechnology Co., Ltd. (Approval No. HLK-20250902-003). This facility was selected through a formal cooperation agreement with Ningbo Medical Center Lihuili Hospital to ensure appropriate ethical oversight and standardized animal experimental procedures.

For all in vivo procedures, mice were anesthetized with isoflurane (3–4% for induction and 1–2% for maintenance in oxygen). At the completion of the experiments, animals were euthanized by carbon dioxide (CO₂) inhalation followed by cervical dislocation to confirm death, in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Tumor Implantation and Grouping

OSRC-2 cells in the logarithmic growth phase were suspended at a concentration of 5×10^6 cells/100 μ L PBS. The cell suspension was inoculated into the subcutaneous tissue of the right axilla of the nude mice under sterile conditions. Tumor-bearing mice were randomly divided into six groups (n=3 per group): Control, Control+US, TiO₂, TiO₂+US, HKUST-1@TiO₂, and HKUST-1@TiO₂+US.

For tumor-bearing nude mice, intratumoral injections of HKUST-1@TiO₂ or TiO₂ were administered (50 μ L per mouse, 200 mg/mL). Ultrasound irradiation (1.0 MHz, 1.0 W/cm², 50% duty cycle) was applied 24 h after injection, with the probe positioned approximately 5 mm above the tumor surface and ultrasound gel as the coupling medium. Treatments were repeated twice weekly for 3 consecutive weeks. The intratumoral dose of 50 μ L at 200 mg/mL was selected based on prior in vitro cytotoxicity results, preliminary pilot studies assessing tolerability, and literature precedent for nanomaterial-based sonodynamic therapy in xenograft models.^{26–28} This concentration ensures effective tumor delivery while avoiding acute systemic toxicity, providing a balance between therapeutic efficacy and safety.

Treatment Protocol

The Control and Control+US groups received an equal volume of PBS injections. The TiO₂ and HKUST-1@TiO₂ groups received corresponding nanomaterials via intratumoral injection (50 μ L per mouse, with a material concentration of 200 mg/mL). Ultrasound treatment was performed within 24 hours after injection for the TiO₂+US and HKUST-1@TiO₂+US groups. All treatments were administered twice a week for 3 consecutive weeks.

Specimen Collection and Processing

On day 21, all mice were euthanized, and tumor tissues were excised. Tumor weights were precisely measured, and the macroscopic morphology of the tumors was recorded by photography. Tumor tissues were divided into two parts: one part was immediately fixed in 4% paraformaldehyde for 12 hours for subsequent histological and immunofluorescence analysis; the other part was stored at -80°C for later use.

Histopathological Examination

Fixed tumor tissues were dehydrated through a gradient alcohol series (70%, 85%, 95%, 100% for 10 minutes each), cleared in xylene (10 minutes $\times$ 2), and embedded in paraffin. Sections (5 μm thick) were cut coronally, stained with Hematoxylin and Eosin (HE), and examined under an optical microscope to observe pathological features, including tumor cell density, necrosis, and inflammatory infiltration.

Ki67 Immunofluorescence Staining

Paraffin sections were subjected to routine deparaffinization and rehydration, followed by heat-induced epitope retrieval (HIER) in citrate buffer (pH 6.0) at 95°C for 10 minutes. Endogenous peroxidase activity was blocked using 3% H_2O_2 for 10 minutes, followed by protein blocking with 5% goat serum for 30 minutes. Ki67 primary antibody (1:200 dilution) was applied and incubated overnight at 4°C . The following day, fluorescence-conjugated secondary antibody (1:500 dilution) was added and incubated for 1 hour at room temperature in the dark. Nuclei were stained with DAPI for 5 minutes. Images were captured under an inverted fluorescence microscope, and five different fields per group were photographed for analysis.

TUNEL Apoptosis Detection

In situ, apoptosis was detected using the Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling (TUNEL) assay. According to the kit instructions, paraffin sections were deparaffinized and rehydrated and then incubated with TUNEL reaction mixture at 37°C for 60 minutes. After washing with PBS (5 minutes $\times$ 3), fluorescence-labeled transferase was added and incubated for 30 minutes. Nuclei were stained with DAPI for 5 minutes. Apoptotic cells were observed and photographed under an inverted fluorescence microscope, and five different fields per group were recorded.

Biocompatibility and Systemic Toxicity Evaluation

To preliminarily evaluate the in vivo safety of the final formulation, major organs (heart, liver, spleen, lung, and kidney) were collected for histopathological examination, and serum biochemical indicators, including ALT, AST, CRE, BUN, and CK, were analyzed at the study endpoint. These assays were used to assess potential hepatic, renal, and muscle-related toxicity following treatment. In the present study, these analyses were intended to provide an initial evaluation of systemic tolerability under the tested dosing conditions.

Serum Sample Collection

At the end of the treatment, all mice were euthanized, and blood was collected via the abdominal aorta. The blood was placed into sterile collection tubes, left to stand for 2 hours, and then centrifuged at 3000 rpm for 10 minutes. The supernatant serum was collected, aliquoted, and stored at -80°C for subsequent biochemical index testing.

Serum Biochemical Indexes Detection

Serum samples were analyzed using an automatic biochemical analyzer, measuring the following parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRE), blood urea nitrogen (BUN), and creatine kinase (CK). These parameters reflect liver function, kidney function, and muscle damage, respectively. All tests were conducted according to the manufacturer's operational protocols, and each sample was tested in triplicate. The average value was taken as the result.

Histopathological Examination of Major Organs

Major organs, including the heart, liver, spleen, lungs, and kidneys, were collected from each group of mice and immediately fixed in 4% paraformaldehyde for 12 hours. After fixation, tissues were dehydrated through a gradient alcohol series (70%, 85%, 95%, 100%, 10 minutes each), cleared in xylene (10 minutes $\times$ 2), and embedded in paraffin. Coronally sectioned samples were stained using the standard Hematoxylin and Eosin (HE) method. Pathological changes in each organ, including cell morphology, tissue structural integrity, inflammation, necrosis, or fibrosis, were observed under an optical microscope.

Image Analysis, Quantification, and Statistical Analysis

Quantitative analysis of all fluorescence images was performed using Image J software. For Ki67 and TUNEL quantification, at least five different fields per slide were statistically analyzed, and the percentage of positive areas was calculated. The average value of the positive area was taken as the result for each sample. All image analyses were performed by trained researchers blinded to the experimental groups.

All experiments were performed with at least three biological replicates ($n \geq 3$ per group), consistent with commonly reported sample sizes in similar preclinical studies.^{29–31} In practice, we often included more than three replicates to account for potential experimental variation and ensure reliable results. Independent technical repeats were performed for each assay, and the data are presented as mean $\pm$ standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered significant.

Results and Discussion

Fabrication and Characterization of HKUST-1@TiO₂

The successful fabrication of the HKUST-1@TiO₂ composite was confirmed through detailed morphological and structural characterization using SEM and TEM. As shown in [Figure 1A](#), the pristine HKUST-1 crystals exhibit a regular and typical octahedral structure, with a smooth, flat surface, well-defined edges.³² In contrast, after the in-situ growth of TiO₂, the surface morphology of the HKUST-1@TiO₂ composite changed significantly ([Figure 1B](#)). The smooth surface of HKUST-1 was uniformly and densely coated with numerous small TiO₂ nanoparticles, significantly increasing the surface roughness.³³ Transmission electron microscopy (TEM) images further revealed the internal structure of the materials. Pure HKUST-1 exhibited a solid, homogeneous structure ([Figure 1C](#)), while the edge of HKUST-1@TiO₂ ([Figure 1D](#)) clearly showed the TiO₂ nanoparticle layer attached to the surface of the MOF, confirming the formation of a core-shell-like heterostructure.³³ Furthermore, energy dispersive X-ray spectroscopy (EDS) elemental mapping ([Figure 1E and F](#)) showed that O (red), Ti (green), and Cu (cyan) elements were evenly distributed throughout the composite particle, with a high degree of overlap in their spatial distribution. This indicates that TiO₂ is not simply physically mixed but is uniformly grown or loaded onto the HKUST-1 framework, forming a tightly coupled composite structure.

