One-Pot Fabrication of Hollow Porphyrinic MOF Nanoparticles with Ultrahigh Drug Loading toward Controlled Delivery and Synergistic Cancer Therapy

Xin Sun, Guihua He, Chuxiao Xiong, Chenyuan Wang, Xiang Lian, Liefeng Hu, Zhike Li, Scott J. Dalgarno, Ying-Wei Yang,* and Jian Tian*

ABSTRACT: Hollow nanostructures have attracted significant research interest in drug delivery systems due to their high capacities for drug loading and unique physicochemical properties, showing great potential in specific biomedical applications. Herein, hollow porphyrinic metal–organic framework (H-PMOF) nanoparticles with a mesoporous spherical shell have been fabricated via a facile self-sacrificial ZIF-8 nanoparticle template strategy. The H-PMOF nanoplatform not only demonstrates a greatly enhanced photodynamic therapy efficacy compared with nonhollow porphyrinic MOF nanoparticles but also can be used as a superior drug carrier to co-load doxorubicin (DOX) and indocyanine green (ICG) with an ultrahigh drug-loading capacity of 635%. Furthermore, cancer cell membrane camouflage of the (DOX and ICG)@H-PMOF composite nanoparticles affords a biomimetic nanoplatform, that is, (DOX and ICG)@H-PMOF@mem (DIHPm for short), with an outstanding homologous tumor-targeting and immune-escaping ability. Interestingly, DIHPm shows both pH-controlled and near-infrared laser-triggered DOX release. Both in vitro and in vivo studies of DIHPm demonstrate an excellent imaging-guided synergistic photodynamic/photothermal/chemotherapy anticancer activity with negligible systemic toxicity. The development of the high-performance H-PMOF nanoplatform provides new insights into the design of MOF-based multifunctional nanomedicines for combination cancer therapy and precise theranostics.

KEYWORDS: hollow nanoparticles, metal–organic framework, cell membrane camouflage, drug delivery, synergistic therapy

1. INTRODUCTION

Photodynamic therapy (PDT) is a minimally invasive anticancer procedure that involves the use of nontoxic photosensitizers (typically porphyrin derivatives), tissue oxygen, and light to induce cell apoptosis and necrosis through the generation of cytotoxic reactive oxygen species (ROS).1,2 PDT is strongly effective for the treatment of superficial cancer but has limited therapeutic efficacy on deep-seated tumors due to the difficulty of light penetration into deep tissue. Moreover, the dependence of PDT on light irradiation also reduces its efficacy for systemically disseminated diseases.3 More efficacious PDT systems and new treatment procedures (e.g., combination therapy) are thus required to strengthen the effect of PDT on both eliminating local tumors and suppressing distant metastases.3,4 Significant efforts have been invested in developing porphyrin-based nanomedicines to improve the phototherapeutic effect of cancer.5 In this regard, porphyrinic metal–organic framework (PMOF) nanoparticles (NPs) have demonstrated great potential as the next generation PDT system.5–11 The constituent porphyrins in porous PMOF structures are well dispersed, meaning that PDT can be greatly enhanced through the prevention of self-quenching from photosensitizer aggregation; the result of this is promoting the diffusion of photogenerated ROS. In addition, PMOF NPs can also be employed as nanocarriers to encapsulate various therapeutic cargoes (e.g., chemotherapy drugs and photothermal reagents) to achieve synergistic cancer therapy.12–14 For instance, Lin and co-workers reported the promising anticancer and antimitastatic effects of indoleamine 2,3-dioxygenase inhibitor (IDOi)-loaded PMOF NPs by combining PDT and IDOi-based immunotherapy.15 In porphyrin-based nanoplatforms, increasing ROS generation efficiency and drug loading play a pivotal role in improving the synergistic effect between PDT and other therapies. Hollow nanostructures with large internal voids and porous shells (e.g., hollow
mesoporous silica NPs) are particularly attractive as superior nanocarriers to realize the most effective loading of therapeutic and imaging agents, the release of which could be precisely controlled by tuning shell structure or function.\textsuperscript{15−17} To our best knowledge, hollow PMOF (H-PMOF) nanostructures with inherent photodynamic activity have not been studied for such a purpose but hold huge potential for biomedical applications.

