Optimized Bionic Drug Delivery Inducing Immunogenic Cell Death and cGAS-STING Pathway Activation for Enhanced Photochemotherapy-Driven Immunotherapy in Prostate Cancer

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Abstract

Prostate cancer (PCa) presents a challenging disease as it is often characterized as an immunologically “cold” tumor, leading to suboptimal outcomes with current immunotherapeutic approaches in clinical settings. Photodynamic therapy (PDT) harnesses reactive oxygen species generated by photosensitizers (PSs) to disrupt intracellular redox equilibrium. This process induces DNA damage in both the mitochondria and nucleus, activating the process of immunogenic cell death (ICD) and cGAS-STING pathway. Ultimately, this cascade of events leads to the initiation of antitumor immune responses. Nevertheless, existing PSs face challenges, including suboptimal tumor targeting, aggregation-induced quenching, and insufficient oxygen levels in tumor regions. To this end, a versatile bionic nanoplatform (M-TPNPs), has been designed for the simultaneous delivery of the aggregation-induced emission PS TPAQ-Py-PF6 and paclitaxel (PTX). The cell membrane camouflage of the nanoplatform leads to its remarkable abilities in tumor targeting and cellular internalization. Upon laser irradiation, the utilization of TPAQ-Py-PF6 in conjunction with PTX showcases a notable and enhanced synergistic antitumor impact. Additionally, the nanoplatform has the capability to initiate the cGAS-STING pathway, leading to the generation of cytokines. The presence of damage-associated molecular patterns (DAMPs) induces ICD and collaborates with these aforementioned cytokines, leading to the recruitment and facilitation of dendritic cells (DCs) maturation. Consequently, this elicits a systemic immune response against tumors. In summary, this promising strategy highlights the use of a multifunctional biomimetic nanoplatform, combining chemotherapy, PDT, and immunotherapy to enhance the effectiveness of anti-tumor treatment.

Introduction

PCa stands as the second most prevalent cancer type to be diagnosed and ranks as the fifth primary factor contributing to cancer-linked fatalities in males. Incidence rates of PCa have been steadily increasing over time.[1] Early-stage localized PCa patients often undergo treatment through surgery or radiation therapy. However, these interventions are associated with various complications, including urinary incontinence, sexual dysfunction, and bladder irritation.[2, 3] Patients typically experience progression to castration-resistant PCa following endocrine therapy and chemotherapy in advanced stages. At this juncture, disease progression speeds up, resulting in a reduced survival duration. [4]

Conventional cancer treatments, including surgery, chemotherapy, and radiotherapy, often exhibit restricted antitumor efficacy, leading to issues such as tumor recurrence and metastasis. In contrast, immunotherapy has already become a transformative strategy in the field of cancer treatment, offering substantial potential for the eradication of malignant tumors.[5, 6] Nevertheless, the practical implementation of immunotherapy encounters a challenge wherein a significant proportion of solid tumors are poorly immunogenic and refractory to immunotherapeutic intervention, categorically termed as “cold tumors”. [7-10] ICD demonstrates great potential in promoting the immunogenicity of cancer cells, thereby facilitating improved antitumor immune reactions. Throughout this mechanism, the tumor cells initiate the release of immunostimulatory DAMPs, such as surface-calreticulin (ecto-CRT), high
mobility group protein B1 (HMGB1), heat shock protein 70 (HSP70), and adenosine triphosphate (ATP). [11-15] The purpose of this coordinated release is to foster T cell infiltration, effectively converting immunologically "cold" tumors into a more responsive and immunogenic "hot" state. Additionally, it is important to highlight that the activation of the tumor immune system has been achieved through the utilization of pattern recognition receptors (PRRs).[16] These PRRs are found within cells of the innate immune system and play a crucial role in recognizing specific patterns, thereby promptly initiating an immune reaction. The cyclic guanosine monophosphate-adenosine monophosphate synthase-interferon gene stimulator signaling (cGAS-STING) pathway, renowned for its capacity to selectively activate immune cells, acts as a well-established PRRs system. [17] The activation of these PRRs results in the stimulation of tumor antigen-specific T cells, enhancing the tumor tissue infiltration and strengthening the immune responses against tumors.

PDT has garnered significant attention and demonstrated substantial progress over the past decades as a noninvasive and efficacious treatment modality for various diseases, including cancer.[18, 19] The therapeutic effects are promoted by PDT through the utilization of localized external light, photosensitizers (PSs), and oxygen. By harnessing these elements, highly toxic reactive oxygen species (ROS) are generated, inducing the desired therapeutic response. [20] In comparison to traditional techniques, PDT presents various benefits, such as remarkable selectivity in space and time, minimal invasiveness, excellent compatibility with the human body, reduced likelihood of drug resistance, and simplified operation, among other notable features. Recent researches have demonstrated that PDT, a type of reaction between PSs and oxygen under light exposure, results in the production of singlet oxygen ($^{1}$O$_2$) and/or ROS. This mechanism serves the dual purpose of directly eliminating cancer cells as well as initiating immunogenicity by effectively inducing ICD. [21-25] Moreover, PDT possesses the potential to disrupt the intracellular equilibrium of redox, leading to harm in mitochondrial and nuclear DNA and subsequent release into the cytoplasm. This stimulation initiates the cGAS-STING cascade, provoking an immune response against tumors while minimizing detrimental repercussions.[26]

As the cornerstone of PDT, the design of a potent and efficient PS capable of generating abundant ROS within the tumor site is paramount. However, the aggregation-caused quenching (ACQ) effect poses a challenge for conventional organic dyes like indocyanine green (ICG) and methylene blue (MB), which are widely used in clinical settings, causing a substantial reduction in fluorescence emission and PDT efficacy.[27, 28] Fortunately, aggregation-induced emission (AIE) has appeared as a novel photophysical strategy for solving the ACQ problem, enables the creation of efficient emitters and PSs with superior performance.[29-32] However, certain AIE luminogens (AIEgens) are challenged by comparatively limited absorption of light, necessitating the use of intense light irradiation to achieve efficient PDT. Additionally, the swift proliferation and uptake of oxygen by cancerous cells contribute to the prominent existence of hypoxia in the surrounding milieu of various malignant solid neoplasms. Unfortunately, this characteristic diminishes the efficacy of PDT in treating solid tumors. Besides, the restricted penetration depth of light constitutes a significant factor affecting phototherapy, including PDT.[33, 34] Penetration of light into tumor tissue is hindered, leading to reduced antitumor impact. As a result, combining phototherapy with
additional therapeutic techniques emerges as a hopeful strategy for bolstering the efficacy of cancer immunotherapy.[35]