To further investigate the surface chemical composition and electronic states of the materials, X-ray photoelectron spectroscopy (XPS) analysis was performed. As shown in [Figure 1G–I](#), the pure TiO₂ sample exhibited characteristic peaks for Ti 2p, O 1s, and C 1s, while the HKUST-1 sample showed typical signals for Cu 2p, O 1s, and C 1s. In the full spectrum of the HKUST-1@TiO₂ composite ([Figure 1I](#)), characteristic signals for Cu, Ti, O, and C elements were observed, confirming that TiO₂ had been successfully incorporated into the MOF system.³⁴ In addition, X-ray diffraction (XRD) patterns further validated the crystalline phase composition of the composite material ([Figure S1A and B](#)). Pure TiO₂ exhibited characteristic diffraction peaks for the anatase phase ([Figure S1A](#)), while pure HKUST-1 displayed the high crystallinity diffraction peaks typical of MOF materials ([Figure S1B](#)). For the HKUST-1@TiO₂ composite material ([Figure S1C](#)), its XRD pattern perfectly retained the characteristic peaks of both components, with no obvious impurity peaks or collapse of the crystal structure. This indicates that during the synthesis process, the metal-organic framework of HKUST-1 maintained high integrity, and it, along with the photoactive anatase TiO₂, formed a stable heterojunction system.³³ Structural integrity is critical for the subsequent synergistic effects in sonodynamic therapy.

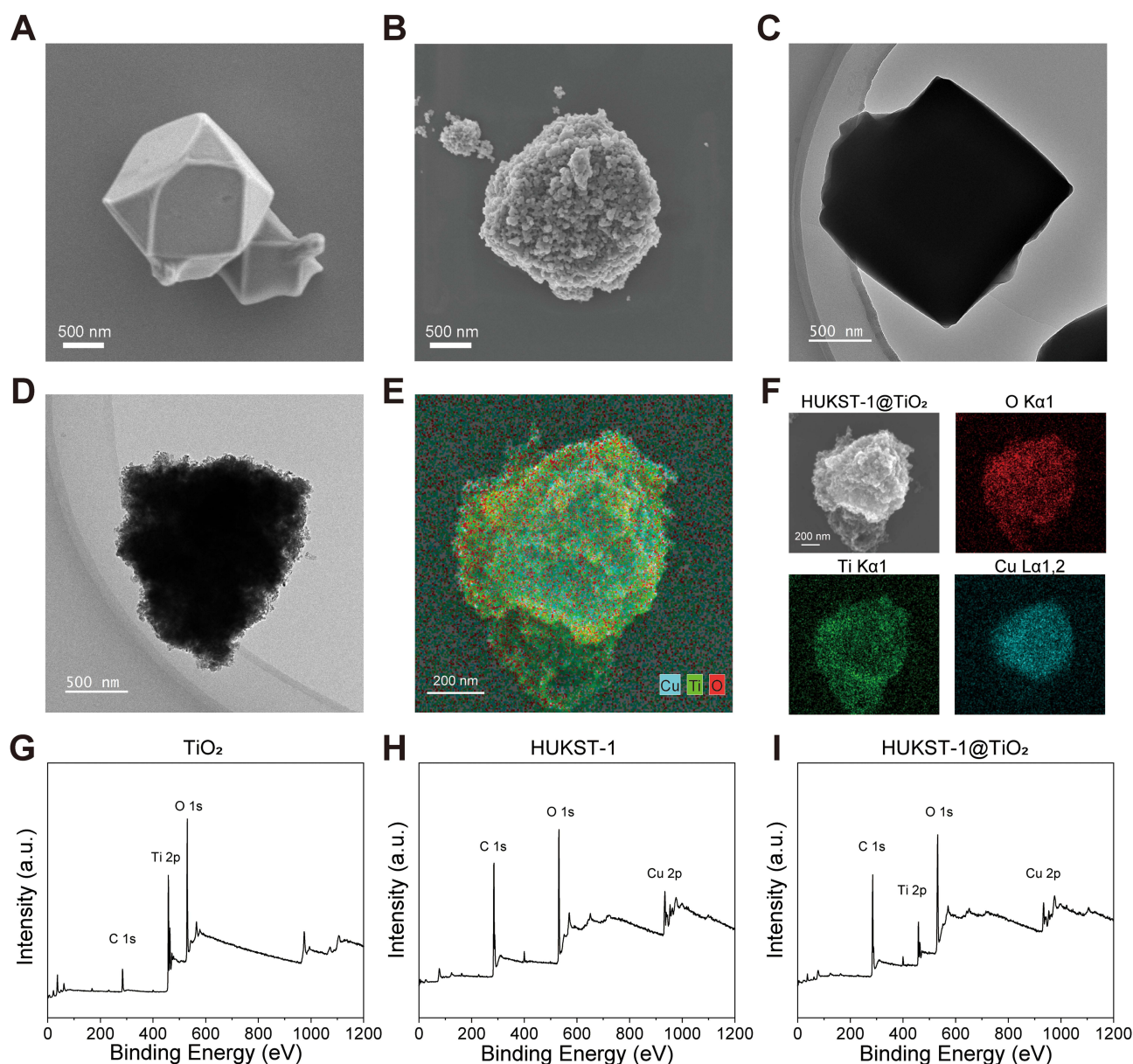


Figure 1 Morphological and Structural Characterization of HKUST-1, TiO_2 , and HKUST-1@ TiO_2 . (A) SEM image of HKUST-1, showing a smooth-surfaced polyhedral structure (particle size approximately 500 nm). (B) SEM image of HKUST-1@ TiO_2 , showing TiO_2 particles uniformly coated on the surface of HKUST-1. (C) TEM image of HKUST-1, exhibiting a regular, solid crystalline structure. (D) TEM image of HKUST-1@ TiO_2 , showing the coating morphology after composite formation. (E) EDS total spectrum of HKUST-1@ TiO_2 , showing uniform distribution of O (red), Ti (green), and Cu (cyan) elements. (F) Single-element mapping of the corresponding regions, confirming the consistent spatial distribution of each element. (G) XPS full spectrum of TiO_2 , showing characteristic peaks for Ti 2p, O 1s, and C 1s. (H) XPS full spectrum of HKUST-1, displaying characteristic signals for Cu 2p, O 1s, and C 1s. (I) XPS full spectrum of HKUST-1@ TiO_2 , containing signals for Ti, Cu, O, and C elements.