In order to maximize the therapeutic effect of porphyrin-based nanoplatforms, it is also highly desirable to realize their abilities to target specific tumors and evade immune clearance. Much effort has been devoted to both decrease systemic clearance and enhance recognition through the conjugation of specific targeting moieties on the NP surface, such as folate, antibodies, and peptides.\textsuperscript{18−20} Complex preparations have frequently restricted such approaches, and expression density of the tumor receptor influences tumor-targeting effects. Furthermore, the surface modification of NPs could result in immune clearance to some extent. There is much evidence demonstrating immune escape and homologous binding abilities of cancer cells (CCs) based on the expression of multiple antigens on the cell membrane.\textsuperscript{21−26} Therefore, decorating porphyrin-based nanoplatforms with the homologous CC membrane could be a facile and efficient approach to obtain ideal nanoplatforms for highly targeted and effective cancer treatment.

In this work, we present the development of a new type of porphyrin-based nanoplatform, namely H-PMOF NPs. The synthesis was achieved via a self-sacrificial ZIF-8 NP template strategy in a one-pot solvothermal protocol (Scheme 1). A prefabricated ZIF-8 NP template was gradually digested by the acidic precursors in solution, and a shell of Zr-based porphyrinic MOF formed via the coordination-driven self-assembly of the tetrakis(4-carboxyphenyl)porphyrin (TCPP) ligand and Zr\textsuperscript{4+} ions, affording hollow spherical NPs comprising a mesoporous MOF shell. Compared with previously reported nonhollow PMOF NPs, mesoporous H-PMOF NPs demonstrated greatly enhanced PDT efficacy due to the thin-shell nanostructure, facilitating the interaction of the constituent porphyrins with surrounding O\textsubscript{2} molecules and the diffusion of \( ^{1}\text{O}_{2} \) upon generation (noting the diffusion length is estimated to be \( \sim 20−220 \) nm in cells). More importantly, the biocompatible H-PMOF NPs are well suited for ultrahigh drug-loading content (LC) due to their hollow structure, thus offering function as superior drug nanocarriers with inherent potent PDT. To realize imaging-guided synergistic cancer therapy, an ultrahigh amount of U.S. Food and Drug Administration (FDA)-approved doxorubicin (DOX) and indocyanine green (ICG), used for chemotherapy and photothermal therapy (PTT), respectively, were successfully encapsulated into the H-PMOF to prepare (DOX and ICG)@H-PMOF NPs (DIHP for short). The ICG molecule not only has a high photothermal conversion efficiency with a concomitant low PDT efficiency but also functions as a near-infrared (NIR) dye for \textit{in vivo} fluorescence imaging-guided therapy.\textsuperscript{27} DIHP NPs were subsequently coated with murine mammary carcinoma (4T1) cell membranes to obtain a biomimetic combined nanoplatform, that is, DIHPm. After intravenous injection into the xenograft 4T1 tumor model, the DIHPm nanoplatform demonstrated an outstanding homologous tumor-targeting ability owing to its 4T1 cell membrane camouflage, while at the tumor site, the synergistic PDT/PTT/chemotherapy showed excellent anticancer and anti-metastasis effects at a very low dose. This work highlights the great potential of H-PMOF NPs as multifunctional PDT nanoplatforms for the codelivery of photosensitizers and other therapeutic or imaging agents to achieve a synergistic effect in cancer theranostics.\textsuperscript{28,29}
2. METHODS AND EXPERIMENTS

2.1. Materials. Zinc nitrate hexahydrate [Zn(NO₃)₂·6H₂O], 2-methylimidazole (2-MIM), polyanlypyrrolidone (PVP, MW = 30,000), zirconium tetrachloride (ZrCl₄), 1,3-diphenylisobenzofuran (DPBF), pyrrole, and methyl p-formylbenzoate were purchased from Energy Chemicals (Shanghai, China). DOX hydrochloride was purchased from Beijing Maisuo Chemical Technology Co., Ltd. (China). ICG was provided by Tokyo Chemical Industry Co., Ltd. (Japan). All chemicals were used as received without further purification. TCPP was purchased from Alpha Chemicals. 2,7'-Dichlorofluorescein diacetate (DCFH-DA), 4,6-diamidino-2-phenylindole (DAPI), calcein-AM, Annexin V-FITC/propidium iodide (PI) cell apoptosis kit, bicinchoninic acid protein assay kit, membrane protein extraction kit, and phenylmethanesulfonyl fluoride (PMSF) were provided by Beyotime Institute of Biotechnology (China). Fetal bovine serum, Dulbecco’s modified Eagle’s medium, and RPMI-1640 were obtained from Gibco Life Technologies (USA).

