

Platelet Membrane–Camouflaged PB-MnO₂ Nanobiomimetic for Enhanced Photothermal Tumor Ablation

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Abstract

Phototherapies have become a hot topic in the field of cancer therapy owing to their excellent advantages of simplicity, noninvasiveness, localized treatment, and high therapeutic efficacy. In this regard, highly efficient photosensitive materials have been extensively investigated in tumor phototherapy. Herein, we developed a photo-activated nanobiomimetic for promoting cancer therapy. Such a nanobiomimetic contains a Prussian blue / magnesium oxide nanoparticle core (PB-MnO₂) encapsulated with a platelet membrane shell (PLT). The fabricated (PLT)PB-MnO₂ nanobiomimetics exhibited excellent photothermal conversion efficiency, tumor-ablation ability, and biocompatibility. *In vitro* studies demonstrated significant photothermal cytotoxicity against cancer cells upon near-infrared irradiation, while *in vivo* experiments in tumor-bearing models showed remarkable tumor growth suppression with minimal systemic toxicity. These results highlighted the synergistic potential of biomimetic platelet membrane coating and photothermal therapy for effective cancer treatment.

1. Introduction

Breast cancer, the most common cancer among women worldwide with a high mortality rate, poses a growing global threat to people's lives. If this trend continues, reports predict over 3 million cases and approximately 1 million deaths annually by 2040¹. Phototherapy has been used for thousands of years to treat conditions such as skin cancer, vitiligo, and psoriasis. In the early 20th century, laser applications in surgery marked a significant advancement in phototherapy. However, laser therapy for cancer has limitations, including a lack of selectivity for target cells, low penetration depth, and the need for high-power density. To improve effectiveness, targeting light to specific tissues and using photosensitizing agents can enhance therapeutic benefits, as these agents selectively accumulate in the target area and absorb light². Traditional cancer treatments often fail due to unwanted side effects, while the complexity of the tumor microenvironment (TME), risk of metastasis, and cancer recurrence further limit therapeutic outcomes³. Over the past decade, considerable developments in nanotechnology have made nanomedicine a promising solution to minimize these drawbacks. Nanotechnology enables the development of multifunctional platforms capable of bypassing physiological barriers, improving tumor targeting, and introducing specific therapeutic effects^{4,5}. Light-sensitive nano-agents are materials with anti-tumor effects that induce endogenous toxic reactions in tumor cells or the TME, or generate heat for tumor ablation under external laser irradiation⁶. Photo-based techniques such as photothermal therapy (PTT) take advantage of the capacity of these nano-agents to convert absorbed light, especially in the near-infrared (NIR) spectrum, into localized heating, resulting in cancer cell destruction and reduced damage to healthy tissues⁷⁻⁹. The NIR window is particularly beneficial because of its deep tissue penetration and low absorption by biological tissues, making it ideal for *in vivo* applications^{8,10}. In addition to the photothermal effect, some nanoplatforms are also able to activate chemodynamic therapy (CDT), which results in the generation of reactive oxygen species (ROS) and oxidative damage in tumor tissues¹¹.

Despite the great potential of photo-based therapies, its clinical translation remains challenged by the poor stability of nanomaterials in biological environments, limited tumor accumulation, and rapid immune clearance^{12–14}. Novel biomimetic coating strategies aim to improve stability during systemic blood circulation, enhance tumor targeting, and provide specific surface characteristics compared with conventional coatings such as PEGylated nanoparticles (NPs)^{15,16}. The concept of biomimetic coating was first reported in 2011 by Hu et al.¹⁷ who coated polymeric nanocarriers with red blood cell membrane vesicles for drug delivery applications. This study led to considerable progress in the utilization of cell membranes as promising biomaterials for coating nano-templates to fabricate therapeutic nano-biomimetic systems¹⁸. The cell membrane is an integral structural and functional component of a cell that, after isolation, can be used as a biomimetic coating. Camouflaging NPs with cell membranes enhances bio interfacing features. Accordingly, biomimetic nano-systems with advantages such as biocompatibility, stealth features, and active targeting have been developed through various physical and chemical techniques^{19–21}. Since their discovery in 1842, platelets, which are derived from megakaryocytes, have attracted attention in biomedical applications due to their intrinsic ability to interact with tumor cells and evade the immune system. Platelet membrane-coated NPs are particularly promising for anti-tumor nanoplatforms because they actively target tumors, enhance NP accumulation at tumor sites, and minimize damage to surrounding healthy cells^{22,23}. Furthermore, platelet membrane-coated NPs overcome limitations of conventional coatings such as polyethylene glycol (PEG) and dextran, while enhancing tumor penetration and improving drug delivery through membrane-associated molecular signaling pathways^{24,25}. Moreover, combining PTT with CDT can further enhance anti-tumor efficacy through synergistic thermal ablation and ROS-mediated oxidative stress²⁶.

Here, (PLT)PB-MnO₂ nanobiomimetics were investigated for synergistic PTT/CDT in a breast cancer model *in vivo*. Prussian blue (PB), an FDA-approved particle, has attracted considerable interest in cancer phototherapy due to its strong NIR absorption, excellent photothermal conversion ability, and suitability for surface modification in complex biological environments^{27,28}.

In addition, MnO₂ was incorporated into PB NPs to further enhance PTT/CDT synergistic therapy efficacy because its multifunctional properties facilitate Fenton-like reactions and ROS generation^{29–31}.

Camouflaging PB-MnO₂ NPs with platelet membranes represents a promising approach to enhance PTT/CDT efficacy for cancer. Therefore, combining the photothermal properties of PB-MnO₂ with the tumor-targeting and immune-escape capabilities of platelet membranes led to the fabrication of a nanobiomimetic platform to overcome the limitations of conventional PTT³². Utilizing a breast cancer animal model, this study investigated the biomimetic and PTT/CDT synergistic therapy potential of (PLT)PB-MnO₂ (Fig. 1).

2. Materials and Methods

HCL (Hydrochloric acid), polyvinylpyrrolidone (PVP), K_3FeCN_6 , and $KMnO_2$ were purchased from Sigma-Aldrich (Germany). Platelet cell membrane was obtained from blood donation center in Zanjan (Iran). Bag dialysis was purchased from Merck (Germany). Cell culture reagents including DMEM high-glucose (DMEM), fetal bovine serum (FBS), penicillin/streptomycin (pen & strep) and the [4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were all purchased from the BIO-IDEA Co (Iran). The 4T1 cell line was prepared from the cell bank of the Pasteur Institute (Tehran, Iran) which was cultured in DMEM medium enriched with 10–12% of FBS and pen & strep (100U/mL and 100 μ g/mL). Further, the cell culture containing 4T1 cells was incubated at 37°C (under CO_2 atmosphere) for *in vitro* and *in vivo* experiments. Female balb/C mice (19 ± 5 gr weaning, 4–6 weeks old) were purchased from the Pasteur Institute (Iran) and animals were kept under care standard condition (12 h of light/dark cycles, 50–60% of humidity, 21–25°C) with free access to adequate water and food. All *in vivo* tests were performed under approval animal care and treatment protocol by Zanjan University of Medical Sciences (ZUMS) university with ethical code of IR.ZUMS.AEC.1402.009.