Physicochemical and Sonocatalytic Properties of HKUST-1@ TiO_2

The hydrodynamic diameter of HKUST-1 was measured at $1.711 \pm 0.702 \mu\text{m}$, with a surface zeta potential of $-17.87 \pm 1.01 \text{ mV}$. Upon loading of TiO_2 nanoparticles, the diameter of HKUST-1@ TiO_2 increased to $1.936 \pm 0.723 \mu\text{m}$, accompanied by a slight shift in zeta potential to $-19.12 \pm 1.14 \text{ mV}$ (Figure 2A), collectively confirming the successful fabrication of the composite. This loading process substantially altered the porous architecture of the material: the BET specific surface area of pristine HKUST-1 was $1411.06 \text{ m}^2/\text{g}$ ($R^2 = 0.99975$, Figure S2A), which decreased markedly to $495.01 \text{ m}^2/\text{g}$ ($R^2 = 0.99970$, Figure S2B) in HKUST-1@ TiO_2 . The pronounced reduction in adsorption capacity (Figure 2B) suggests that TiO_2 incorporation restricts the accessibility of the MOF pore channels, providing further evidence for the successful formation of the composite structure.³⁵

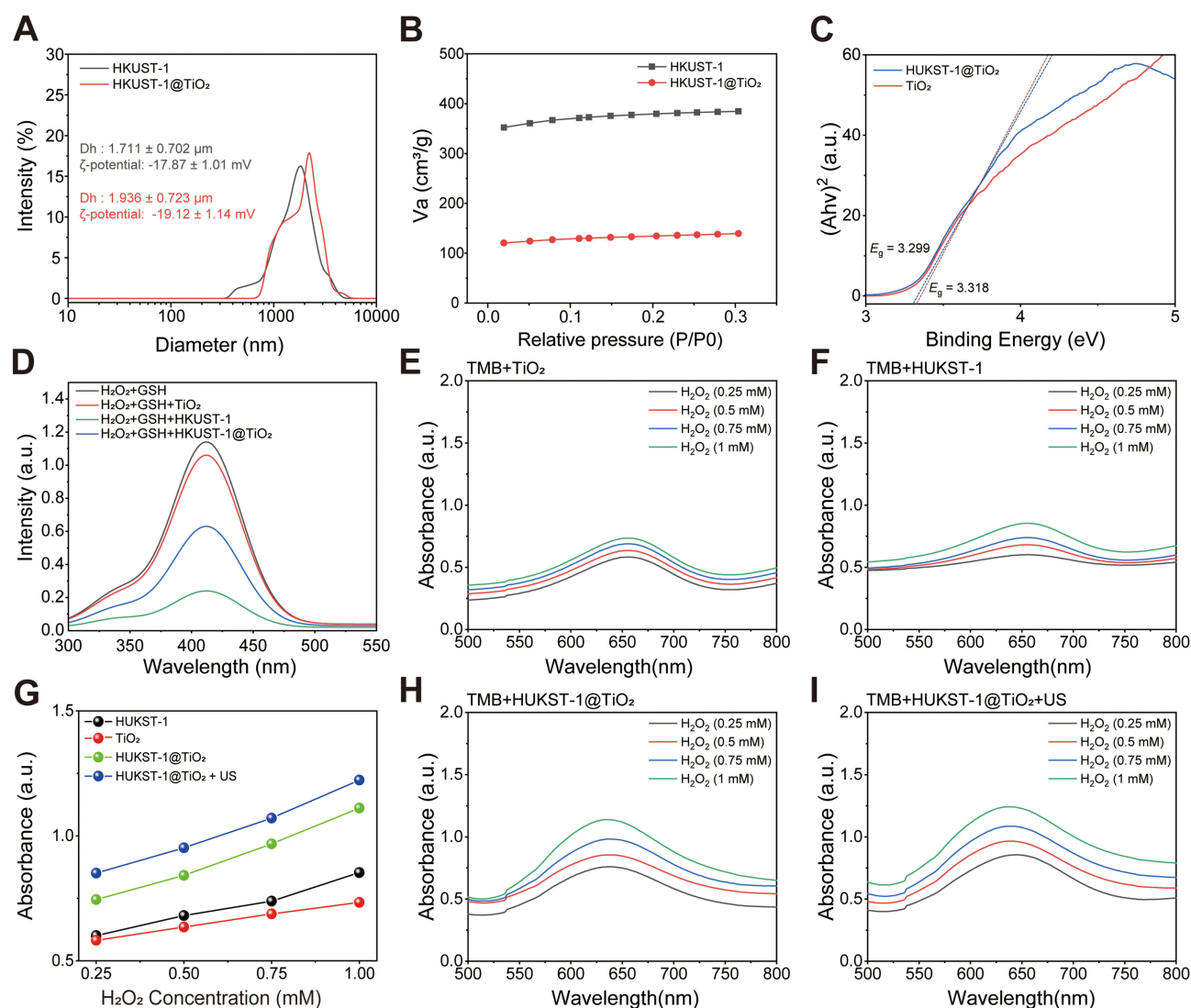


Figure 2 Physicochemical Characterization and Multienzyme-like Catalytic Mechanisms of HKUST-1@TiO₂ Nanocomposites. **(A)** Hydrodynamic diameter Dh distribution and zeta-potential of HKUST-1 and HKUST-1@TiO₂ nanoparticles measured by DLS. **(B)** N₂ adsorption isotherms of HKUST-1 and HKUST-1@TiO₂. **(C)** Tauc plot analysis, showing that the band gap of HKUST-1@TiO₂ (3.299 eV) is narrower than that of TiO₂ (3.318 eV), which facilitates electron transitions. **(D)** Comparison of glutathione (GSH) depletion capacities for various groups determined by the DTNB assay at 412 nm. **(E)** UV-Vis absorption spectra of the TiO₂ system at different H₂O₂ concentrations. **(F)** UV-Vis absorption spectra of the HKUST-1 system at different H₂O₂ concentrations. **(G)** Linear relationship between the absorbance at 652 nm and H₂O₂ concentration (0.25–1.0 mM) for each system, confirming the synergistic enhancement effect of ultrasound and the composite material. **(H)** UV-Vis absorption spectra of the HKUST-1@TiO₂ system as a function of H₂O₂ concentration. **(I)** UV-Vis absorption spectra of the HKUST-1@TiO₂ system (ultrasound-assisted) as a function of H₂O₂ concentration, showing the highest catalytic activity.

UV-Vis diffuse reflectance spectroscopy was performed to characterize the optical properties of the materials. As shown in [Figure S3](#), HKUST-1@TiO₂ exhibited stronger light absorption in the visible region compared to pristine TiO₂. Tauc plots derived from the Kubelka–Munk function ([Figure 2C](#)) revealed optical bandgaps of approximately 3.318 eV for pristine TiO₂ and 3.299 eV for HKUST-1@TiO₂. This bandgap narrowing lowers the energy threshold for electronic transitions, thereby facilitating the excitation and separation of photogenerated or sonogenerated charge carriers and providing a physical basis for the enhanced sonocatalytic activity.³⁶

Given that GSH is overexpressed in the tumor microenvironment and serves as a major antioxidant barrier that limits CDT efficacy,^{37,38} the GSH depletion capability of each system was evaluated using the DTNB assay, which quantifies GSH concentration by monitoring the absorbance of the TNB product at 412 nm. As shown in [Figure 2D](#), the absorbance at 412 nm in the H₂O₂ + GSH + TiO₂ group remained comparable to that of the H₂O₂ + GSH control, confirming that TiO₂ alone possesses negligible GSH oxidation activity. In contrast, both HKUST-1 and HKUST-1@TiO₂ induced

a substantial reduction in absorbance, with HKUST-1 exhibiting the most pronounced depletion effect, indicating that Cu^{2+} released from the copper-based framework is the primary driver of GSH oxidation.³⁹ The relatively attenuated depletion observed for HKUST-1@TiO₂ may be attributed to partial surface coverage by TiO₂, which moderately restricts Cu^{2+} release. Importantly, such GSH consumption not only disrupts the antioxidant defense of tumor cells but also drives the reduction of Cu^{2+} to Cu^+ , supplying the electron donors necessary to sustain the Fenton-like reaction and generate cytotoxic hydroxyl radicals ($\cdot\text{OH}$), thereby potentiating CDT efficacy.⁴⁰