2.2. Preparation of H-PMOF NPs. H-PMOF NPs were prepared via a self-sacrificial metal–organic framework NP (ZIF-8) template strategy. ZIF-8 NPs were first prepared according to the reported method with slight modifications. Brieﬂy, Zn(NO₃)₂·6H₂O (297 mg) in methanol (20 mL) was mixed with 2-MIM (328 mg) in methanol (20 mL) and stirred at room temperature for 30 min. The products were collected by centrifugation (10,151 g, 10 min), followed by washing with methanol several times. The resulting ZIF-8 NPs were dispersed in dimethylformamide (DMF, 20 mL) for further surface modification of PVP. PVP (500 mg) in DMF (20 mL) was added into the ZIF-8 NP suspension, and the mixture was stirred at room temperature for 12 h. The PVP-stabilized ZIF-8 NPs were collected by centrifugation (10,151 g, 30 min) and washed with DMF several times to remove the excessive free PVP and redispersed in DMF.

To obtain H-PMOF, PVP-stabilized ZIF-8 NPs were introduced to a ZnCl₂ solution containing ZrCl₄ and TCPP. Typically, ZrCl₄ (12 mg) and TCPP (4.5 mg) were dissolved in DMF (16 mL). The resulting solution was sonicated for 5 min. Subsequently, PVP-stabilized ZIF-8 NPs (25 mg) were added to the above mixture. The mixture was heated at 120 °C for 5 h and then cooled to room temperature. The resultant material was collected by centrifugation (10,151 g, 30 min) and washed with DMF and water twice, followed by drying under vacuum at room temperature to afford the desired H-PMOF NPs. Yield: 72% based on TCPP. Fresh H-PMOF samples show a signiﬁcant weight loss of solvent up to ~75.4% as revealed by thermogravimetric analysis (Figure S1). Anal. Calc H-PMOF (based of TCPP, 1 mL). Then the mixture was irradiated with laser (750 nm, 10 min) under magnetic stirrer.

2.3. Drug Loading. To quantify the drug LC of H-PMOF, DOX, ICG, and a mixture of DOX and ICG were, respectively, loaded by drying under vacuum at room temperature to a constant weight loss of solvent up to ~75.4% as revealed by thermogravimetric analysis (Figure S1). Anal. Calcd H-PMOF (based of TCPP, 1 mL) for 12 h. The PVP-stabilized ZIF-8 NPs were collected by centrifugation (10,151 g, 30 min) and washed with DMF (20 mL) and stirred at room temperature for 30 min. The products were collected by centrifugation (10,151 g, 10 min), followed by washing with methanol several times. The resulting ZIF-8 NPs were dispersed in dimethylformamide (DMF, 20 mL) for further surface modification of PVP. PVP (500 mg) in DMF (20 mL) was added into the ZIF-8 NP suspension, and the mixture was stirred at room temperature for 12 h. The PVP-stabilized ZIF-8 NPs were collected by centrifugation (10,151 g, 30 min) and washed with DMF several times to remove the excessive free PVP and redispersed in DMF.