2.1. Preparation of (PLT) PB- MnO_2 Nanobiomimetic

(PLT) PB- MnO_2 nanomimetics fabrication consists of three distinct steps: (1) Isolation of platelet membranes, (2) Preparation of the PB- MnO_2 core, and (3) Preparation of platelet membrane-coated PB- MnO_2 nanoparticles.

2.1. Isolation of Platelet Membranes

Platelet membranes were isolated from fresh whole blood using a differential centrifugation strategy adapted from established protocols. Briefly, blood was collected into sterile tubes containing 3.2% (w/v) sodium citrate as an anticoagulant and subjected to an initial low-speed centrifugation at $200 \times g$ for 12 min at room temperature to pellet erythrocytes and leukocytes. The supernatant, constituting platelet-rich plasma (PRP), was carefully aspirated and transferred to a fresh tube, followed by a second centrifugation at $800 \times g$ for 15 min to pellet the platelets. The platelet pellet was washed twice with Tyrode's buffer (pH 7.4) supplemented containing 5 mM glucose and freshly added BSA (3 mg/mL) to inhibit premature platelet activation and preserved with a protease inhibitor cocktail³³.

Membrane extraction was performed using the glycerol-lysis technique, followed by osmotic lysis, as previously described³⁴. The resulting membrane-enriched pellet was collected by ultracentrifugation at $100,000 \times g$ for 1 h at 4°C. To remove intracellular contaminants, the crude membrane pellet was further purified using sucrose density gradient ultracentrifugation. The purified platelet membranes were washed, pelleted, resuspended in sterile PBS, and either utilized immediately or stored at $-80^\circ C$ until further use.

2.1.2. Preparation of PB- MnO_2 NPs

PB-MnO₂ NPs were prepared through hydrothermal method based on the previous reports with some modifications to optimize the process³⁵. A typical procedure for the preparation of PB-NPs is described briefly as follows; first, 1.5 g PVP was dissolved in deionized water that contained 129 µL concentrated hydrochloric acid (HCl, 36% w/v) at room temperature. Next, 66 mg K₃[Fe(CN)₆] and 52 mg KMnO₄ were added successively to the PVP mixture. After the quite dissolution of the materials, the resulting brown-black mixture was transferred to an oil bath that had been heated to a temperature 86°C. The reaction was sustained for 24h. To collect the PB-NPs, the resulting mixture was centrifuged at 16000 rpm for 30 minutes. The collected PB-MnO₂ NPs were washed by deionized water and ethanol (96%) for three times to remove the unreacted materials. The purified PB-NPs were re-suspended in deionized water and stored at a 4°C temperature for next tests.

2.1.3. Preparation of Platelet Membrane-Coated PB-MnO₂ Nanoparticles

To fabricate (PLT)PB-MnO₂ biomimetic, the platelet membranes were coated on the prepared PB NPs, through the mechanical procedure. Briefly, platelet-derived membranes (125 µL) were added to 500 µL PB-MnO₂ cores (1 mg/mL) and 375 µL deionized water. The mixture was vortexed and sonicated to facilitate efficient membrane core fusion while preserving the integrity of membrane proteins. The final mixture was transferred into a dialysis membrane (Molecular Weight Cut-Off, 12–14 kD), which was subsequently immersed in a beaker containing distilled water and subjected to slow rotation. Following a period of 24 hours, the contents of the dialysis bag were carefully emptied into a sterile tube and stored at 4°C for further analysis and experimentations.

2.2. Characterization of PB-MnO₂ NPs and (PLT)PB-MnO₂ Nanobiomimetics

2.2.1 Western Blot Analysis of Platelet and Platelet-Membrane Coated MnO₂ Nanoparticles

To evaluate the composition of the platelet membrane coating on the (PLT)PB-MnO₂ nanoparticles, Western blot analysis was performed using antibodies against β-actin and P-selectin (platelet surface membrane marker). Protein lysates from PLT and (PLT)PB-MnO₂ samples in equal amounts (20–50 µg) were separated by SDS-PAGE on 10% polyacrylamide gels. Proteins were transferred onto PVDF membranes at 120 V for 90 min. Membranes were blocked with 3% BSA in TBS-T, then incubated overnight at 4°C with primary antibodies against P-Selectin (mouse monoclonal CTB201, sc-8419, 1:200; Santa Cruz) and β-Actin (mouse monoclonal 2A3, sc-517582, 1:300; Santa Cruz) as a loading control; negative control membranes were incubated without primary antibody to assess non-specific binding. After washing, membranes were incubated with HRP-conjugated anti-mouse secondary antibody m-IgGkBP-HRP: sc-516102 (1:1000 : Santa Cruz).

2.2.2 Physicochemical Characterization of Platelet-Membrane Coated MnO₂ Nanoparticles

The hydrodynamic diameters and zeta-potential of prepared PB-MnO₂ and (PLT)PB-MnO₂ were measured with dynamic light scattering instrument (DLS, Malvern, Germany). Further, the Fourier-transform infrared spectroscopy (FTIR, Bruker, Germany) and energy dispersive X Ray analysis (EDX) analysis were conducted for the evaluation of the specific functional groups and the major elemental composition of prepared samples to confirm PB-MnO₂ and (PLT)PB-MnO₂ formation. The FTIR spectra of samples were measured in the 4000 – 400 cm⁻¹ wavelength region at room temperature. The (PLT)PB-MnO₂ nanomimetics were characterized for morphological and microstructural properties by using Transmission electron microscopy (TEM, Philips, USA) and AFM (JFK Nanowizard, Germany) techniques.

2.3. Photothermal Properties Evaluation

To evaluate the photostability of PB-MnO₂ and (PLT)PB-MnO₂, *in vitro* photothermal study was performed using portable near-infrared laser (NIR, model 3J-IR-808 nm, China). For this aim, 1 mL aqueous suspension of samples was exposed by 4 cycles of NIR laser irradiation (808 nm) on-off where laser on for 5 min per cycle. In all cases, the distance between the irradiated samples and laser output was kept constant at 2 cm which in, the hot area temperature was screened with thermovision camera (WINTACT WT3320, China) continuously at the power output of 1.5W/cm² at the temperature range of 28–70°C.

Further, to estimate the photothermal conversion efficiency, 1 mL aqueous suspensions containing (PLT)PB-MnO₂ nanobiomimetics in various concentrations (100, 200 and 300 µg/mL) were illuminated using an 808 nm laser (output power of 0.5 and 1.5W/cm²) at a distance of 2 cm. Under laser illumination, the temperature of any sample was recorded every 1 min using thermovision camera.

2.4. Cell Viability Assay

To evaluate the *in vitro* cytotoxicity of PLT, PB-MnO₂, and (PLT)PB-MnO₂ against 4T1 murine breast carcinoma cells, an MTT assay was performed. 4T1 cells were seeded in 96-well plates and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Upon reaching approximately 90% confluency, the culture medium was replaced with 100 µL of PLT, PB-MnO₂, and (PLT)PB-MnO₂ at various concentrations (1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) and incubated for 24 and 48 hours at 37°C. Following incubation, 100 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well, and the plates were further incubated for 4 hours at 37°C to form purple formazan crystals. Afterward, the produced formazan crystals were dissolved in 150 µL of dimethyl sulfoxide (DMSO) and the absorbance was measured using an ELISA microplate reader (Tecan, USA) at 570 nm. The cell viability was calculated by the following formula:

$$Cell\ Viability\ (\%) = \frac{As - An}{Ap - An} \times 100$$

where A_s is the absorbance of treated samples, and A_n and A_p are the absorbance of negative and positive controls, respectively.