The peroxidase-like (POD) activity and sonodynamic performance of each system were evaluated using 3,3',5,5'-tetramethylbenzidine (TMB) as a colorimetric substrate in the presence of H₂O₂.⁴¹ The photograph in [Figure S4](#) visually confirms that HKUST-1@TiO₂ + US produced the deepest blue coloration among all groups, indicating the highest ROS generation and catalytic activity. UV–Vis absorption spectra were further employed to quantitatively track the characteristic TMB oxidation peak at 652 nm. As shown in [Figure 2E–I](#), the absorbance of all systems increased proportionally with H₂O₂ concentration (0.25–1.0 mM), consistent with substrate-dependent catalytic behavior. Among the individual components, TiO₂ ([Figure 2E](#)) and HKUST-1 ([Figure 2F](#)) exhibited relatively modest activity. In contrast, the HKUST-1@TiO₂ heterojunction ([Figure 2H](#)) demonstrated markedly superior catalytic performance even in the absence of ultrasound, suggesting that heterojunction formation facilitates interfacial charge transfer and enhances intrinsic catalytic activity. Upon introduction of ultrasound irradiation ([Figure 2I](#)), the absorbance increased substantially, surpassing all other groups. As summarized in [Figure 2G](#), the linear fits of absorbance versus H₂O₂ concentration confirm a catalytic activity ranking of HKUST-1@TiO₂ + US > HKUST-1@TiO₂ > HKUST-1 > TiO₂, providing strong evidence for a synergistic interplay between acoustic cavitation and the heterojunction structure. Ultrasound irradiation not only promotes the generation of electron–hole pairs within the sonosensitizer but may also enhance mass transfer in the Fenton-like reaction via thermal and mechanical effects, collectively driving efficient TMB oxidation.⁴²

To ensure the feasibility of the heterojunction for biological applications, the long-term stability and degradation behavior of HKUST-1 and HKUST-1@TiO₂ were evaluated in physiological environments. First, structural stability was investigated in PBS buffer. As shown in [Figure S5A](#), bare HKUST-1 exhibited significant surface erosion and structural deformation after 1-day incubation in PBS, which is attributed to the high affinity of phosphate ions for Cu^{2+} centers, leading to the collapse of the MOF framework.⁴³ In contrast, HKUST-1@TiO₂ ([Figure S5B](#)) maintained its integrated spherical architecture despite a rougher surface, suggesting that the TiO₂ coating serves as a robust protective shield. This was further quantified by the BET surface area measurements ([Figure S6](#)). While bare HKUST-1 experienced a sharp decline in surface area (from ~1400 to ~500 m²/g within 24 hours), the degradation rate of HKUST-1@TiO₂ was significantly attenuated, retaining a more stable porous structure over the 14-day observation period.

Furthermore, colloidal stability was examined in various biological media to simulate the conditions of the subsequent cellular assays ([Figure S7](#)). In PBS, both materials displayed a narrow size distribution. When transitioned to RPMI 1640 medium and eventually to medium supplemented with 10% FBS, a gradual increase in the hydrodynamic diameter (Dh) was observed. Specifically, the Dh of HKUST-1@TiO₂ increased from 1.937 μm in PBS to 2.601 μm in serum-containing medium. This size increment, accompanied by a shift in zeta potential, provides clear evidence of the formation of a protein corona, which is known to stabilize nanoparticles against aggregation in complex biological fluids.⁴⁴

In vitro Experimental Results

The results of the live/dead cell staining assay are shown in [Figure 3A](#). In the Control and Control+US groups, the green fluorescence area was the largest, and red fluorescence was almost undetectable, indicating high cell survival. After TiO₂ treatment, the green fluorescence area significantly decreased, and red fluorescence signals appeared, suggesting cell death in a portion of the cells. After HKUST-1@TiO₂ treatment, the green fluorescence further decreased, and red fluorescence significantly increased. In the HKUST-1@TiO₂+US group, red fluorescence was the strongest, and the green fluorescence area was the smallest, indicating that this treatment induced the most cell death. The cytotoxicity hierarchy observed in live/dead staining supports the synergistic mechanism of HKUST-1@TiO₂. The Control+US group showed no significant difference in tumor cell killing compared to the Control group, indicating that the applied ultrasound parameters alone do not effectively induce tumor cytotoxicity without a sonosensitizer. TiO₂ shows moderate cytotoxicity, enhanced under ultrasound due to its sonocatalytic ROS generation. Notably, HKUST-1@TiO₂ exhibits superior killing efficiency, further amplified by ultrasound,