To obtain H-PMOF, PVP-stabilized ZIF-8 NPs were introduced to a ZnCl₂ solution containing ZrCl₄ and TCPP. Typically, ZrCl₄ (12 mg) and TCPP (4.5 mg) were dissolved in DMF (16 mL). The resulting solution was sonicated for 5 min. Subsequently, PVP-stabilized ZIF-8 NPs (25 mg) were added to the above mixture. The mixture was heated at 120 °C for 5 h and then cooled to room temperature. The resultant material was collected by centrifugation (10,151 g, 30 min) and washed with DMF and water twice, followed by drying under vacuum at room temperature to afford the desired H-PMOF NPs. Yield: 72% based on TCPP. Fresh H-PMOF samples show a signiﬁcant weight loss of solvent up to ~75.4% as revealed by thermogravimetric analysis (Figure S1). Anal. Calc H-PMOF (based of TCPP, 1 mL). Then the mixture was irradiated with laser (750 nm, 10 min) under magnetic stirrer.

2.4. Preparation of DIHPm and H-PMOF@mem NPs. CC membrane fragments were obtained according to the previously reported literature. Murine mammary carcinoma (4T1) cells were incubated in the T175 culture flask until they were over 80%. The cells were harvested, dispersed in membrane protein extract supplemented with PMSF hypotonic lysate, and placed in an ice bath for 15 min after gently and thoroughly mixed. Furthermore, liquid nitrogen freezing was used to destroy the plasma membrane 3 times. The mixture was centrifuged (700g, 10 min) at 4 °C, and the supernatant was again centrifuged (14,000g, 30 min). The final product, white cell membrane fragments, was quantified by lyophilization. Thereafter, CC-vesicles were acquired by extruding through a 400 nm polycarbonate porous membrane for 11 passes on a mini extruder. Afterward, H-PMOF@mem NPs were prepared by co- extruding H-PMOF with CC-vesicles (mass ratio = 5:1) 11 times. DIHPm was obtained in the same way as H-PMOF@mem via co-extruding DIHP with CC-vesicles.

2.5. Generation and Detection of ROS. Singlet oxygen was detected by measuring the attenuation of DPBF absorbance by UV–vis spectrophotometer. In a typical method, a DPBF/dimethyl sulfoxide solution (10 mM, 10 µL) was mixed with the aqueous solution that includes H-PMOF, PCN-222, or PCN-224 NPs (10 µg mL⁻¹ in terms of TCPP, 1 mL). Then the mixture was irradiated with a 660 nm laser (75 mW cm⁻²) for various periods. Absorbance change of DPBF at 410 nm was detected at regular time points. Single oxygen sensor green (SOSG), a highly sensitive fluorescence probe for O₂ detection, was also employed to detect photogenerated singlet oxygen. A certain amount of SOSG (2.5 µM) was added to H-PMOF, PCN-222, or PCN-224 NPs solutions (10 µg mL⁻¹ TCPP, 1 mL). After being irradiated under 660 nm laser (5 mW cm⁻²) for various periods, the generated ROS of each nanophotosensitizer was determined by measuring recovered SOSG fluorescence under 504 nm excitation. To detect the generation of intracellular ROS, an intracellular fluoroscent microscope was used to detect the fluorescence change of the indicator DCFH-DA. Generally, after 4T1 cells were incubated with phosphate-buffered saline (PBS), free TCPP, PCN-222 NPs, PCN-224, or H-PMOF with the same concentration (40 µg mL⁻¹) for 24 h, the medium was removed. Subsequently, the cells were incubated with DCFH-DA for 30 min and then were irradiated with 660 nm laser (75 mW cm⁻²) for 9 min.

2.6. Photothermal Effect of DIHPm NPs. DIHPm solutions (50 µL) of different concentrations (0, 5, 10, 25, and 50 µg mL⁻¹), the concentration of DIHPm is shown herein in terms of the concentration of ICG) were, respectively, added to 200 µL plastic centrifuge tubes. The solution was irradiated under different powers of 808 nm laser, and the thermal infrared (IR) imaging camera was used to monitor the temperature of DIHPm solutions. The PBS, H-PMOF (25 µg mL⁻¹), DOX (25 µg mL⁻¹), and ICG (25 µg mL⁻¹) aqueous solutions were used as control samples.