2.5. Apoptosis /Necrosis Evaluation by Flowcytometry Assays

For this assay, 0.8×10^4 4T1 cells/well were seeded into each well. Confluent cells were incubated with 250 $\mu\text{g/mL}$ of PB-MnO₂ and (PLT)PB-MnO₂ with or without NIR irradiation (1.5W/cm², 8 min). After a 3-hour incubation period, the media were removed, cells were washed with phosphate buffered saline (PBS), and harvested carefully. Treated cells were washed with PBS and double stained with 3 μL annexin-V-FITC and 1.5 μL propidium iodide (PI) solution in 500 μL of binding buffer provided by the kit (Rosh Azma apoptosis detection kit). The Annexin V-FITC and PI was determined in the FL-1 channel the FL-3 channel using flow cytometer BD Accuri C6 plus and the FlowJo software (version 7.6.1). All experiments were performed in triplicate.

2.6. Intracellular ROS Detection

The intracellular ROS detection was performed with ROS Fluorometric assay Kit (Kiazist, Iran). In brief, 0.8×10^4 cells were seeded into each well of a black 96-well plate. Confluent cells were incubated with 150 μL DCFDA reagent (20 μM) in the dark for 45 min before treatment. Then, cells were treated with 250 $\mu\text{g/mL}$ of PBMnO₂ and (PLT)PB-MnO₂ with or without NIR irradiation (1.5W/cm², 8 min). Afterward, the fluorescence intensity of ROS induced oxidized DCFDA in cells was measured in Fluorescence microplate reader in Ex/Em = 485 nm/535 nm.

2.7. In Vivo Anticancer Performance of (PLT)PB-MnO₂

To establish a mice breast cancer model, 1×10^3 4T1 cells suspended in 500 μL of PBS were injected the left lateral lobes of the livers. After tumor growth, the mice were randomly divided into 3 groups ($n = 5$, control, (PLT)PB-MnO₂(-NIR) and (PLT)PB-MnO₂(+ NIR) and treated groups intratumorally injected with 100 μL PB-MnO₂ and (PLT)PB-MnO₂ (250 $\mu\text{g/mL}$).

Two hours post injection, the tumors in the (PLT)PB-MnO₂(+ NIR) group exposed to 808 nm laser at 1W/cm² for 8 min to reach 47°C temperature. Tumor size and body weight were screened at 1, 4, and 14 days. At day 14, the experiment was finished, all animals were euthanized by mixture of Ketamine (40–80 mg/kg) and xylazine (5–10 mg/kg) administered as Intraperitoneal injection and sacrificed. The following, the tumors and five vital organs (kidney, liver, heart, spleen and lung) were obtained. To evaluate cancer efficacy and systemic toxicity of prepared nanomimetics, tumor volumes were measured by vernier caliper, and the organs pretreated with 10% buffered formalin were examined for

Hematoxylin/Eosin (H&E) staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assessments (Olympus Bx51, Japan).

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0.2 software at least 3 independent replications for any test. All data are reported as mean $\pm$ SD. The statistical significance was evaluated at the probabilities of $p \leq 0.05$ via ANOVA analysis.

3. Results and Discussion

3.1. Characterization of PB-MnO₂ NPs and (PLT)PB-MnO₂ Nanobiomimetics

Specific interaction between functional groups of prepared NPs was investigated via FTIR spectroscopy (Fig. 2A) ³⁶. In the spectrum of both PB-MnO₂ and (PLT)PB-MnO₂ a wide absorption band was observed at 2700–3500 cm⁻¹ region because of the –OH stretching vibration of surface- adsorbed water molecules in these structures. The observed bands at 1800–2400 cm⁻¹ range were also attributed to the Fe^{II-III}-C $\equiv$ N network of PB. The absorption bands at 2081, 1429 cm⁻¹ were due to the -C $\equiv$ N stretching vibration of K₃FeCN₆, and –OH and HOH bending mode of hydroxylated and hydrated form of PB, respectively. The absorption bands at 923–1224 cm⁻¹ range, and 646 cm⁻¹ appeared due to the vibration mode of Fe-C and NCO groups of cyano complex. Stretching mode for C–H and C–H₂ asymmetric and symmetric vibrations at 2817–3024 cm⁻¹ range of PVP were also visible in the FTIR spectrum of the PB-MnO₂ and (PLT)PB-MnO₂. The comparison of PB-MnO₂ and (PLT)PB-MnO₂ FTIR spectra indicated the emergence of similar absorption bands, confirming successful synthesis of these nanostructures ³⁷. The DLS analysis was consistent with zeta potential results, after coating the PB-MnO₂ NPs with PLT, an increased hydrodynamic diameter around 95 nm was observed (Fig. 2B). Moreover, coating the PB-MnO₂ NPs increased the surface zeta potential of NPs from – 21.2 mV to -11.8 mV, suggesting the successful camouflaging of negative charge of NPs by the more negative charge of PLT membrane (Fig. 2C). TEM and AFM tools were used to visualize the morphological properties and topography of samples. Compared with the PB-MnO₂ NPs that are not platelet membrane-coated, (PLT)PB-MnO₂ nanobiomimetics indicate a core–shell structure, and the thickness shell of the platelet cell membrane. Further, the AFM imaging exhibited that the (PLT)PB-MnO₂ were well dispersed with a homogeneously shape of NPs (Fig. 2D, E). Herein, we reported that the particle size and the zeta potential of (PLT)PB-MnO₂ was larger than that of uncoated PB-MnO₂, confirmed by DLS and TEM analyses. Element mapping analysis or EDX also displayed the homogeneous distribution of the C, N, O, Mn, Fe, P and S elements in the structure of (PLT)PB-MnO₂ that revealed the prosperous fabrication of coated NPs (Fig. 2F). Furthermore, the presence of the N, P and S in the EDX pattern can be due to the glycoprotein structure of platelet membrane. In short, successful platelet cell membrane coating and

structural properties could be confirmed through FTIR, DLS, TEM, AFM and EDX. For example, the FTIR pattern of the characteristic peaks of the functional groups of the raw materials and protein structure of the platelet cell membrane in the PB-MnO₂ NPs and (PLT)PB-MnO₂ exhibit successful attachment of the platelet membrane and formation of these nanostructures. In coated NPs, the presence of platelet cell membrane in the surface of the NPs could increase the particle size and surface charge of nanobiomimetics, as confirmed by TEM and DLS results that might be due to the activating of the hydrolyze reactions particularly in the amide groups of glycoproteins of platelet membrane. Since the cell membrane commonly consisted of phospholipid bilayers, the presence of specific elements, such as P, S, C and O, in the elemental composition analyses of (PLT)PB-MnO₂, proving that the platelet cell membrane was successfully coated on the NPs. Retaining the surface proteins of cell membranes is critical for ensuring that coated NP functions effectively. So, the presence of functional membrane proteins was confirmed by Western blotting. Western blot analysis provides a useful tool for distinguishing target protein markers in the samples. P-Selectin, a characteristic protein expressed by platelets, was detected in both native platelet lysates and (PLT)PB-MnO₂ nanobiomimetics, while uncoated PB-MnO₂ NPs showed no detectable expression (Fig. 3A). β -actin was used as a loading control to ensure equal protein loading. The presence of P-Selectin in the coated nanoparticles, with a similar pattern to the source platelet membrane, confirms the successful preservation and transfer of functional platelet membrane proteins during the extraction and coating process. Additional UV–Vis absorption characterization data of the prepared nanoparticles are provided in the Supporting Information (**Figure S1**).