Certain chemotherapeutic drugs, such as PTX and doxorubicin (DOX), have been identified for their immunomodulatory capabilities.[36-38] More specifically, the stimulation of ICD effects by PTX can result in the induction of endogenous vaccines. However, the use of small molecule drugs is associated with systemic toxicity owing to their limited targeting ability to the site of the disease. Nanotechnology-based drug delivery systems offer a solution by enhancing drug accumulation at the disease site. The concentration of chemotherapeutic agents at the tumor site can be increased through the enhanced permeability and retention (EPR) effect of the nanodrug delivery system, it may also spread to additional organs, specifically the liver and spleen within the reticuloendothelial system (RES). This imposition may burden these organs and potentially lead to detrimental consequences. Therefore, employing a strategy of cell membrane coating for biomimetic camouflage of nanoparticles (NPs) has become an effective approach to enhance the targeting and biosafety of nanomedicines.[39, 40]

In the present investigation, a versatile biomimetic nanoplatform M-TPNPs incorporating chemotherapy, PDT, and immunotherapy was developed for the treatment of PCa (Scheme 1). In brief, a therapeutic agent specifically targeting tumors is created through the encapsulation of the AIEgen and PTX within NPs. This is subsequently camouflaged using the cell membrane of macrophages. Consequently, enhanced accumulation at the tumor location is observed. The simultaneous application of chemotherapy and PDT has significantly activated immune functions. PDT induced ICD, generating a cascade of DAMPs, leading to the recruitment and stimulation of DCs maturation, and promoting the infiltration of cytotoxic T lymphocytes. At the same time, cellular oxidative stress is provoked by PDT, resulting in DNA damage within the mitochondria and nucleus. Consequently, the cGAS-STING pathway is activated, thereby initiating an immune response against tumors. Additionally, in the hypoxic regions of tumors where PDT efficacy is compromised due to oxygen deficiency, the chemotherapeutic agent PTX can exert excellent tumor-killing effects. Furthermore, it continues to induce ICD in tumor cells, achieving a more comprehensive destruction of the tumor. Hence, the innate combination therapy of PDT and PTX, a chemotherapeutic agent, possesses the capability to induce a potent immune response against tumors, leading to the suppression of the primary tumor and the inhibition of distant tumor growth. In summary, this combined PDT and chemotherapy approach may be an effective strategy for cancer treatment, holding significant importance for the complete eradication of tumors and improvement of patient prognosis.

Materials And Methods

Materials

All chemicals and reagents were provided by commercial sources.

Roswell Park Memorial Institute (RPMI) 1640 culture medium, Dulbecco's Modified Eagle's Medium (DMEM) culture medium, fetal bovine serum (FBS), and penicillin streptomycin were purchased from
Characterizations

$^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectra were recorded on Bruker-DPX 400 spectrometer. High-resolution mass spectra (HRMS) were conducted on a Bruker AutoflexIII LRF200-CID mass spectrometer in matrix assisted laser desorption ionization time of flight (MALDI-TOF) mode. Absorption spectra were obtained using a Shimadzu UV-1800 spectrometer. Photoluminescence (PL) spectra were recorded with a Horiba Fluorolog-3 spectrofluorometer. Transmission electron microscope (TEM) images were captured using a Talos L120C G2 TEM (FEI, Czech). Dynamic light scattering (DLS) measurements were conducted with a Malvern Zeta sizer Nano ZS-90. In vivo imaging was performed using the NightOWL II LB983 system for fluorescence imaging of animals and organs.

Extraction of macrophage cell membrane

Phosphate-buffered saline (PBS) was utilized to harvest RAW 264.7 cells by means of scraping, followed by a 5 min centrifugation at 700 g. Subsequently, the cells were suspended in a combination of cryopreservation medium (Hyclone) and complete culture medium, maintaining a ratio of 50:50. The cell suspension was stored at -20 °C. To obtain the plasma membrane, harvested cells were defrosted and rinsed using a pH 7.0 buffer containing 30 mM tris-HCl, 0.0759 M sucrose, and 0.225 M D-mannitol. Following the wash, cells were suspended in the same buffer supplemented with 0.5 mM egtazic acid and a phosphatase/protease inhibitor cocktail. Differential centrifugation was employed with a Beckman Coulter Optima L-90K Ultracentrifuge to ultimately isolate the cell membrane. To achieve mechanical disruption, a Kinematica Polytron PT 10/35 probe homogenizer was utilized at 70% power for 10 passes. Initially, the cell homogenate underwent centrifugation at 10,000 g for 25 min, and the resulting supernatant was subsequently subjected to centrifugation at 150,000 g for 35 min to pellet the membrane material. The isolated membrane was preserved in 0.2 mM ethylenediaminetetraacetic acid in water at -20 °C for subsequent applications. Quantification of total protein content was accomplished using a BCA protein assay kit.

Preparation of PNPs, TPNPs, and M-TPNPs

Accurately weigh 1 mg of TPAQ-Py-PF$_6$, 1 mg of PTX and 10 mg of Pluronic F-127, and then fully dissolve their mixture in 1 ml of tetrahydrofuran (THF). This solution was then mixed with 10 mL of deionized water after which it underwent continuous sonication for a duration of 3 min. To eliminate the THF present in the solution, a stirring process was conducted overnight within a fume hood. Afterward, the resulting TPNPs were obtained by performing centrifugation utilizing centrifuge filters with a molecular weight cutoff (MWCO) of 100 kDa. To prepare M-TPNPs, prepared TPNPs (200 μL, 0.5 mg mL$^{-1}$...
1, based on TPAQ-Py-PF₆) were conjugated with 0.4 mg of macrophage membranes under ultrasonication (80 W, 60 Hz, 20 min). The resulting mixture was further processed by extrusion through a polycarbonate membrane to generate M-TPNPs. prepared M-TPNPs were stored at 4 °C and used as quickly as possible.