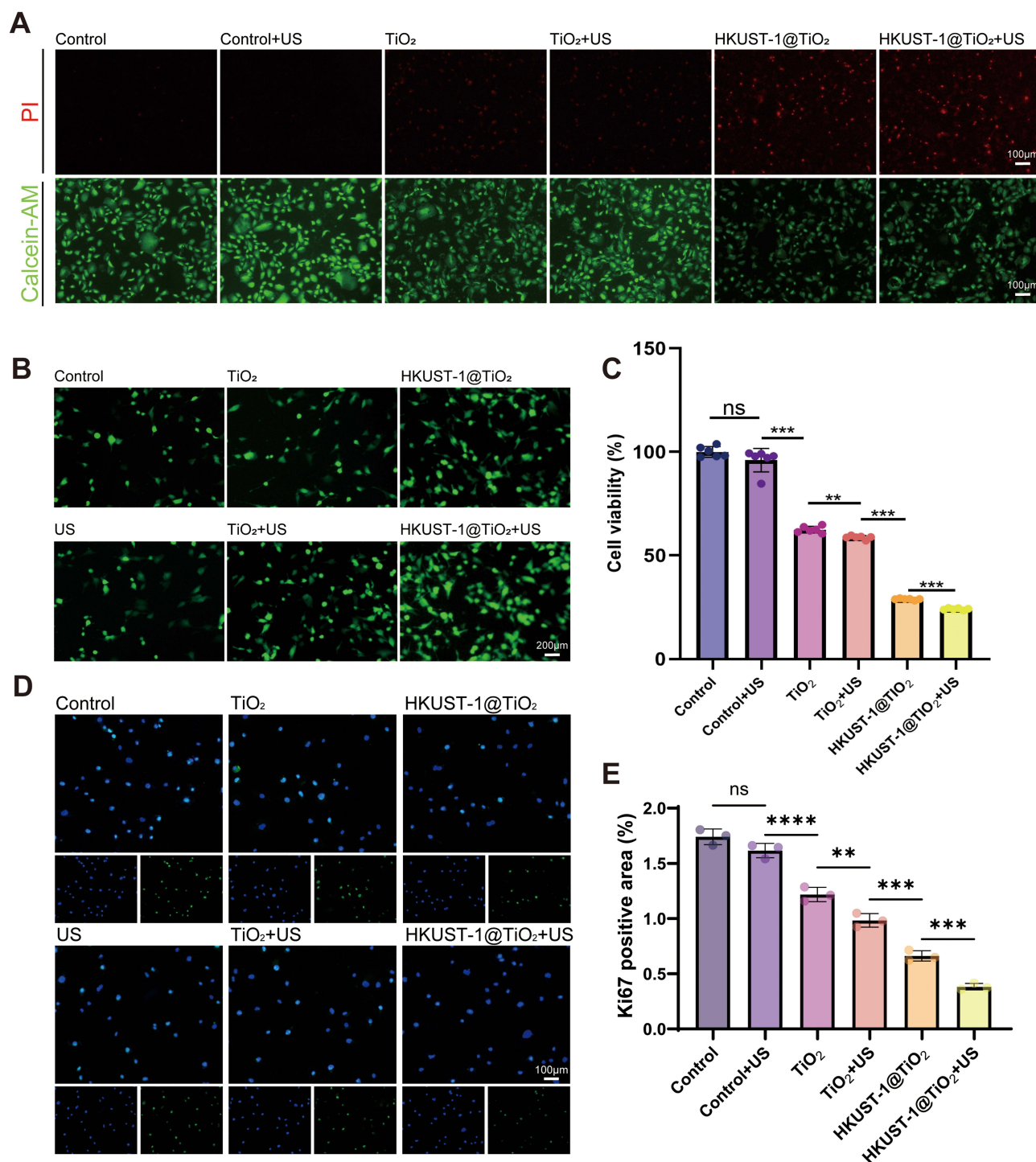


Figure 3 In Vitro Experiments: (A) Cell viability and death staining results (scale bar = 100 μ m); (B) The results of DCFH-DA staining; (C) CCK-8 assay to detect cell viability in each group; (D) Ki67 immunofluorescence staining results (scale bar = 100 μ m); (E) Quantitative analysis of Ki67 immunofluorescence staining. Data are presented as mean $\pm$ standard deviation (mean $\pm$ SD) (n = 3). **p < 0.01, ***p < 0.001, ****p < 0.0001 indicate statistically significant differences, "ns" indicates no statistically significant difference.

indicating that the heterojunction facilitates effective charge separation and reduces electron–hole recombination. Additionally, the Cu-based redox cycle in HKUST-1 enables glutathione depletion and Fenton-like $\cdot$ OH production, providing a complementary CDT effect. This explains why HKUST-1@TiO₂ alone outperforms TiO₂+US, demonstrating a dual SDT–CDT therapeutic synergy.^{45,46}

Cell viability was first evaluated by CCK-8 assay in a concentration-dependent manner, and the results demonstrated that both TiO_2 and HKUST-1@TiO_2 induced dose-dependent reductions in OSRC-2 cell viability. Based on the dose-response profile, 200 mg/mL was selected as the representative working concentration for subsequent group comparisons, as this dose produced a clear biological effect while still allowing discrimination among different treatment groups. Under this concentration, the results are shown in [Figure 3C](#). The Control and Control+US groups maintained cell viability close to 100%, with no statistically significant difference between them, indicating that ultrasound treatment alone had no notable cytotoxic effect. Compared with the control group, TiO_2 treatment significantly reduced cell viability ($P < 0.001$), which was further decreased in the TiO_2 +US group ($P < 0.01$). The HKUST-1@TiO_2 group exhibited a markedly greater reduction in cell viability compared with the TiO_2 +US group ($P < 0.001$), while the HKUST-1@TiO_2 +US combination treatment resulted in the lowest cell viability, which was significantly lower than that of the HKUST-1@TiO_2 group alone ($P < 0.001$). These results collectively indicate that HKUST-1@TiO_2 in combination with ultrasound exerts the most potent cytotoxic effect among all treatment groups. The dose-dependent cytotoxicity of HKUST-1@TiO_2 and the selection of 200 mg/mL align with SDT studies that favor intermediate concentrations to preserve a clear therapeutic window. Negligible toxicity in the Control+US group confirms that ultrasound alone does not induce apoptosis without a sensitizer. A stepwise decrease in viability ($\text{TiO}_2 < \text{TiO}_2$ +US $< \text{HKUST-1@TiO}_2 < \text{HKUST-1@TiO}_2$ +US) demonstrates synergistic effects between heterojunction engineering and ultrasound activation, consistent with other MOF–semiconductor systems. The pronounced effect of HKUST-1@TiO_2 +US suggests that acoustic cavitation enhances ROS generation and membrane permeability, promoting intracellular Cu ion activity and amplifying CDT, characteristic of sonoenhanced chemodynamic therapy.^{19,47}

The biocompatibility was evaluated on HUVECs via Live/Dead staining and CCK-8 assay. Live/Dead staining using Calcein-AM and PI revealed predominantly green fluorescence with negligible red signal across all experimental groups, including TiO_2 , TiO_2 +US, HKUST-1@TiO_2 , and HKUST-1@TiO_2 +US, indicating minimal cytotoxicity ([Figure S8](#)). Consistently, CCK-8 assay demonstrated that cell viability remained comparable across all treatment groups relative to untreated control, with no statistically significant differences observed ($P > 0.05$) ([Figure S9](#)). These findings collectively confirm that HKUST-1@TiO_2 , even in combination with ultrasound irradiation, exhibits excellent biocompatibility toward normal vascular endothelial cells, supporting its potential for safe biomedical application. The selective cytotoxicity of HKUST-1@TiO_2 +US toward OSRC-2 cells while sparing HUVECs highlights its tumor-specific therapeutic potential. This selectivity arises from tumor microenvironment features: elevated H_2O_2 levels in RCC cells favor Cu^+ -mediated $\cdot\text{OH}$ generation, while high GSH accelerates the $\text{Cu}^{2+} \rightarrow \text{Cu}^+$ redox cycle, enhancing CDT. In contrast, normal cells lack these conditions. Additionally, ultrasound provides spatial control, restricting ROS activation to the tumor site. Together, these factors indicate a microenvironment-responsive mechanism, supporting HKUST-1@TiO_2 +US as a promising tumor-selective nanomedicine strategy.^{47,48}

DCFH-DA staining revealed a significant increase in intracellular ROS in OSRC-2 cells treated with HKUST-1@TiO_2 + ultrasound, compared with controls or single treatments, supporting that the heterojunction material effectively generates intracellular ROS, contributing to observed apoptosis and proliferation inhibition in tumor cells ([Figure 3B](#)). DCFH-DA fluorescence provides direct intracellular evidence that HKUST-1@TiO_2 links sonocatalytic and chemodynamic processes to ROS-mediated cytotoxicity. After intracellular hydrolysis, DCFH is oxidized by ROS to fluorescent DCF, reflecting oxidative stress. Strong fluorescence in the HKUST-1@TiO_2 +US group indicates enhanced ROS generation via heterojunction-assisted charge separation. The combined production of $\text{O}_2^{\cdot-}$, $^1\text{O}_2$, and $\cdot\text{OH}$ overwhelms cellular defenses, triggering apoptosis, consistent with subsequent proliferation and apoptosis results.^{17,49}

Ki67 is a marker protein for cell proliferation, reflecting the proliferative capacity of cells. As shown in [Figure 3D](#), immunofluorescence staining revealed the expression of Ki67 in cells from each group. The Control and Control+US groups showed the highest Ki67 expression levels. After TiO_2 treatment, Ki67 expression decreased. HKUST-1@TiO_2 treatment significantly inhibited Ki67 expression, while the HKUST-1@TiO_2 +US group showed the lowest Ki67 expression.