The *in vitro* photothermal stability of the PB-MnO₂ NPs and (PLT)PB-MnO₂ nanobiomimetic was evaluated under NIR laser 808 nm illumination for four heating/cooling cycles. As shown in Fig. 3B, no meaningful differences were observed during the on-off four circles of NIR illumination on photothermal ability of the PB-MnO₂ NPs and (PLT)PB-MnO₂ nanobiomimetic as well as favorable thermal stability. This perfect result showed that (PLT)PB-MnO₂ nanobiomimetic can be used as an excellent photosensitive agent for enhanced photothermal tumor therapy. The full-length Western blot images, together with additional photothermal characterization data of fabricated nanoparticles, have been provided in the Supporting Information (**Figures S2 and S3**).

3.2. Cell Toxicity/ ROS and Apoptosis/Necrosis Evaluation

The *in vitro* biocompatibility of PB, PB-MnO₂, and (PLT)PB-MnO₂ nanobiomimetics was assessed against HEK-293 and NIH-3T3 non-tumorigenic cell lines using the MTT assay after 48 and 72 hours of incubation. As shown in Fig. 4A-B, at concentrations up to 500 μ g/mL, all three nanobiomimetics did not induce significant cytotoxicity; rather, they increased cell viability to more than 100% relative to the control in both cell lines. This proliferative effect was most notable for PB-MnO₂ after 72 hours of incubation confirmed with earlier reports demonstrating the intrinsic catalase activity and catalase-mimetic activity of MnO₂ NPs at moderate concentrations, thereby reducing intracellular oxidative stress. At the highest tested concentration (1000 μ g/mL), the (PLT)PB-MnO₂ nanobiomimetics showed

slightly lower viability compared to uncoated particles, possibly due to mild activation of platelet receptors (e.g., GPVI or CLEC-2) expressed on fibroblasts and HEK cells. In contrast, striking differences were observed in the murine breast cancer cell line 4T1. In the absence of laser irradiation (48 hours), PB and PB-MnO₂ induced significant concentration-dependent cytotoxicity, especially in PB-MnO₂, in agreement with the known pro-oxidant behavior of both PB and MnO₂ in the acidic and ROS-rich tumor microenvironment. Additionally, (PLT)PB-MnO₂ nanobiomimetics significantly promoted 4T1 cell proliferation up to 500 µg/mL (up to 100% of control). This phenomenon has been repeatedly documented for platelet membrane-coated nanoparticles and is attributed to strong homotypic binding between platelet P-selectin and CD44 overexpressed on 4T1 cells, resulting in enhanced nanoparticle adhesion and transient pro-survival signaling. Upon near-infrared laser irradiation (808 nm, 1.5 W/cm², 5 min), only the platelet membrane-coated nanoparticles exhibited dramatic phototoxicity, with cell viability dropping to < 15% at 250 µg/mL and higher concentrations. No significant phototoxic effect was observed for PB or PB-MnO₂ under irradiation conditions. The selective phototoxicity of (PLT)PB-MnO₂ nanobiomimetics is explained by substantially higher cellular uptake mediated by platelet membrane camouflage, enabling efficient intracellular delivery of both photothermal MnO₂ and photodynamic PB moieties, leading to synergistic hyperthermia and ROS generation exclusively in cancer cells. These results collectively demonstrate that platelet membrane coating confers excellent biocompatibility in normal cells while dramatically enhancing tumor-specific accumulation and on-demand phototoxicity, making (PLT)PB-MnO₂ a highly promising theranostic platform for photothermal/photodynamic therapy of breast cancer.

As shown in Fig. 4D, the ROS production of the NIR treated PB-MnO₂ and (PLT)PB-MnO₂ groups were significantly higher than the same group without NIR and control. These results can be explained by a dual mechanism of the photothermal and chemodynamic effects of the PB-MnO₂ NPs under NIR laser illumination and platelet membrane coating approaches. It was found that the Mn²⁺ ions can catalyze the Fenton-like reactions, resulting in the formation of higher hydroxyl radicals (•OH) and the increased heat-induced ROS levels in the NIR treated NPs. Further, the generation of ROS in the absence of laser irradiation, by the PB-MnO₂ NPs, by which the catalytic activity of Fenton-like alone could be proficiently affected instead of being NIR absorption^{38,39}. Moreover, the platelet membrane coating provides a biomimetic barrier, which decreases the ROS production by limiting energy/electron transfer, light absorption and access of substrates (e.g., H₂O₂ or O₂) to the core of NPs. This causes a physical distance effect, scatters NIR light, and possibly allows ROS scavenging through membrane elements, leading to a reduction of ROS even compared to NIR and non-irradiated uncoated groups. Non-irradiated coated NPs exhibit the least ROS because of the reduced catalytic activity without the photothermal activation^{40–42}. For apoptosis, all treatment groups induced early apoptosis in the range of 35–50%. The (PLT)PB-MnO₂(+ NIR) group showed the early apoptosis level of 51%, significantly higher than other groups. Moreover, necrosis and late apoptosis rates of all groups were in the range of 1–2% and 16–28%, respectively. The (PLT)PB-MnO₂(+ NIR)-mediated higher levels of early apoptosis, resulting in synergistic hyperthermia-induced mitochondrial damage and ROS-generated oxidative stress, which this

may be beneficial for triggering caspase-dependent pathways. Additionally, the very low necrosis rate indicated that (PLT)PB-MnO₂ nonbiomimetic might be used as a biocompatible photomaterial for PTT of cancer with controlled cell death and minimized inflammatory side effects (Fig. 4E) ^{43,44}.

3.3. *In Vivo* Studies

To evaluate photothermal effects of PB-MnO₂ NPs and (PLT)PB-MnO₂ nano biomimetics on tumor suppression, *in vivo* photothermal studies were performed in mice bearing 4T1 tumor model. Animals in the control and (PLT)PB-MnO₂ treatment group were subjected to NIR laser irradiation. A substantial photothermal effect was observed in (PLT)PB-MnO₂-treated animals upon NIR laser exposure (1.5 W/cm², 5 min), with the local temperature increasing by roughly 18°C over the room temperature baseline (25–27°C). 27°C), whereas the control group presented only a modest increase of 5°C. Further, PTT promotes cancer cell death while may cause indirect damage to normal cells at elevated temperatures, so PTT with moderate temperature ($\leq 45^{\circ}\text{C}$) has been investigated. As shown in Fig. 5A-D, the control group showed a significant increase in tumor growth, confirmed by digital photographs of tumor area and evaluation of relative volume of tumor remaining over the screening time-points of 0, 1, 4 and 14 days. At 14 days post-administration, tumor volumes in the PB-MnO₂ and (PLT)PB-MnO₂ groups were significantly reduced to $200 \pm 2 \text{ mm}^3$ and $70 \pm 5 \text{ mm}^3$, respectively, versus $400 \pm 2 \text{ mm}^3$ in the control group. The proposed mechanism of action PTT relies on an induction of thermal apoptosis via heat produced from (PLT)PB-MnO₂ nanobiomimetics upon irradiation with NIR laser that allows highly efficient killing of 4T1 tumor cells. The body weights of mice were also evaluated, showing no significant statistical differences in weight of the control and treated groups (Fig. 5E).