**Cells and animals**

The RM1 cell line and RAW264.7 cells were procured from the Cells Bank of the Chinese Academy of Sciences in Shanghai, China. The cells were grown in a controlled environment maintained at 37 °C with 5% CO₂.

Male C57BL/6 mice (7 weeks old) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). Tumor volume was calculated using the formula: Volume = (Length × Width × Width)/2. All animal experiments conducted in this study were approved by the Dushu Lake Hospital, which is affiliated with Soochow University (P. R. China). The laboratory animals' studies were conducted in accordance with the guidelines provided in the Guide for the Care and Use of Laboratory Animals.

**Cytotoxicity study**

RM1 cells were inoculated into 96-well plates and incubated with different concentrations of TPNPs or M-TPNPs for 24 h at 37 °C. Subsequently, for PBS + L group, TPNPs + L group and M-TPNPs + L group, the cells experienced white light illumination (0.1 W·cm⁻²) for a duration of 3 min. In contrast, for PBS group, and M-TPNPs group remained in darkness without any light exposure. The relative cell viability was evaluated 24 h post-irradiation using a standard (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay.

**ROS detection in RM1 cells**

RM1 cancer cells were first placed in a confocal chamber and cultured in serum-free DMEM medium. The culture was supplemented with MNPs, PNPs, TNPs, or M-TPNPs (5 μg mL⁻¹) and maintained at 37 °C for 24 h. Subsequently, the cells underwent a washing step before being stained with 20 μM of 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) in DMEM medium lacking serum. Following staining, the cells were subjected to a white light exposure of 0.1 W·cm⁻² for 3 min. To establish a baseline, cells cultured with PBS were included for comparison. For confocal laser scanning microscopy imaging, the excitation wavelength was set at 488 nm, with emission at 530 ± 20 nm.

**Characterization of immunologic cell death**

In order to evaluate the concentrations of extracellular ATP and HMGB1, RM1 cancer cells were seeded into 6-well plates with a cell density of 1.5 × 10⁵ cells/mL. Cells were then incubated with serum-free culture medium containing MNPs, PNPs, TNPs, or M-TPNPs (5 μg mL⁻¹) for 24 h. Subsequently, white light irradiation (0.1 W/cm²) was applied for a duration of 3 min. Following light stimulation for 12 h, the
Culture supernatants were centrifuged at 13,500 g at 4 °C for 10 min. The HMGB1 ELISA assay was performed to quantify the HMGB1 released in the supernatant in accordance with the instructions provided by the manufacturer. Furthermore, the ATP bioluminescent assay kit was used to measure the levels of secreted ATP, following the instructions provided.

**Western Blot Assay:**

The washed cells undergoing treatment were lysed on ice, and the resulting supernatant was obtained through centrifugation (12,000 g, 30 min). To denature the protein, the loading buffer was added and boiled at 100 °C for 10 min. Following electrophoresis, membrane transfer, and blocking, the primary antibodies (at a dilution of 1:1000) were added and left to incubate overnight at 4 °C. Subsequently, the secondary antibodies (at a dilution of 1:10,000) were applied and incubated at room temperature for 1 hour, followed by imaging using a chemiluminescent imaging system. The antibodies used for the ICD pathway were anti-GAPDH (ab9485), anti-HMGB1 (ab18256) and anti-HSP70 (ab2787). The Rabbit IgG (H+L) Secondary Antibody was obtained from Invitrogen. cGAS-STING pathway related antibodies used in the experiment included anti-TBK1 (ab40676), anti-STING (ab288157), and anti-IRF-3 (ab68481), were procured from Abcam. Anti-p-TBK1 (Ser172), and anti-p-STING (Ser365), and anti-p-IRF3 (Ser396), were procured from Cell Signaling Technology.

**In vitro BMDCs maturation study**

To obtain BMDCs, we utilized the tibia and femur of male C57BL/6 mice aged 7 weeks, using well-established techniques. To initiate BMDC differentiation, we employed RPMI 1640 culture medium, which was supplemented with 20 ng mL\(^{-1}\) of GM-CSF, and 10 ng mL\(^{-1}\) of IL-4. In order to examine the maturation of BMDCs in vitro, we seeded RM1 cancer cells on the upper chamber of transwell, cultured them overnight, and subsequently subjected them to various treatments. In the next step, a quantity of 5 × 10\(^5\) BMDCs were placed in each well of the lower transwell chamber, and they were allowed to incubate for a period of 24 h. Following this, the BMDCs were gathered and subjected to staining with antibodies against CD11c, CD80, and CD86. The subsequent analysis was conducted using flow cytometry to determine the outcomes.

**In vivo anti-tumor study**

The bilateral RM1 tumor model was established to assess the abscopal effect. Initially, C57BL/6 mice were subcutaneously inoculated with RM1 cells (1 × 10\(^6\) per mouse) into the left flank to develop primary tumors for various therapeutic interventions. After six days, RM1 cells (5 × 10\(^5\) per mouse) were subcutaneously inoculated into the right flank without treatment to create distant tumors, mimicking cancer metastasis. Following this, mice harboring bilateral RM1 tumors were randomly assigned to seven groups (n = 5 mice): G1: PBS; G2: PNPs; G3: TNPs + L; G4: M-NPs; G5: TPNPs + L; G6: M-TPNPs; G7: M-TPNPs + L. During day 0 and day 3, light irradiation was conducted only on the primary tumor, 8 h after each intravenous dose of distinct NPs. The light irradiation had an intensity of 0.3 W/cm\(^2\) and lasted for
10 min. The dose for TPAQ-Py-PF$_6$-based NPs was 150 μL at a concentration of 0.4 mg mL$^{-1}$, and for PTX-based NPs it was 1 mg mL$^{-1}$. Following the treatment, the sizes of the primary tumor and distant tumors were observed and measured.