2.7. PTT-Promoted Drug Release. Drug-release curves of DIHPm (containing 0.67 mg H-PMOF) were measured in a constant temperature shaker by putting 1 mL of sample solution into dialysis bags (MWCO, 14,000) that were immersed into PBS (pH = 7.4 or 5.0, 200 µL) as a release medium. These experiments were carried out with or without 808 nm laser irradiation (1 W cm⁻², 10 min) at the beginning of the experiment. The concentration of DOX in the release medium was measured by ﬂuorescence spectroscopy with excitation at 496 nm and emission at 596 nm. Both the excitation and emission slit widths were set to 5 nm. Intracellular photothermal promotion of DOX release was monitored by confocal laser scanning microscopy (CLSM). After the cells and DIHPm (10 µg mL⁻¹) were incubated for 8 h, the experimental group was irradiated with laser light (808 nm, 0.5 W cm⁻², 10 min) after the medium was changed. The control group was kept in the dark. Then the medium was discarded and the cell nuclei were stained with DAPI for 12 min. The ﬂuorescence intensity of DOX and ICG was observed.

2.8. In Vitro Cellular Uptake. Three types of cells were used to evaluate the speciﬁc targeting of DIHPm. 4T1, U87MG, and A549 cells were seeded separately in a 6-well plate and incubated at 37 °C for 24 h. Next, cells were treated with DIHPm (10 µg mL⁻¹) for 24 h and then washed, digested, and collected for flow cytometry analysis. In addition, RAW 264.7 and 4T1 cells were, respectively, seeded in glass-bottom culture dishes and cultured for 24 h. DIHP and DIHPm were added at the equivalent dose of H-PMOF NPs (10 µg mL⁻¹). After culturing for 8 h, the cell nuclei were stained with DAPI for 15 min. Subsequently, the cells were treated for CLSM observation.

2.9. In Vitro Antitumor Activity. The cell cytotoxicity study of DIHPm in vitro was tested by the methyl thiazolyl tetrazolium (MTT)
method, similar to the MTT assay procedure that was used to assess biocompatibility. The difference was that after the cells were treated with DIHPm (0, 0.125, 0.25, 0.5, 1, 2, and 4 μg mL⁻¹) for 24 h, the medium was replaced with fresh one and laser treatment was performed next. After the laser treatments were completed and the cells were incubated for another 12 h, the medium was removed and MTT was added. The subsequent steps were also the same as the above experiment. For singlet PDT based on H-PMOF, 4T1 cells were irradiated with 660 nm laser (75 mW cm⁻², 9 min). For ICG-based PTT, 4T1 cells were irradiated with 808 nm laser (1 W cm⁻², 5 min). For combined PDT and PTT, 4T1 cells were irradiated with the 808 nm laser, followed by the 660 nm laser. For the control group, 4T1 cells were cultivated in the dark.

Apoptosis analysis against 4T1 cells was carried out by flow cytometry assay with an Annexin V-FITC and PI double staining kit. Cells were seeded in 6-well plates and treated with DIHPm (10 μg mL⁻¹). After 24 h of culture, the medium was refreshed. For the phototherapy (PDT or PTT or PDT + PTT), the cells were irradiated at the same conditions as described above. 4T1 cells were stained with Annexin V-FITC/PI for 20 min followed by being harvested for flow cytometry measurements. A live/dead cell staining assay against 4T1 cells was performed using an inverted fluorescence microscope. The previous drug and laser irradiation treatments were the same as apoptosis analysis. After the laser treatment was finished, the cells were incubated for another 12 h. Subsequently, the cells were stained with calcein-AM (8 μM) and PI (8 μM) solution in PBS buffer and observed by the inverted fluorescence microscope. The calcein-AM and PI excitation lasers were 488 and 543 nm, respectively.

2.10. In Vivo Imaging and Biodistribution. All animal experiments were performed under the approval of the Institutional Animal Care and Use Committee of Wuhan University, China. To model the 4T1 tumor-bearing mice, 4T1 cells (1×10⁶ cells) were implanted subcutaneously into the right hind leg region of female Balb/c mice (6–8 weeks). After the tumor volume reached 80–100 mm³, DIHP or DIHPm was administrated intravenously at an equivalent dose (0.4 mg kg⁻¹). The in vivo NIR fluorescence imaging was performed using the NIR-II fluorescence imaging system at the desired times after dosing (for ICG, Ex: 808 nm). They were sacrificed 24 h post-injection, and the major organs were imaged for biodistribution. Photothermal imaging was monitored with an IR thermal camera (FOTRIC 225).