3.4. Histopathology and Immunohistochemical Evaluation

To further evaluate the effect of (PLT)PB-MnO₂-activated PTT on breast cancer calls, mice were euthanized on day 14, and tumor tissue from the lesion site was excised for histological analysis. H&E staining revealed that the thickness of the tumor section in the (PLT)PB-MnO₂-treated group was thinner than in the control groups (including untreated tumors and those treated with non-coated PB-MnO₂ NPs), which was consistent with the result presented in Fig. 6A. As shown in the H&E staining images, the tumor tissue in the untreated and non-coated PB-MnO₂ groups had dense cellularity with minimal signs of necrosis. Red blood cells (RBCs) that flowed out from ruptured vessels were observed at some sites. Similarly, although the number of necrotic regions in other control groups was small, a certain degree of viable tumor cells was observed. In contrast, extensive regions of necrosis with pyknotic nuclei, cellular shrinkage, and loss of cellular integrity indicative of effective photothermal-induced tumor cell ablation can be observed in the (PLT)PB-MnO₂ groups tumor tissue due to its excellent photothermal ablation properties. The improved tumor accumulation of (PLT)PB-MnO₂, which was attributed to the biomimetic properties of the PLT membrane, probably contributed to the localized and efficient heat generation under NIR laser irradiation, resulting in marked tumor tissue damage. Furthermore, no significant inflammatory infiltrates and damage of the surrounding healthy tissue were observed in the treated

group, suggesting high biocompatibility and specificity of the (PLT)PB-MnO₂ nanosystem for targeted PT. In short, histopathology images proved that the (PLT)PB-MnO₂ group demonstrate the highest extent of tumor necrosis, suggesting highly enhanced therapeutic outcome.

The TUNEL assay, which detects apoptosis, showed that the (PLT)PB-MnO₂ (+ NIR) group effectively induced programmed cell death in cancer cells compared to the control and (PLT)PB-MnO₂ (-NIR) treated. It is hypothesized that the localized hyperthermia generated by PTT of (PLT)PB-MnO₂ probably caused damage to cellular membranes, proteins, and DNA, triggering apoptotic pathways. Additionally, MnO₂ may induce to apoptosis by generating ROS in the TME, thereby enhancing oxidative stress and cell death. Besides, the Ki67 protein immunohistochemical staining of the tumor tissue, a marker of cell proliferation, exhibited a obvious reduction in cancer cell proliferation in its proliferative cells in the (PLT)PB-MnO₂ treated group exposed to NIR illumination compared with others (Fig. 6B). This suggests that the (PLT)PB-MnO₂ nanobiomimetics under NIR irradiation can effectively inhibit tumor cell growth, resulting from the direct thermal destruction of cancer cells or indirect effects such as disruption of the cell cycle caused by heat stress or ROS production of nanobiomimetics. The significant results of the TUNEL and Ki67 assays can be attributed to the synergistic effects of PB NPs photothermal properties, ROS generation through MnO₂ photocatalytic potential, and the platelet membrane's role in enhancing tumor targeting and biocompatibility. It seems that the PLT membrane coating probably enhanced the nanoparticle ability to escape immune surveillance and achieve homotypic targeting. Such nanobiomimetics, by mimicking platelet-specific protein interactions (e.g., CD47), may reduce phagocytosis by macrophages, thereby increasing nanoparticle accumulation in tumor tissue. In short, these combined mechanisms induce effective induction of apoptosis and suppression of tumor cell proliferation.

4. Conclusion

The conventional breast cancer treatments mainly include surgery, chemotherapy, radiotherapy, or hormonal therapy. However, repeated applications of chemotherapy may lead to drug resistance in tumor cells. In addition, the treatment efficiency of common methods can be potentially reduced due to the TME barriers and non-specific systemic distribution on-specific systemic distribution of therapeutic agents. We hypothesize that the treatment with photothermal-enabled NPs, regarded as a minimally invasive and tumor-targeted approach, can effectively ablate breast cancer lesions. Nanoplatforms serving as the delivery vehicle for photothermal agents or treatment substances to address tumor growth have been studied. In the current investigation, we introduce photo-activated nanobiomimetic material to PTT that not only respond to NIR laser irradiation for efficient photothermal ablation of cancer cells but also are able to regulate the TME through promote ROS production as well as homotypic targeting^{27,45,46}. In summary, we have developed a biomimetic and effective nanoplatform to treat breast cancer through the combination of photocatalytic nanomaterials with biological membranes. The camouflage of PB/MnO₂ NPs with PLT for PTT offers several advantages, including enhanced photothermal efficiency, oxygen generation in the TME, biocompatibility, and biodegradability. MnO₂ NPs

can enhance heat production in the photothermal technique due to their catalytic properties and active reactivity in the acidic TEM. While PB, as an FDA-approved dye, acts as an NIR light-absorbing material, this combination improves the light-to-heat conversion efficiency, resulting in boosted eradication of cancer cells. Further, MnO_2 NPs as a material, which can regulate the oxygen generation through reacting with hydrogen peroxide (H_2O_2) in the TME, addressing tumor hypoxia and increasing PTT efficiency via facilitating the ROS production. Also, MnO_2 NPs degrade into manganese ions (Mn^{2+}) in the acidic TEM, which are quickly removed from the biological system, providing high biocompatibility. In short, we believe that the development such PTT-enabled nanobiomimetics not only can be utilized to treat breast cancer efficiently but also may be investigated to manage other cancers.

Declarations

Funding Declaration

This work was supported by the Zanjan University of Medical Sciences (Grant No. A-12-1143-8 and A-12-2038-2).

Clinical trial number:

not applicable.

Ethics approval

All animal experiments were conducted in accordance with institutional guidelines and approved by the Zanjan University of Medical Sciences (ZUMS) ethics committee (IR.ZUMS.AEC.1402.009).

Consent to participate

Not applicable. This study does not involve human participants.