Further quantitative analysis results are shown in [Figure 3E](#). There was no significant difference in the Ki67-positive area between the Control and Control+US groups ($p > 0.05$, ns). TiO_2 treatment reduced the Ki67-positive area ($p < 0.01$). HKUST-1@TiO_2 treatment further decreased the Ki67-positive area ($p < 0.001$). The HKUST-1@TiO_2 +US treatment had

the most significant inhibitory effect on Ki67 expression ($p < 0.001$). These results indicate that the combination of HKUST-1@TiO₂ and ultrasound significantly suppressed cell proliferative capacity. Ki67, a hallmark proliferation marker expressed in active cell-cycle phases, decreased progressively across treatments, with strongest suppression in HKUST-1@TiO₂+US. This trend aligns with ROS findings, as excessive ROS induces DNA damage and activates DDR pathways, causing G2/M arrest and reduced Ki67 expression. The absence of change in Control+US confirms ultrasound alone is ineffective. These results support ROS-mediated proliferation inhibition as a key mechanism of HKUST-1@TiO₂-based SDT.⁵⁰

In vivo Experimental Results

To evaluate the in vivo anti-tumor effects of the HKUST-1@TiO₂ nanomaterial combined with ultrasound treatment, the appearance and growth of tumors in each group of nude mice were first observed. As shown in Figure 4A, after 21 days of treatment, the Control and Control+US groups exhibited rapid tumor growth, with tumor volumes significantly larger than those in the other groups. The TiO₂ and TiO₂+US treatment groups showed slight reductions in tumor volume. The HKUST-1@TiO₂ treatment group showed a significant reduction in tumor volume, while the HKUST-1@TiO₂+US group had the smallest tumors, which were lighter in color, indicating the most significant therapeutic effect.

Quantitative analysis of tumor volume is shown in Figure 4B. There was no statistically significant difference between the tumor volumes of the Control and Control+US groups ($p > 0.05$, ns), indicating that ultrasound treatment alone did not significantly inhibit tumor growth. TiO₂ treatment resulted in a reduction in tumor volume ($p < 0.001$). TiO₂+US treatment further reduced tumor volume ($p < 0.01$). The combination of HKUST-1@TiO₂ and ultrasound treatment showed the strongest inhibitory effect, with tumor volume significantly lower than that of the Control group. The gradient of tumor suppression, from TiO₂ to TiO₂+US, and finally HKUST-1@TiO₂+US, highlights the stepwise contribution of each mechanism. Ultrasound alone had no significant anti-tumor effect, consistent with previous findings.⁵¹ TiO₂+US efficacy stems from its sonocatalytic properties, but the wide bandgap limits ROS yield due to rapid electron-hole recombination.^{52,53} HKUST-1@TiO₂'s superior performance arises from synergistic sonodynamic and chemodynamic effects: enhanced ROS generation through charge transfer and Cu²⁺/Cu⁺ redox-driven ·OH amplification, overcoming tumor cell antioxidant defenses.^{16,20,54}

The excised tumors were precisely weighed, and the results are shown in Figure 4C. There was no significant difference in tumor weight between the Control and Control+US groups ($p > 0.05$, ns), suggesting that ultrasound treatment alone did not significantly affect tumor weight. TiO₂ treatment led to a reduction in tumor weight ($p < 0.01$). TiO₂+US treatment further reduced tumor weight ($p < 0.05$). The HKUST-1@TiO₂+US group had the lowest tumor weight ($p < 0.001$). The correlation between tumor weight and tumor volume further confirmed the significant anti-tumor effect of the HKUST-1@TiO₂+ultrasound treatment. The consistency between tumor volume and weight measurements across groups validates the anti-tumor findings and eliminates concerns about edema or necrotic fluid influencing results, important in xenograft models with variable tumor composition.⁵⁵ The significant tumor weight reduction in the HKUST-1@TiO₂+US group aligns with prior MOF-based SDT reports, such as enhanced tumor suppression by Z-scheme MOFs and Cu-MOF nanoparticles combining CDT and SDT, supporting the translational value of Cu-based MOF systems.^{19,20}

HE staining results are shown in Figure 4D. Tumor tissue in the Control group exhibited typical malignant tumor morphology, with closely packed tumor cells, large, darkly stained nuclei, high nuclear-to-cytoplasm ratio, and numerous mitotic figures, with no significant necrosis observed. The Control+US group showed similar pathological features, with no significant change in tumor cell density and very few necrotic areas. In the TiO₂ treatment group, partial necrosis appeared in the tumor tissue, with a decrease in tumor cell density and some inflammatory cell infiltration. In the TiO₂+US treatment group, the necrotic areas expanded, and tumor cell density further decreased. In the HKUST-1@TiO₂ treatment group, more necrotic foci and fibrotic changes were observed, with a significant decrease in tumor cell numbers and obvious inflammatory cell infiltration. The HKUST-1@TiO₂+US group exhibited the most severe structural damage in tumor tissue, with large areas of necrosis and hemorrhage, abnormal morphology of remaining tumor cells, progressive nuclear condensation, numerous apoptotic bodies, and the most extensive inflammatory cell infiltration, indicating the strongest cytotoxic effect induced by this treatment. Histopathological analysis showed progression from intact tumor architecture in controls to extensive necrosis, nuclear condensation, and apoptotic bodies in the HKUST-1@TiO₂+US group, indicating ROS-mediated tumor destruction. Nuclear condensation and apoptotic bodies align with mitochondrial membrane permeabilization and apoptosis.⁵⁶ Hemorrhagic necrosis