2.11. In Vivo Antitumor Evaluation. The 4T1 tumor-bearing mice were modeled with the above-described method. When the tumor volume reached about ~100 mm³, 40 mice were randomly
Figure 2. In vitro photodynamic effects of H-PMOF NPs. (a) Singlet oxygen generation evaluated by DPBF consumption with 660 nm laser irradiation of PBS, PCN-222, PCN-224, and H-PMOF NPs at the same TCPP concentration. (b) Cytotoxicity of PCN-222, PCN-224, and H-PMOF NPs to 4T1 cells under 660 nm laser irradiation. Intracellular ROS detection under 660 nm laser irradiation using DCFH-DA as the sensor: (c) inverted fluorescence microscope images and (d) corresponding MFI values of 4T1 cells after treatment with PBS, free TCPP, PCN-224, PCN-222, and H-PMOF NPs. Scale bar: 100 μm. Data are presented as means ± standard deviation (sd) (n = 3) (**P < 0.05, ***P < 0.001).

3. RESULTS AND DISCUSSION

3.1. Fabrication and Characterization of H-PMOF. H-PMOF NPs were prepared in a one-pot solvothermal reaction using a prefabricated self-sacrificial ZIF-8 NPs template.30 ZIF-8 NPs with a diameter of ~90 nm were prepared according to a reported procedure (Figure 1a).31 followed by the surface modification of PVP to increase their dispersibility in solution and affinity with MOF precursors. The resulting well-dispersed ZIF-8@PVP NPs were then used as cores of the heterogeneous nucleation for the growth of external PMOF layers driven by the coordination self-assembly between the TCPP ligand and Zr4+ ions, while the cores were gradually digested by the acidic precursors in the solvothermal synthesis (Figure S2). Transmission electron microscopy (TEM) was used to monitor the growth of H-PMOF NPs. After ~10 min of the solvothermal reaction, ZIF-8 NPs were etched away, and regular hollow spheres with respective cavity size and shell thickness of ~90 and ~14 nm were formed (Figure 1c). A further increase in reaction time to 5 h showed no obvious shell thickness change, likely as a result of the exhaustion of MOF precursors (Figures S3 and S4). The H-PMOF NPs with ~90 nm cavity size and ~24 nm shell thickness were selected for use in the following studies due to the aforementioned properties but also for the reason that elemental mapping based on high-angle annular dark-field scanning TEM (HAADF-STEM) revealed a clear contrast of element distribution between the cavity and the shell (Figure 1d). It should be noted that the H-PMOF morphology could be further regulated in terms of cavity size/shell thickness by varying the size of the ZIF-8 NP sacrificial starting materials as well as the solvothermal reaction time (Figure S5), giving access to a highly controllable synthetic strategy. Moreover, powder X-ray diffraction (PXRD) patterns showed that H-PMOF NPs had the same characteristic peaks with reported patterns of Zr-based PMOF PCN-222 NPs and the simulated
indicating the successful formation of hollow NPs without altering their crystallinity (Figure 1e). The optical properties of H-PMOF were studied by UV−vis and fluorescence spectrum (Figure S6). UV−vis absorption spectroscopy of H-PMOF showed a Soret band at 419 nm and four Q-bands at 519, 552, 592, and 650 nm, similar to that of isostructural PCN-222 NPs. Besides, the H-PMOF exhibited 660 nm excited fluorescence emission with a maximum at ∼724 nm, revealing its potential as a general theranostic platform. The porosity of H-PMOF was evaluated by nitrogen adsorption−desorption studies at 77 K (Figure 1f). H-PMOF NPs show the presence of significant volumes of macropores ascribed to the hollow cavity and mesopores (∼3.4 nm) and micropores (∼1.3 nm) of the outer shell with the PCN-222 type structure (Figure 1g). The surface area of the H-PMOF NPs was determined to be ∼120 m²/g using the Brunauer−Emmett−Teller method. All of these results verified the formation of H-PMOF NPs possessing a large cavity and a mesoporous shell.