Consent for publication

Not applicable. This study does not contain data from human participants.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Author Contribution

Anahita Barooj Kiakalaiy: Formal analysis ,Visualization, InvestigationLaleh Salarilak: Formal analysis ,Visualization, InvestigationShayesteh Bochari: Formal analysis ,Visualization, InvestigationAli Kalantari-Hesari: Formal analysis ,Visualization, InvestigationAbdolreza Esmaeilzadeh: Writing – review & editing, Methodology Sophia Taheri: Formal analysis ,Visualization, InvestigationKiana Nazari Nasi: Software Faezeh Almasi: Writing – review & editing,Mitra Khalili: Writing – review & editing, Methodology Fatemeh Hakimi: Conceptualization, Writing – review & editing, Writing – original draft , VisualizationAziz Maleki: Conceptualization, Project administration, Validation, Writing – review & editing, Supervision, Funding acquisition , Methodology

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Figures

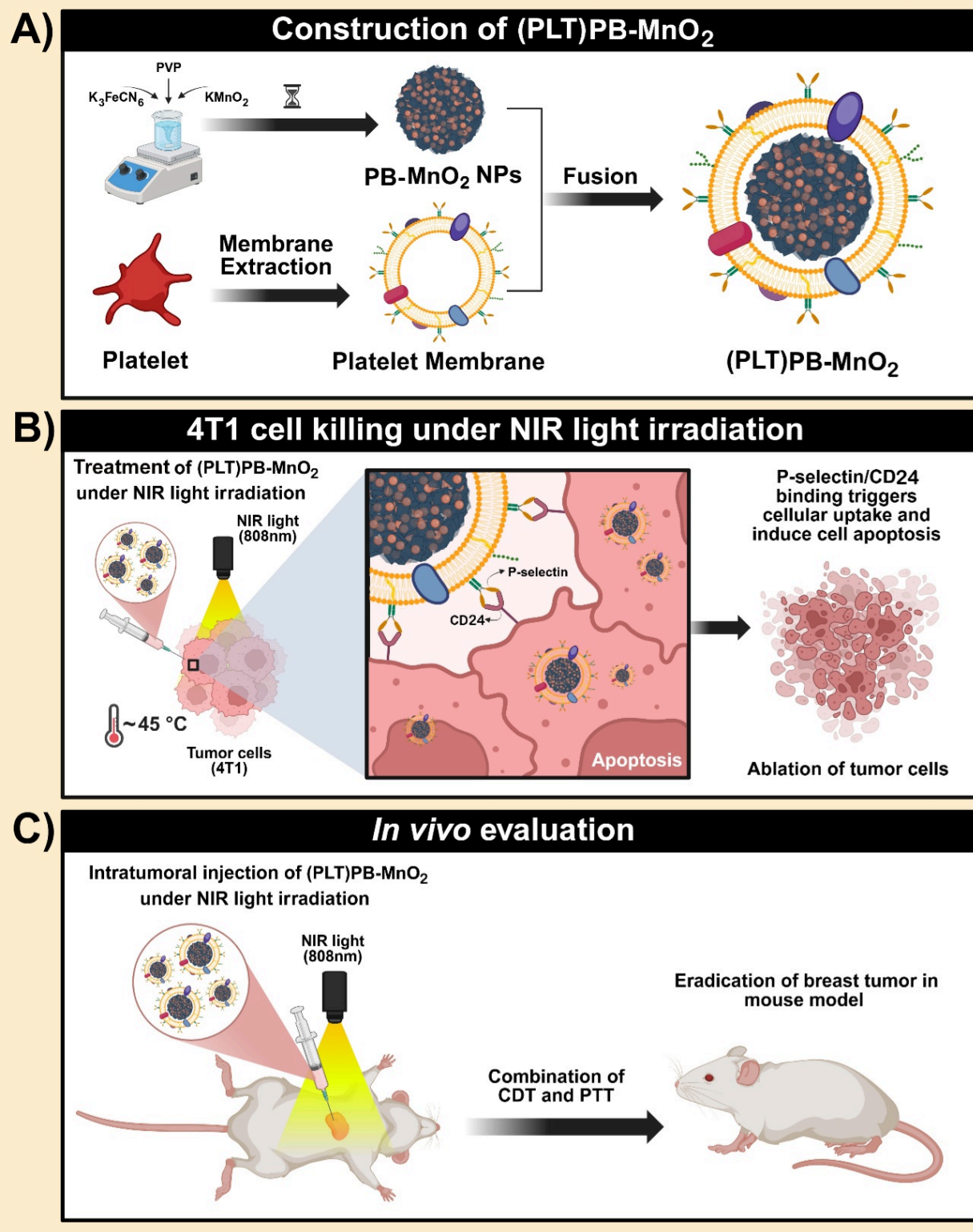


Figure 1

Schematic illustration of the fabrication and therapeutic mechanism of (PLT)PB-MnO₂ nanobiomimetics for PTT/CDT of cancer.

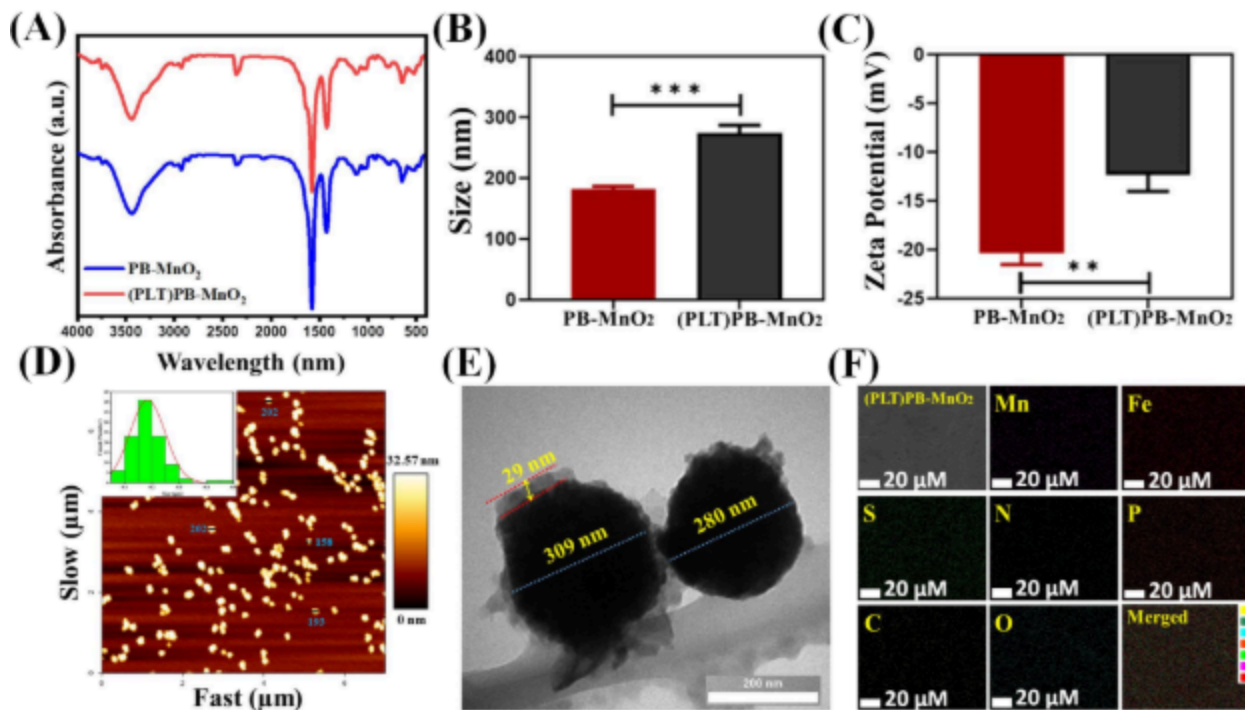


Figure 2

Physicochemical characterization of (PLT)PB-MnO₂ nanobiomimetics. (A) FTIR spectra, (B) Hydrodynamic size distribution, (C) Zeta potential analysis, (D) AFM image, (E) TEM image, and (F) EDX elemental mapping.