**Ex Vivo Analysis of Immune Cells:**

Following the treatment, single-cell suspensions were prepared from the tumor tissue, lymph nodes, and spleen of the mice. The tissues were dissected, minced, and homogenized. The resulting material was filtered through a 70 μm mesh to obtain a single-cell suspension. For spleen tissues, RBC Lysis Buffer was applied to eliminate red blood cells. Afterward, the cells were moved to a flow tube for the purpose of staining. The detection of DC maturation in lymph nodes followed established protocols, utilizing anti-CD11c-PE, anti-CD80-BV421, and anti-CD86-APC-fire750 antibodies. In accordance with well-established guidelines, the infiltration of CD$4^+$ T cells and CD$8^+$ T cells in the tumor was examined by employing anti-CD3-PE594, anti-CD4-Alexa Fluor 700, and anti-CD8-PE-Cy7 antibodies. The CD$8^+$ TEM and CD$8^+$ TCM in the spleen were detected using anti-CD3-APC, anti-CD8-PE, anti-CD44-Alexa Fluor 700, and anti-CD62L-PE594 antibodies in accordance with established protocols. Moreover, cytokines (TNF-α and IFN-β) in the serum were assessed following the instructions provided in the ELISA kit.

**In vivo biocompatibility evaluation**

To assess the biocompatibility of M-TPNPs in vivo, we conducted a toxicity examination using healthy C57BL/6 mice. The experiment involved administering M-TPNPs (150 μL, 0.4 mg mL$^{-1}$ concentration of TPAQ-Py-PF$_6$) intravenously to a group of three mice on both day 0 and day 3. Control group mice, also healthy, were treated with PBS (n = 3 mice). All mice were grouped sequentially as follows: G1: PBS; G2: PNP; G3: TNPs + L; G4: M-NPs; G5: TPNPs + L; G6: M-TPNPs; G7: M-TPNPs + L. After a period of 10 days, all mice were sacrificed, and we collected and prepared sections of the major organs (heart, liver, spleen, lung, and kidney) from both the control and M-TPNPs-treated mice for subsequent hematoxylin and eosin (H&E) staining.

**Statistical Analysis:**

We utilized OriginPro 9.0 and GraphPad Prism 8.0.2 for the execution of data analysis and visual depiction. The software FlowJo v10.8.1 was employed for the analysis of flow cytometry data. Survival curves were generated through Kaplan-Meier analysis, and statistical comparisons were performed using both Student’s t-tests (between two groups) and one-way ANOVA (among multiple groups). The presentation of results is in the form of mean ± standard deviation (SD), and statistical significance was determined at a threshold of P < 0.05.

**Results And Discussion**

Supplementary Figure 1 depicts the synthetic pathway employed for the creation of TPAQ-Py-PF$_6$. [41] TPAQ-Py was formed using a conventional structure of D-A-A', where TPA acts as the electron
bestower, pyridine assumes the role of the electron-receiving component, and anthraquinone serves as the auxiliary A. TPAQ-Py acquires the AIE property due to the peculiar arrangement of its propeller-shaped structure. The redox cycling reaction of the anthraquinone fragment induces the process of photoinduced self-electron transfer. This process forms anthraquinone anion radicals, subsequently generating \( \text{O}_2^- \) and \( \text{OH}^- \).[42, 43] Subsequently, the methylpyridine hexafluorophosphate group replaced the pyridine group, thereby enhancing its ability to accept electrons and leading to the creation of TPAQ-Py-PF\(_6\). Analyses using \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, and high-resolution mass spectroscopy (HRMS) were conducted to validate the chemical structures of the compounds. (Supplementary Figure 2-4).

First, we experimentally evaluated the basic photophysical properties of TPAQ-Py-PF\(_6\). As depicted in Figure 1b, c, we detected the absorption and emission spectra of TPAQ-Py-PF\(_6\) (in hexane). The absorption spectrum exhibited maxima at 490 nm, while the emission spectrum displayed peaks at 540 nm. Concurrently, the AIE characteristics of the NPs were further explored by employing tetrahydrofuran (THF) as a favorable solvent and hexane as an unfavorable solvent. As depicted in figures 1d, the fluorescence intensities of both AIE NPs exhibited an increment with increasing hexane ratio (\( f_{\text{hex}} \)). In pure THF, TPAQ-Py-PF\(_6\) displayed minimal fluorescence emission owing to its strong ICT feature, coupled with outstanding solubility in THF. At \( f_{\text{hex}} = 90\% \), a great fluorescence intensity enhancement of TPAQ-Py-PF\(_6\) was captured compared to that in pure THF, indicating a typical AIE feature for the molecules. Subsequently, in order to improve the solubility and biosafety of TPAQ-Py-PF\(_6\), we co-loaded TPAQ-Py-PF\(_6\) and PTX to form self-assembled TPNPs using Pluronic F127 as a facilitating agent. As a control, NPs were prepared by assembling PTX or TPAQ-Py-PF\(_6\) with Pluronic F127, named PNPs or TNPs, respectively. To confer tumor-targeting capability to the NPs, we subsequently coated the surface of TPNPs with macrophage membrane, resulting in the biomimetic M-TPNPs. The macrophage membrane, and its related membrane proteins, was designed to have the function of a "camouflage" to avoid being cleared by the reticuloendothelial system (RES). Additionally, it was envisioned to serve as a guide to direct the tumor-targeting NPs towards the inflamed tumor tissue, thereby increasing their accumulation in that area. The illustration of the membrane-coated TPNPs preparation is depicted in Figure 1a. In the synthesis of M-TPNPs, RAW 264.7 cell membranes (RM) were acquired and subsequently applied to coated the TPNPs surface through a 20-minute sonication process in an ice water bath. Various methods were employed to corroborate the effective membrane coating of RM. Transmission electron microscopy (TEM) images revealed uniform spherical structures, with the surface of M-TPNPs showing the presence of a thin layer of cell membrane while TPNPs exhibited a bare structure (Figure 1f-h, j). To evaluate the dimensions and structure of various NPs, we employed dynamic light scattering (DLS) and TEM analyses. The results, presented in Figure 1f-h and supplementary figure 6, indicated average diameters of 87.2 nm for PNPs, 72.3 nm for TNPs, 149.7 nm for RAW 264.7 cell membrane vesicle, 92.7 nm for M-NPs (RAW 264.7 cell membrane coated F127 NPs), 77.9 nm for TPNPs, and 92.6 nm for M-TPNPs, respectively. And the protein constituents of RM, RAW 264.7 cell membrane vesicle (RMV), and RAW 264.7 cell membrane-coated TPNPs (M-TPNPs) were assessed through SDS polyacrylamide gel electrophoresis (SDS-PAGE), with the results depicted in Figure 1e. The protein components originating
from both RM and RMV were effectively preserved in M-TPNPs. This not only confirmed the successful fusion of RM with TPNPs but also demonstrated that the membrane fusion and RM coating procedures had no discernible impact on the protein composition inherited from the original cells. The western blotting analysis revealed that both the macrophage membrane and MTPNPs expressed CD86 and CD44, which are typical markers for macrophages. This validates the successful encapsulation of the macrophage membrane onto the surface of M-TPNPs (Figure 1i). The zeta potentials of all NPs were consistently below -10 eV (Figure 1k). Furthermore, these NPs exhibited robust colloidal stability, with minimal changes in diameter was detected after 5 days of incubation in 10% serum (Supplementary Figure 7).