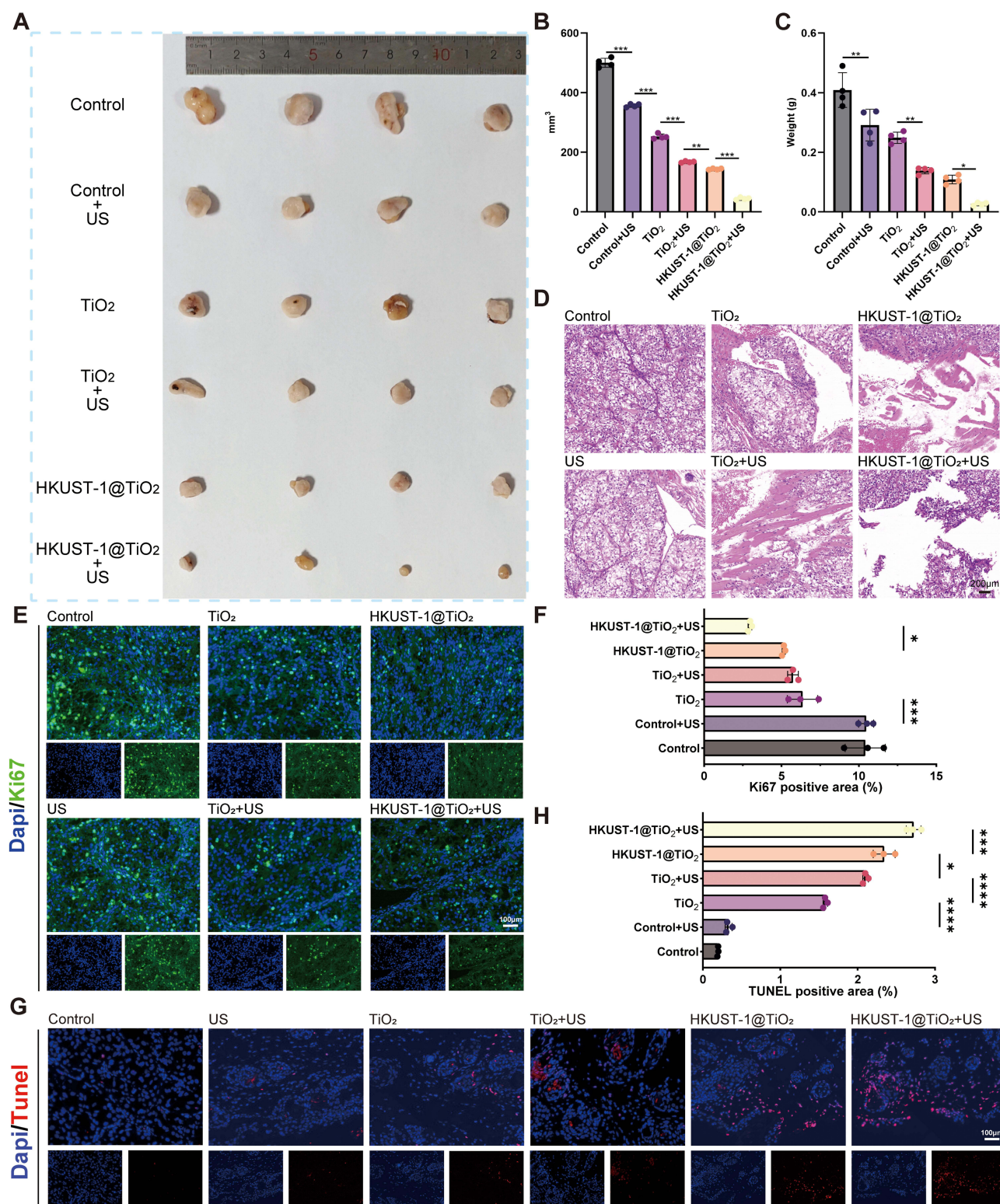


Figure 4 Animal Experiments: (A) Tumor growth in each group after subcutaneous implantation in nude mice for three weeks; (B) Tumor volume changes in each group; (C) Tumor weight in each group; (D) Hematoxylin and eosin (HE) staining results of tumors (scale bar = 200 μ m); (E) Ki67 immunofluorescence staining results of tumors (scale bar = 100 μ m); (F) Quantitative analysis of Ki67 immunofluorescence staining; (G) TUNEL immunofluorescence staining results of tumors (scale bar = 100 μ m); (H) Quantitative analysis of TUNEL immunofluorescence staining. Data are presented as mean $\pm$ standard deviation (mean $\pm$ SD) ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ indicate statistically significant differences.

suggests vascular disruption, while inflammatory infiltration in HKUST-1@TiO₂ groups may indicate immunogenic cell death and DAMP release, potentially activating immune responses, warranting further exploration in immunocompetent models.^{57,58}

Ki67 is a nuclear protein closely related to cell proliferation, and its expression level directly reflects the proliferative activity of the tumor. Ki67 immunofluorescence staining results are shown in Figure 4E. In the Control and Control+US groups, Ki67-positive cells were widely distributed with high density, indicating strong tumor cell proliferation activity. After TiO₂ treatment, the number of Ki67-positive cells significantly decreased. TiO₂+US treatment further reduced Ki67 expression. The number of Ki67-positive cells further decreased in the HKUST-1@TiO₂ treatment group. The HKUST-1@TiO₂+US treatment group had the fewest Ki67-positive cells, with the weakest green fluorescence signal.

Quantitative analysis of Ki67 expression is shown in Figure 4F. There was no significant difference in the Ki67-positive area between the Control and Control+US groups ($p > 0.05$, ns), indicating that ultrasound treatment alone did not significantly affect cell proliferation. TiO₂ treatment reduced the Ki67-positive area ($p < 0.001$). TiO₂+US treatment also reduced the Ki67-positive area ($p > 0.05$, with no significant difference compared to TiO₂ treatment). The combination of HKUST-1@TiO₂ and ultrasound treatment showed the most significant inhibition of Ki67 expression. These results suggest that the combination of HKUST-1@TiO₂ and ultrasound treatment effectively suppressed tumor cell proliferation. The progressive reduction in Ki67-positive area highlights that HKUST-1@TiO₂-mediated SDT impairs tumor cell proliferation in addition to inducing cell death. Ki67, a marker for actively proliferating cells, decreases through ROS-induced DNA damage, activating DDR pathways and enforcing cell cycle arrest.^{59,60} The lack of significant Ki67 reduction with TiO₂+US alone reflects its limited ROS output. In contrast, HKUST-1@TiO₂+US enhances ROS production, leading to greater DNA damage and stronger antiproliferative effects, consistent with in vitro results and other MOF-semiconductor SDT studies.¹⁷

TUNEL staining specifically labels apoptotic cells with fragmented DNA. TUNEL immunofluorescence results are shown in Figure 4G. In the Control group, very few TUNEL-positive cells were observed, almost undetectable, indicating a very low apoptosis rate. In the Control+US group, there was no significant increase in the number of TUNEL-positive cells ($p > 0.05$, ns), indicating that ultrasound treatment alone did not effectively induce apoptosis. In the TiO₂ treatment group, a small number of TUNEL-positive cells were observed, with enhanced red fluorescence signals. In the TiO₂+US treatment group, the number of TUNEL-positive cells significantly increased. The HKUST-1@TiO₂ treatment group showed further increased TUNEL-positive cells. The HKUST-1@TiO₂+US treatment group had the most TUNEL-positive cells, with the strongest red fluorescence signals and the widest distribution, indicating the strongest apoptotic response induced by this treatment.

Quantitative analysis of TUNEL results is shown in Figure 4H. In the Control group, the apoptotic cell area ratio was very low, with no statistical significance compared to the Control+US group ($p > 0.05$, ns). TiO₂ treatment increased the apoptotic cell area ratio ($p < 0.0001$). TiO₂+US treatment further increased the apoptotic area ($p > 0.05$, with no significant difference compared to TiO₂ treatment). The combination of HKUST-1@TiO₂ and ultrasound treatment resulted in the highest cell apoptosis. The TUNEL results correlate with tumor growth inhibition and reduced Ki67 expression, suggesting that the HKUST-1@TiO₂+ultrasound treatment exerts its significant anti-tumor effects primarily through promoting tumor cell apoptosis and inhibiting cell proliferation via a dual mechanism. The strong TUNEL signal in the HKUST-1@TiO₂+US group confirms extensive DNA fragmentation, a hallmark of apoptotic and necroptotic pathways, linked to mitochondrial outer membrane permeabilization (MOMP) induced by ROS accumulation.^{61,62} TiO₂+US did not significantly increase TUNEL positivity compared to TiO₂ alone, indicating insufficient ROS production. In contrast, HKUST-1@TiO₂ depletes GSH and amplifies $\cdot\text{OH}$ production via Fenton chemistry, enhancing apoptosis. These findings, supported by Ki67, tumor volume, and weight data, confirm the superior efficacy of the heterojunction SDT strategy.¹³

Safety Evaluation

To determine whether the final formulation was sufficiently well tolerated in vivo application, we evaluated systemic toxicity by combining histopathological examination of major organs with serum biochemical analysis after treatment. Under the present dosing regimen, no evident treatment-related abnormalities were observed in the heart, liver, spleen, lungs, or kidneys, and the measured biochemical markers remained within normal ranges, supporting acceptable short-term in vivo tolerability (Figure 5A).