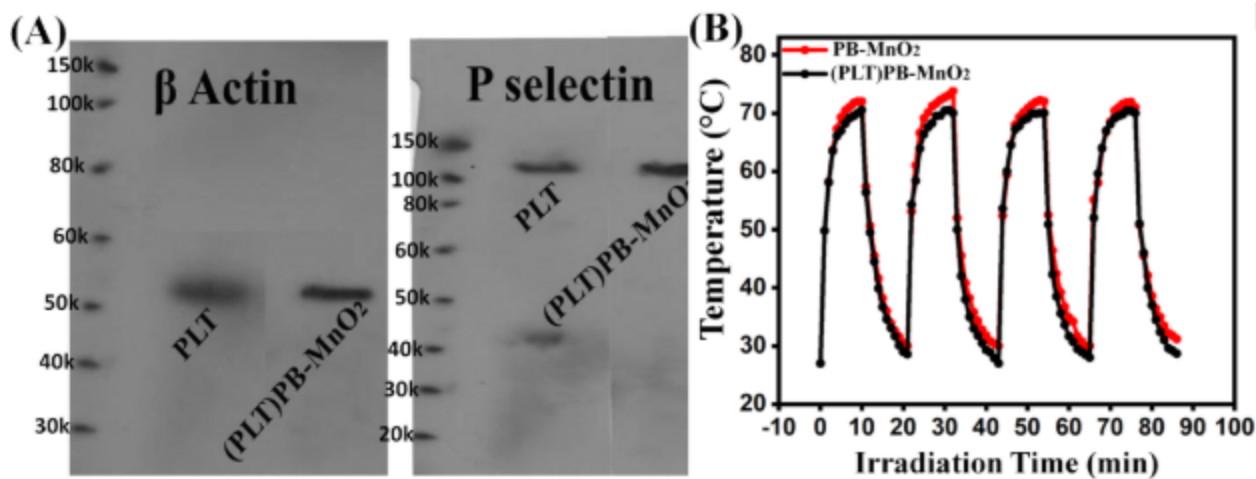


Figure 3

(A) Western blot analysis of P-selectin in PLT and (PLT)PB-MnO₂ groups after coating on PB-MnO₂ nanoparticles, (B) Photothermal conversion stability of (PLT)PB-MnO₂ under repeated on/off laser irradiation cycles (1.5 W/cm²).

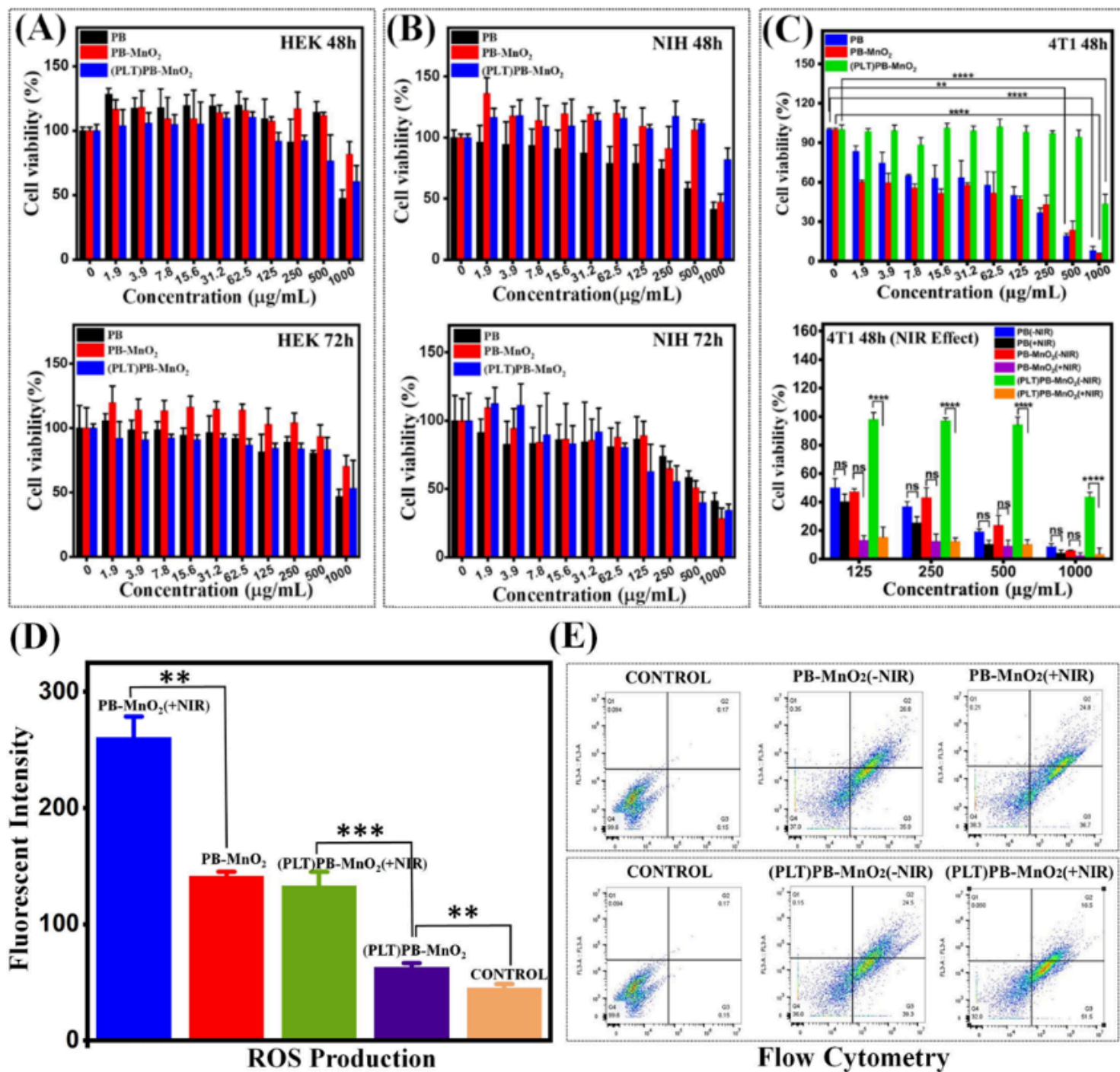


Figure 4

In vitro cytotoxicity evaluation of (PLT)PB-MnO₂ and PB-MnO₂ (A,B) Cell viability of HEK293 cells after 48 h and 72 hours treatment. (C) Cell viability of 4T1 cancer cells after 48 hours treatment with different concentrations of (PLT)PB-MnO₂ and PB-MnO₂ with or without NIR irradiation(1.5 W/cm²)(D) Intracellular ROS production, and (E) Flow cytometry analysis of apoptotic 4T1 cells treated with 125 μg/mL of (PLT)PB-MnO₂ with or without NIR. *P* value<0.05 was considered statistically significant in this study. Data are representative of three independent experiments.