Subsequently, we investigated the ability of TPAQ-Py-PF₆ to generate ROS under light irradiation and its photostability. Initially, 2′,7′-dichlorodihydrofluorescein (DCFH) served as a comprehensive ROS indicator. It undergoes irreversible oxidation by various ROS, leading to the formation of green-emissive dichlorofluorescin (DCF, Em = 525 nm). The exceptional ability of AIE PSs to generate ROS is showcased by Figure 1 l-m, where the green fluorescence of DCF significantly increased (~23-fold) when irradiated with white light (0.1 W·cm⁻²) with the presence of TPAQ-Py-PF₆. With increasing light duration, we observed a gradual decrease of the absorption of ABDA (¹O₂ indicator), and the absorption intensity decreased by about 90% after 10 mins of irradiation (Figure 1 n, o). To further confirm the stability of the nanomedicine, we also compared the absorption changes of M-TPNPs and ICG after light exposure (Figure 1 p, q). The results show that the absorption curve of M-TPNPs was remain unchanged, while the absorption curve of ICG decreases continuously with the increase of light exposure time and the optical properties become unstable. The results confirm that the constructed M-TPNPs possesses stable optical properties.

Given the impressive capability of M-TPNPs to generate ROS upon light exposure and their effectiveness in chemotherapy, we proceeded to assess their in vitro anti-tumor potential. Initially, the cellular uptake of both TPNPs and M-TPNPs was examined using the confocal laser scanning microscope (CLSM). Figure 2a illustrates the CLSM images of RM1 PCa cells after incubation with TPNPs or M-TPNPs at a concentration of 5 μg mL⁻¹ for a duration of 4 h at 37 °C. The results clearly show that M-TPNPs were internalized by RM1 cancer cells to a greater extent than TPNPs that were not modified by macrophage membranes. Quantitative analysis reveals the intensity of red fluorescence in cells treated with M-TPNPs is approximately twice that of cells treated with TPNPs, indicating the enhanced ability of the membrane camouflage to target tumor cells (Figure 2c). Nevertheless, upon incubation with RAW264.7 macrophage cells for a duration of 4 h at 37 °C, contrasting results were observed in CLSM images. The M-TPNPs-treated cells exhibited a diminished fluorescence signal compared to the cells treated with regular TPNPs (Figure 2b). This may be due to the fact that the camouflage of macrophage membranes assisted M-TPNPs to successfully escape recognition and internalization by macrophages. Then, we explored the killing ability of M-TPNPs on tumor cells. Following the incubation of RM1 cells with PBS, TPNPs, or M-TPNPs at varying concentrations, 3-(4,5-Dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assays were conducted to assess cellular toxicity in various conditions, including both untreated and
light-exposed conditions. Although the sole exposure to light showed minimal toxicity to cells, the viability of cells in the other groups showed a reduction that was dependent on the concentration. Specifically, the cell viability in the M-TPNPs plus light group was markedly lower than that in both the M-TPNPs and the TPNPs + L groups (Figure 2d). To investigate the impact of different NPs treatments on cell clone formation and cell migration ability of RM1 cells, we conducted cell clone formation assays (Figure 2e) and Transwell migration assays (Figure 2f). In comparison to the control group, a more significant reduction in the number of cell clusters and migrating cells in the M-TPNPs groups was observed. This reduction was even more pronounced in the TNPs + L group. Notably, the M-TPNPs + L group exhibited the most significant decrease in the number of cell clusters and migrating cells, confirming the effective growth inhibition of RM1 tumor cells by M-TPNPs + L. Collectively, these findings demonstrated that M-TPNPs, combining PDT, chemotherapy, and tumor cell-targeting capabilities, represent potent agents for efficiently eliminating cancer cells and inhibit PCa progression upon light activation.