3.2. Photodynamic Effect of H-PMOF NPs. To assess the photodynamic activity of H-PMOF NPs, DPBF was used as a probe molecule since it shows a decreased absorption intensity at ∼410 nm in the presence of 1O₂. The slope of the decay curve of DPBF absorption is observed to be proportional to the efficiency of 1O₂ generation. As shown in Figure 2a, the H-PMOF sample provided a steeper decline compared with previously reported nonhollow Zr-based PMOF NPs (PCN-222 and PCN-224) within a 9 min 660 nm laser irradiation, indicating a stronger capability of 1O₂ generation. Notably, the H-PMOF sample approached the equilibrium of 1O₂ generation quickly, implying very efficient interactions of photosensitizers with oxygen molecules presumably due to its thin-shell nanostructure. The fluorescence intensity of SOSG, a single oxygen fluorescent probe, also confirmed the PDT activity of H-PMOF NPs to be about 1.7-fold of that of PCN-224 NPs and more than 2-fold that of the isostructural PCN-222 NPs (Figure S7).
4T1 cells compared with the well-studied PCN-222 and PCN-224 NPs (~140 nm, Figure S9) at the same mass concentration (Figure 2b). The hexagonal-inhibitory concentration (IC<sub>50</sub>) of H-POMOF is 9.9 μg mL<sup>−1</sup>, far smaller than those of PCN-224 (48.9 μg mL<sup>−1</sup>) and PCN-224 NPs (28.5 μg mL<sup>−1</sup>). It was noted that the temperature of the H-POMOF aqueous solution only changed slightly following 5 min of 660 nm laser irradiation, excluding the photothermal effect of H-POMOF NPs (Figure S10). To investigate the intracellular ROS generation of H-POMOF NPs for PDT, DCFH-DA was adopted as the ROS-specific probe. Free TCPP, PCN-222, and PCN-224 NPs have also been investigated for comparison. After light irradiation, 4T1 cells incubated with free TCPP just showed very weak green fluorescence corresponding to a low amount of ROS production, which might be attributed to the poor solubility of TCPP in the aqueous phase. However, 4T1 cells incubated with H-POMOF showed significant green fluorescence and the fluorescence intensity was noticeably stronger than that found on treatment with PCN-222 or PCN-224 NPs, indicating that H-POMOF has a stronger ROS production capability (Figure 2c,d), which is consistent with the results of DPBF and SOSG assay. Compared to nonhollow PMOF NPs, the thin-shell nanostructure of H-POMOF could allow more efficient interactions of constituent photosensitizers with surrounding oxygen molecules, as well as a shorter diffusion distance of generated O<sub>2·</sub>, considering that O<sub>2·</sub> has a small action range (~20 nm). Therefore, the H-POMOF NPs platform possesses a much stronger PDT effect and can generate significantly more ROS, rendering it very beneficial for the promotion of PDT as a drug nanocarrier.

### 3.3. Drug Loading of DOX and ICG and Cell Membrane Camouflage

Inspired by the large pore volume of the hollow nanostructure, the drug-loading behaviors of H-POMOF NPs were investigated with DOX and ICG as model drugs that were, respectively, used for chemotherapy and PTT. As shown in Figures 3a and S11, through simply mixing DOX and H-POMOF NPs for 1 h at the feeding ratio of 6:1, the drug LC and EE of DOX were calculated to be 485 wt % and 80.8%, respectively, which are significantly higher than those achieved by DOX loading in nonhollow PCN-222 NPs with a LC of 109 wt % and an EE of 54.5%. Notably, such an ultrahigh LC of DOX is among one of the highest drug-loading capacities in reported hollow nanostructures. The loading curves of H-POMOF NPs toward DOX, ICG, and DOX and ICG were further studied (Figure S12). A very fast loading behavior of DOX and DOX and ICG is found within the first 10 min, followed by a slight increment until the adsorption balance at ~60 min. An extremely high drug loading together with fast loading kinetics demonstrates the great potential of H-POMOF NPs as platforms for multimodal treatment. Next, DIHPm was fabricated by co-extruding DIHP and CC membrane vesicles 11 times through a mini extruder and were characterized by TEM (Figure 3c). All DIHPm NPs showed particularly pronounced cavity structures and two-layer outer structures, affirming the coating of DIHP NPs by thin CC membrane shells. Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter and the ζ potential change. After CC membrane coating, the surface charge of DIHPm changed to approximately that of CC membrane vesicles, while its average hydrodynamic size (~235 nm) was virtually unchanged; the latter may be attributable to the increased water dispersibility of DIHPm upon coating (Figures 3d,e and S13). Previous work has shown that homotypic targeting and immune escaping capabilities of CCs were primarily ascribed to abundant antigens on their cell membranes. To evaluate membrane proteins on DIHPm, protein components were analyzed using gel electrophoresis. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that the protein profile of DIHPm was near-identical to that of the purified CC membrane (Figure 3f), indicating that the membrane protein remained intact after fusion. With DIHPm surface coating confirmed, it was expected that DIHPm would inherit the advantages of the constituent cell membranes, which could give DIHPm immune-escaping and homotypic binding properties.