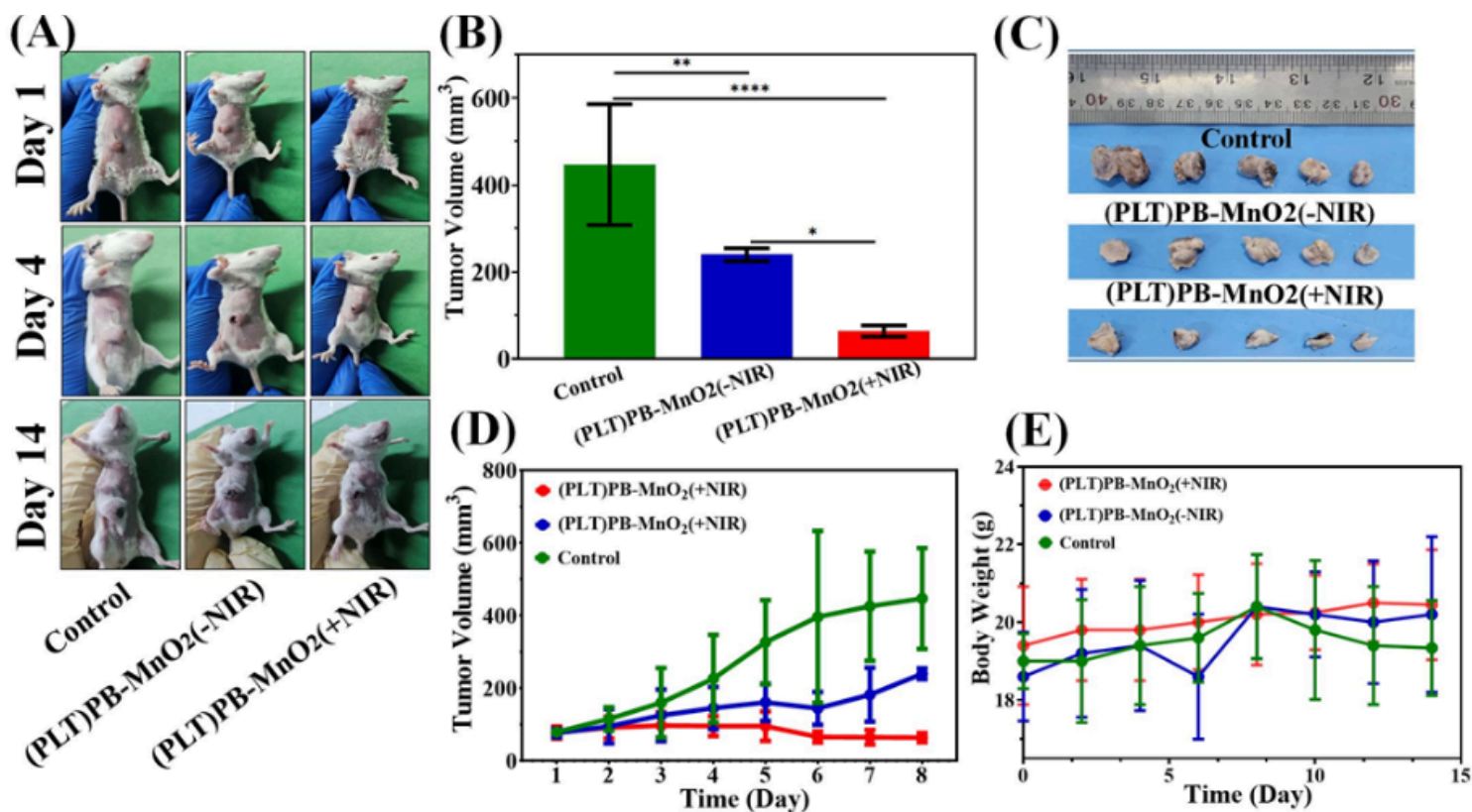


Figure 5

In vivo antitumor efficacy in 4T1 tumor-bearing mice. (A) Representative photographs of mice in different treatment groups on days 1, 4, and 14, (B) Tumor volume changes in different treatment groups, (C) Photographs of tumors in different treatment groups of control, (PLT)PB-MnO₂ (-NIR) and (PLT)PB-MnO₂(+NIR) on day 14, (D) Tumor volume changes over time in various treatment groups. (E) Body weight monitoring of mice during the treatment period. *P* value<0.05 was considered statistically significant in this study.

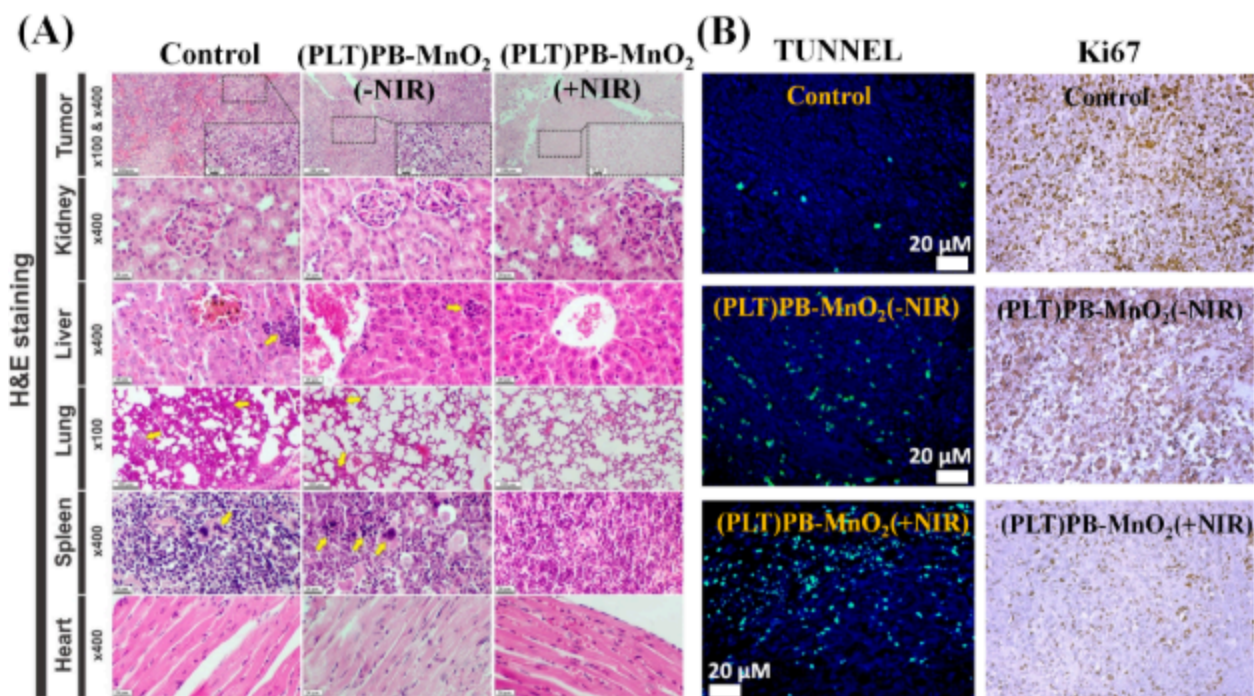


Figure 6

Histopathological and immunohistochemical analysis of major organs and tumor tissues. (A) H&E-stained sections of heart, liver, spleen, lung, kidney, and tumor tissue after treatment with different groups. (B) TUNEL assay for apoptosis detection and Ki-67 staining for cell proliferation assessment in tumor sections.

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