To assess the intracellular capability of various NPs in generating ROS, we employed DCF-DA. After irradiation with white light for 3 min (0.1 W·cm\(^{-2}\)), both TPNPs and M-TPNPs (5 \(\mu\)g mL\(^{-1}\)) exhibited a remarkable ability to produce a significant concentration of ROS. This was evidenced by the noticeable bright green fluorescence signal observed in the treated RM1 cells, which resulted from the conversion of DCF-DA (Figure 3a). These findings validate the exceptional PDT efficacy of TPAQ-Py-PF\(_6\). Since free dsDNA fragments released by nuclear damage play an important role in the activation of the cGAS-STING pathway.[44, 45] Then, we performed immunostaining of dsDNA in RM1 cells using \(\gamma\)-H2AX (green), a specific indicator of cellular nuclear damage. The results demonstrated that compared to the barely visible green fluorescence signal in the PBS and TNPs groups, a more intense green signal was captured in the PNPs and M-TPNPs groups. Conversely, the TNPs + L group exhibited a stronger green signal, while the most pronounced green signal was detected in the M-TPNPs + L group (Figure 3b). RM1 cells treated with different NPs groups were collected, and proteins were extracted for western blotting experiments to validate the expression level of cGAS-STING pathway-associated proteins. In line with the findings of nuclear damage, a significant increase in p-STING, p-IRF3, and p-TBK1 protein expression was observed exclusively in the TNPs + L group and M-TPNPs + L group compared with the control group (Figure 3c). This observation confirms that macrophage membrane-targeted PTX chemotherapy combined with PDT induces more pronounced nuclear damage, which subsequently triggers the activation of the cGAS-STING pathway.

Furthermore, we explored the potential of M-TPNPs as a robust inducer of ICD to augment tumor immunogenicity. Western Blotting revealed elevated HMGB1 and HSP70 expression in the culture supernatant treated with either PNPs or TNPs + L, indicating that both the individual chemotherapeutic drug and PDT components were capable of inducing ICD (Figure 3d). In order to conduct a quantitative analysis of the enhanced effectiveness of M-TPNPs + L-induced ICD, we evaluated the HMGB1 levels in the RM1 cell culture supernatant following various NPs treatments through ELISA (as shown in Figure 3e). Furthermore, we employed an ATP assay kit to measure the ATP levels in the RM1 cell culture supernatant after subjecting it to different NPs treatments (Figure 3f). The experimental results
consistently demonstrate that the culture supernatant of RM1 cells exposed to M-TPNPs + L contained an increased quantity of other DAMPs, such as HMGB1 and ATP. These findings indicate that M-TPNPs + L possess a robust capability to induce ICD in tumor cells, a crucial factor for promoting tumor antigen presentation and activating T lymphocytes. To validate the ICD effect triggered by M-TPNPs, further evaluations were carried out on the maturation of dendritic cells (BMDCs) derived from bone marrow. As shown in Figure 3h, well-established protocols were utilized to extract BMDCs from the femur and tibia of male C57BL/6 mice aged 7 weeks. Subsequently, RM1 cancer cells were cultured in the upper chamber of transwells and subjected to various treatments, including PBS, TNPs, PNPs, M-TPNPs, TNPs + L, and M-TPNPs + L. After this step, the culture of BMDCs took place in the transwell compartment located below. Later, the BMDCs were collected, subjected to staining utilizing anti-CD11c, anti-CD80, and anti-CD86 antibodies, and then analyzed by flow cytometry. It is important to mention that when BMDCs were co-cultured with RM1 cancer cells, which were previously exposed to M-TPNPs + L, both CD80 and CD86 expression levels were significantly increased (Figure 3i, g). This observation suggests that M-TPNPs + L can induce a strong maturation of DCs.

In vivo tumor imaging capability as well as biodistribution of M-TPNPs were subsequently investigated. Following the intravenous administration of TPNPs (DID labelled) or M-TPNPs (DID labelled) into the tail vein of RM1 tumor-bearing mice, we conducted imaging experiments utilizing an in vivo imaging system (IVIS). A distinct fluorescence signal was identified at the location of the tumor, with the intensity of fluorescence reaching its maximum around 8 h after injection (Figure 4a). This observation indicates the ideal moment for tumor photoimaging and therapy. Significantly, the fluorescence signal observed in mice subjected to M-TPNPs treatment was about 1.55-fold greater compared to mice administered with TPNPs (Figure 4b). This result implies a superior tumor accumulation capability facilitated by the coating of macrophage membrane. Moreover, even 24 h post-administration, the tumor site continued to emit robust fluorescent signals, underscoring that NPs have a long-lasting in vivo tumor imaging capability. The biodistribution of M-TPNPs was investigated by intravenously injecting the NPs into tumor-bearing mice for 8 h. Upon resection and imaging of the main organs and tumors using IVIS, it was evident that the tumors from M-TPNPs-treated mice displayed a substantially elevated fluorescence signal compared to those treated with TPNPs (Figure 4c, d).

We subsequently explored whether the robust anti-tumor immune response triggered by M-TPNPs combined with light exposure would elicit an abscopal inhibitory effect on untreated distant tumors. To accomplish this objective, a bilateral model of RM1 tumor was created according to the steps outlined in Figure 5a. To be more specific, we injected $1 \times 10^6$ RM1 cells under the skin of the left side of every C57BL/6 mouse in order to generate the primary tumor. After a duration of six days, inoculation of a second tumor was performed on each mouse (right side) to mimic the presence of metastatic tumors. After the initial tumor inoculation, exactly one week later, the mice harboring bilateral RM1 tumors were divided randomly into seven groups, with each group comprising of five mice ($n = 5$). The groups were labeled as follows: PBS, PNPs, TNPs + L, M-NPs, TPNPs + L, M-TPNPs, and M-TPNPs + L. These mice received intravenous injections of various NPs (150 µL, 400 µg mL$^{-1}$) on days 0 and 3. White light
treatment (0.3 W·cm$^{-2}$, 10 min) was applied exclusively to the primary tumors in groups with "L" at 8 h post-NPs administration, while the distant tumors remained untreated. As illustrated in Figure 5b, c, light-irradiated TPNPs and non-light-irradiated M-TPNPs demonstrated no significant inhibitory effect on both sides of the tumor growth. The findings propose that relying exclusively on either PDT or chemotherapeutic agents is not sufficient to effectively inhibit tumor progression. However, the M-TPNPs + L treatment resulted in substantial inhibition of both primary and distant tumors. Remarkably, the distant tumors' average volumes on day 14 followed a distinct trend among the groups, with TPNPs + L, M-TPNPs, and PBS exhibiting volumes 2.18, 6.15, and 9.62 times larger, respectively, compared to the M-TPNPs + L group (Figure 5c). Notably, none of these treatments exerted discernible effects on the mice's body weight (Figure 5e). H&E and TUNEL staining of distant tumors further indicated a greater necrotic area as well as increased apoptosis of cancer cells in the M-TPNPs + L group (Figure 5h, i). These findings underscore the advantageous outcome of combining robust PDT efficacy with PDT-accelerated chemotherapy, resulting in effective suppression of primary and distant tumors.