Sections of the heart showed neatly arranged myocardial fibers, characterized by centrally located nuclei and intact tissue architecture, and were free from any signs of inflammation or necrosis. The pathological findings were consistent

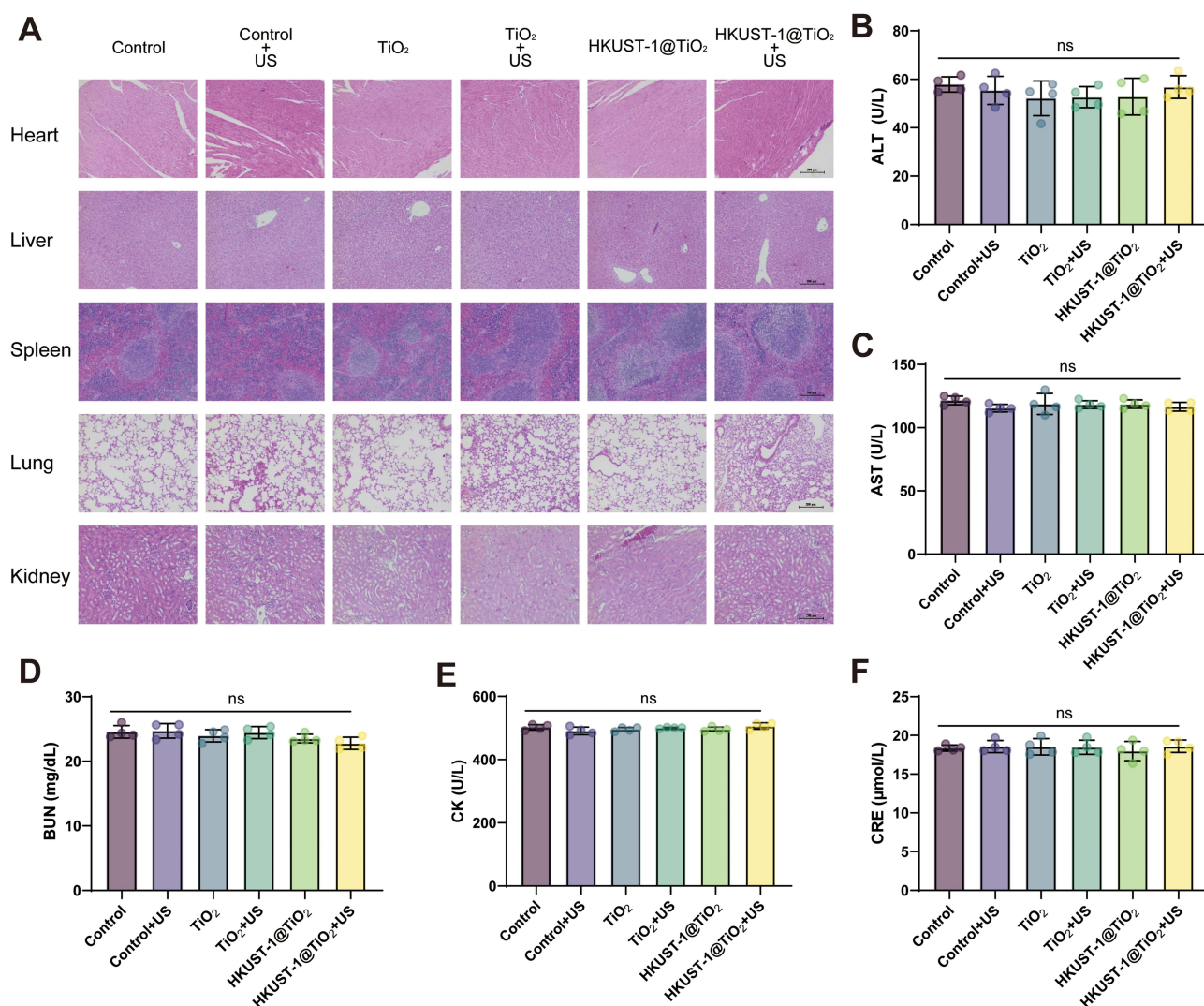


Figure 5 Safety Validation: (A) Hematoxylin and eosin (HE) staining results of heart, liver, spleen, lung, and kidney tissues (scale bar = 200 μm); (B–F) Biochemical marker measurements for ALT, AST, BUN, CK, and CRE in each group. Data are presented as mean ± standard deviation (mean ± SD) (n = 3). “ns” indicates no statistically significant difference.

across all groups, with no toxic reactions observed. Liver tissue maintained normal lobular structure, with hepatocytes arranged in a regular manner, round nuclei, clear blood sinusoids, and no significant inflammation or fatty degeneration. Spleen tissue exhibited normal splenic corpuscle structures, with clear boundaries between the red and white pulp and a uniform distribution of lymphocytes. No lymphocyte proliferation or apoptosis was observed.

Examination of the lung tissue revealed an intact alveolar structure with clear alveolar cavities, thin alveolar walls, and rich capillaries. No alveolar collapse, bleeding, or inflammation was observed in any of the treatment groups. Kidney tissue examination showed that the structure of the nephron was normal, with intact glomerular morphology, clear capillary networks, and orderly arrangement of renal tubules. No mesangial proliferation or epithelial cell shedding was observed.

This study also evaluated the effects of each treatment on liver, kidney, and muscle function through serum biochemical indicators. Regarding liver function, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured. The results indicated that the ALT and AST values in all groups were within the normal physiological range (ALT: 30–120 U/L, AST: 40–160 U/L), with no significant differences between the groups, suggesting that the treatments had no obvious impact on liver function (Figure 5B and C).

For kidney function, the levels of creatinine (CRE) and blood urea nitrogen (BUN) were measured. The results showed that CRE and BUN values in all groups were within the normal range (CRE reference value: 0.4–0.8 mg/dL, BUN reference value:

7–20 mg/dL), with no significant differences between the groups, indicating that the HKUST-1@TiO₂ nanomaterial combined with ultrasound treatment did not cause damage to kidney function (Figure 5D and E).

Regarding muscle injury, creatine kinase (CK) levels were assessed. The results showed that the CK values in all treatment groups were within the normal range (reference value: 24–204 U/L), with no significant differences between the groups, suggesting that none of the treatments caused noticeable muscle tissue damage (Figure 5F).

Taken together, the biosafety results indicate that HKUST-1@TiO₂-mediated sonodynamic treatment was well tolerated under the experimental conditions used in this study. No obvious histological injury was detected in major organs, and no significant abnormalities were found in serum markers associated with liver, kidney, or muscle function. These findings provide preliminary in vivo evidence that the formulation can be administered without overt systemic toxicity at the tested dose, although more comprehensive biosafety studies in normal cells and long-term models will be valuable in future investigations.

Conclusions

This study demonstrates that HKUST-1@TiO₂ heterojunction composites act as effective sonosensitizers for renal cell carcinoma, enhancing ultrasound-triggered ROS generation and inducing apoptosis while suppressing tumor cell proliferation. The results provide mechanistic and preclinical evidence that heterojunction engineering can significantly improve the therapeutic performance of MOF-based sonodynamic therapy. Importantly, the formulation showed favorable short-term tolerability in vivo, supporting its potential for translational development. Nevertheless, this study has limitations: in vitro biocompatibility was assessed only in tumor cells, and long-term systemic toxicity remains to be evaluated. Future work will focus on evaluating normal-cell compatibility, optimizing dosage and ultrasound parameters, and conducting more comprehensive in vivo toxicology studies to facilitate clinical translation.

Overall, our findings highlight a rational strategy for enhancing SDT efficacy and provide a foundation for future development of MOF-semiconductor heterojunction platforms in cancer therapy.

Data Sharing Statement

The data that supports the findings of this study are available from the corresponding author Zeming Weng upon reasonable request.

Ethics Approval

The study (no.: HLK-20250902-003) was approved by the Animal Care and Use Committee of Wuhan Myhalic Biotechnology Co., Ltd.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The authors appreciate financial support from the Medical and Health Research Project of Zhejiang Province (No. 2023KY1035).

Disclosure

The authors report no conflicts of interest in this work.

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