To investigate the factors responsible for the increased effectiveness of MTPNPs in fighting tumors, a flow cytometry analysis was conducted on day 14. The evaluation focused on the maturation process of DCs in the spleen and the presence of immune cells relevant in the tumors. Our findings consistently indicated that M-TPNPs, particularly under light irradiation, significantly elevated the proportions of mature DCs (CD80$^+$ CD86$^+$ cells) in lymph nodes (Figure 6a, i). In the M-TPNPs + L group, the proportions of CD4$^+$ T cells and CD8$^+$ T cells exhibited a notable increase in both primary and distant tumors (Figure 6b, c and Supplementary Figure 8, 9). According to Figure 6g, the proportions of CD4$^+$ T cells in distant tumors were increased by 1.74-fold and 4.48-fold in the M-TPNPs + L group compared to the TPNPs + L group and the M-TPNPs group, respectively. Similarly, the proportions of CD8$^+$ T cells in distant tumors were elevated by 1.53-fold and 2.82-fold in the M-TPNPs + L group compared to the TPNPs + L group and the M-TPNPs group, respectively (Figure 6h). These findings suggest that M-TPNPs + L can effectively stimulate anti-tumor immune responses. In addition to inducing an immune response to eradicate the tumor, it is also crucial to establish immune memory to effectively prevent tumor recurrence. Thus, we evaluated the establishment of immune memory in bilaterally tumor-bearing mice during different nanomedicine treatments by detecting changes in the expression levels of T$_{em}$ cell (effector memory T cell, CD44$^+$ CD62L$^-$ cell) in the spleens of the mice. Thus, we assessed whether immunological memory was established in mice bearing bilateral tumors and treated with M-TPNPs + L by gauging the expression of T$_{em}$ cells in the spleens of the mice. Fortunately, flow cytometry analysis showed a significant increase in the percentage of CD8$^+$ T$_{em}$ cell in the spleens of the M-TPNPs + L group (35.3%) compared to the PBS-treated group (5.82%), confirming the formation of immune memory (Figure 6d, j). According to the flow cytometry data, the level of IFN-β and TNF-α expression was most significantly increased in the M-TPNPs + L group compared to the PBS group (Figure 6k, l). These findings indicate that M-TPNPs serve as potent inducers of ICD and cGAS-STING pathway, capable of eliciting robust anti-tumor immune responses.
Ultimately, the in vivo safety profile of M-TPNPs was systematically assessed. A randomized division of healthy mice into seven groups (n = 3) was conducted. On days 0 and 3, PBS or M-TPNPs was injected intravenously, while detailed pathological analysis was conducted on day 10 to collect major organs including the heart, liver, spleen, lung, and kidney. The histopathological examination of major organs from mice treated with both PBS and M-TPNPs revealed minimal signs of damage or impairment (Figure 7). Collectively, with these results, it is shown that M-TPNPs exhibit favorable biocompatibility.

Conclusion

In summary, this investigation focuses on the creation of a multifunctional bionic nanoplatform designed for the combined treatment of PCa, integrating both PDT and chemotherapy. The nanoplatform consists of the PS TPAQ-Py-PF$_6$ which is possessing AIE properties and chemotherapeutic drug PTX. To enhance its performance, the nanoplatform is enveloped with a macrophage cell membrane. M-TPNPs demonstrate the capability to generate ROS efficiently when exposed to laser irradiation. The ROS production leads to DNA damage in tumor cells and activates the cGAS-STING pathway, triggering the release of type I interferon (IFN-β) to induce maturation of DCs. The combination PDT and chemotherapy can also enhance the effect of ICD and further facilitate the maturation of DCs. This synergistic approach aims to transform the immunogenic status of the tumor from "cold" to "hot." This dual-pronged strategy, combining ICD and the cGAS-STING pathway, demonstrates a significant capacity to enhance the infiltration of cytotoxic T cells in PCa. The approach induces potent anti-tumor immunity, effectively suppresses primary tumor growth and establishes enduring immune memory. In summary, the developed nanoplatform achieves synergistic anti-tumor effects on multiple fronts, providing valuable insights for advancing the combined application of PDT, chemotherapy, and immunotherapy. This holds great significance for achieving comprehensive tumor eradication and improving the prognosis of patients.

Declarations

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Author contributions

C. W and D. F: experiment design and article writing. J. H: supervision. C. W, H. X, and Z. H: doing experiments in vivo and in vitro. C. W and D. F: statistical analysis. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and the Supplementary Information.
Ethics approval and consent to participate

All animal experiments conducted in this study were approved by the Dushu Lake Hospital, which is affiliated with Soochow University (P. R. China). The protocols followed for these animal studies adhered to the guidelines provided in the Guide for the Care and Use of Laboratory Animals.

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Consent for publication

The authors declare no conflict of interest.

Competing interests

Authors declare no competing interests.

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References


Scheme 1 is available in the Supplementary Files section

Figures

Figure 1
Design and characterization. a Illustration of the membrane-coated TPNPs preparation. b UV-Vis absorption and c PL spectra of TPAQ-Py-PF₆ spectra in THF/hexane mixture with 1 vol % THF. d PL spectra of TPAQ-Py-PF₆ in THF/hexane mixture with various hexane fractions. e SDS-PAGE protein analysis of markers, RAW264.7 cell membrane, RAW 264.7 cell membrane vesicle and RAW 264.7 cell membrane coated TPNPs. Representative DLS results and TEM images (inset) of f TPNPs and g Membrane vesicles h M-TPNPs. i Representative western blots of CD44 and CD86 expression in different formulations (I: TPNPs; II: Membrane vesicles; III: M-TPNPs). j, k Average sizes and zeta potentials of various NPs. Data are presented as mean ± SD (n = 3 independent experiments). Time-course charts of l, m DCFH fluorescence enhancement and n, o ABDA decomposition under light irradiation (0.1 W·cm⁻²). For i-o, M-TPNPs at a concentration of 20 μM. UV-Vis absorption of p M-TPNPs (10 μM) and q ICG (10 μM) after different times of light exposure (0.1 W·cm⁻²).
Figure 2

**In vitro cellular uptake and cell growth inhibition of M-TPNPs.** Representative CLSM images of **a** RM1 cells and **b** RAW cells upon incubation with TPNPs or M-TPNPs (5 μg mL⁻¹). Scale bars: 20 μm. **c** Quantitative data showing the mean fluorescence intensity (MFI) based on Figure 2a, b. Data are presented as mean ± SD (n = 3 independent experiments). Statistical significance was determined using two-tailed Student's t test. **d** Cell viabilities of RM1 cancer cells treated with various NPs without or with
light irradiation. Data are presented as mean ± SD (n = 3 independent experiments). e Cell clone formation assay and f Transwell migration assay of RM1 cells upon incubation with different treatments (5 μg mL⁻¹). L: White light exposure (0.1 W·cm⁻², 3 min). *p < 0.05, **p < 0.01, ***p < 0.001.
In vitro activation of cGAS-STING pathway, ICD activation, and maturation of DCs.  

**a** Representative CLSM images showing the ROS generation in RM1 cancer cells with different treatments as indicated (DCF-DA was nonfluorescent but switched to fluorescent DCF with green signal when oxidized by ROS). Scale bars: 20 µm.  

**b** Representative CLSM images of nuclear damage in RM1 cells with different treatments. Green signal: γ-H2AX. Blue signal: DAPI. Scale bars: 50 µm.  

**c** cGAS-STING pathway-associated proteins were evaluated in RM1 cells following various treatments.  

**d** Western blotting of HMGB1 and HSP70 release levels of RM1 cells after different treatments.  

**e, f** Quantitative analysis of the HMGB1 and ATP concentration in cell supernatants of RM1 cancer cells received various treatments as indicated.  

**h** Schematic diagram of BMDCs in vitro stimulation maturation experiment.  

**g** DCs maturation (CD80⁺ and CD86⁺ cells) and **i** representative flow cytometry data after different treatments in vitro stimulation maturation experiment. Error bars: mean ± SD (n = 3). The illustration was created with BioRender.com. L: White light irradiation (0.1 W·cm⁻², 3 min). Statistical analyses were performed by one-way ANOVA or Student’s t-test. *p < 0.05, **p < 0.01 and ***p < 0.001.

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**Figure 4**

**Targeting and biological distribution of M-TPNPs in vivo.**  

**a, b** Representative fluorescence images of RM1 tumor-bearing mice and corresponding fluorescence intensities in the tumor regions at various time points post i.v. injection of TPNPs or M-TPNPs.  

**c, d** Fluorescence imaging of vital organs and tumors extracted from mice, post intravenous administration of TPNPs or M-TPNPs at the 8-hour mark, along
with the respective fluorescence intensities, are presented as representative ex vivo data. Data are presented as mean ± SD (n = 3 mice). Statistical significance was determined using one-way ANOVA.

Figure 5

**In vivo tumor growth inhibition.** a The diagram demonstrates the process to assess the immune responses and anti-tumor effect generated by different treatments in a mouse model with bilateral RM1 tumors. The abbreviation "i.v." corresponds to "intravenous injection". The illustration was produced using BioRender.com. Plots of b primary tumor volume, c distant tumor volume, d survival curves, and e weight monitoring of RM1 subcutaneous tumor-bearing mice after receiving different treatments. Data are presented as mean ± SD (n = 5 mice). f-g Individual tumor growth curve of primary tumors and distant tumors. h-i Representative H&E staining and TUNEL staining of distant tumor sections harvested from the
mice receiving different treatments on day 14. Scale bars: 50 μm. L: White light exposure (0.3 W·cm$^{-2}$, 3 min). Statistical significance was determined using one-way ANOVA. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Figure 6
In vivo anti-tumor immune activation. a, i Representative flow cytometry analysis and quantitative data of the proportion of DCs maturation (CD11c+ CD80+ CD86+) in lymph nodes after various treatments on day 14. Representative flow cytometry plots and quantitative data of the percentages of CD8+ T cells in b, f primary tumors and c, h distant tumors collected from bilateral RM1 tumor-bearing mice after various treatments on day 14. d, j Representative flow cytometry analysis and quantitative data of the population of T_{em} cell (CD44+ 62L) in lymph nodes after various treatments on day 14. Quantitative data of the percentages of CD4+ T cells in e primary tumors and g distant tumors collected from bilateral RM1 tumor bearing mice after various treatments on day 14. k-i Detection the level of IFN-β and TNF-α in mice serum. All data are presented as mean ± SD (n = 3). For G1-G7, they respectively stand for: G1: PBS; G2: PNPs; G3: TNPs + L; G4: M-NPs; G5: TPNPs + L; G6: M-TPNPs; G7: M-TPNPs + L. L: White light exposure (0.3 W·cm^-2, 10 min). Statistical significance was determined using one-way ANOVA. *p < 0.05, **p < 0.01 and ***p < 0.001.

Figure 7

Biosafety analysis. H&E staining images of the main organs (heart, liver, spleen, lung, and kidney) collected from sacrificed mice. Scale bar: 50 μm. For G1-G7, they respectively stand for: G1: PBS; G2: PNPs; G3: TNPs + L; G4: M-NPs; G5: TPNPs + L; G6: M-TPNPs; G7: M-TPNPs + L.

Supplementary Files
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- Scheme1.png
- Supplementaryinformation.docx