The colloidal stability of DIHPm in water, N-(2-hydroxyethyl)piperazine-N′-ethanesulfonic acid (HEPES), and PBS buffer was evaluated by the DLS measurements (Figure S14). It was observed that the hydrodynamic size and polydispersity index of DIHPm did not change significantly after being dispersed in water for 8 days or 24 h in HEPES and PBS solutions, confirming that DIHPm possesses satisfactory stability at normal physiological conditions.

### 3.4. Photothermal Effect of DIHPm and pH and Photothermal-Triggered Release of DOX

Although ICG, an FDA-approved bifunctional drug, has been used in noninvasive bioimaging and phototherapy (primary PTT and minor PDT), its clinical use is hindered by its concentration-dependent aggregation, photobleaching, and other unfavorable properties in aqueous solutions. In addition, ICG can be rapidly cleared from the body as a result of its facile binding to nonspecific plasma proteins. Several nanosized drug delivery systems (e.g., lipid, MOF, and super-paramagnetic iron oxide-based nanoplates) have been developed to protect the stability of ICG and facilitate its PTT application in vivo.

The fluorescence intensity of free ICG in H<sub>2</sub>O dropped significantly to 12% after 1 week in our experiments (Figure S15), while the fluorescence intensity of DIHPm remained at around 80%. It is inferred that the fluorescence stability of ICG was markedly improved upon loading into DIHPm due to the protective effect of cell membrane coating. The photothermal effect of DIHPm was investigated using an 808 nm NIR laser (1 W cm<sup>−2</sup>) irradiation. As shown in Figure 3g, the temperature of DIHPm aqueous suspension with an ICG concentration of 25 μg mL<sup>−1</sup> increases steadily by ~52 °C.
within 150 s of irradiation. However, the temperature of the free ICG solution with the same concentration started to decrease after 1 min of irradiation due to its poor photostability. In comparison, the control samples including PBS, DOX suspension, and H-PMOF suspension only gave a temperature increase of less than 3 °C. Moreover, the temperatures of DIHPm suspension can be finely controlled by varying DIHPm concentration (in terms of ICG) and irradiation laser power (Figures 3h and S16). These results demonstrated that DIHPm had an outstanding and tunable photothermal effect.

The release of DOX from DIHPm in the physiological environment and tumor microenvironment is of great interest. A release study was conducted in PBS buffer at pH 7.4 and 5.0. As shown in Figure 3i, the overall release content of DOX reached 54% after 140 h incubation at pH 5.0. This is in contrast to the final release of only 24% observed at pH 7.4. Notably, the release of DOX after 808 nm laser irradiation increased significantly to 65% at pH 5.0. Therefore, we propose that the acidic environment and photothermal effect would promote the release of DOX within DIHPm.43−45 Next, we investigated the intracellular release of DOX in 4T1 cells treated with DIHPm. Following 808 nm laser irradiation the intracellular fluorescence intensity of DOX increased significantly as observed by CLSM (Figure 4a). The promoted intracellular release of DOX was a consequence of the photothermal effect of DIHPm NPs, boosting the diffusion of loaded DOX from DIHPm as a result.

3.5. Homologous Targeting and Immune Escaping. The homologous targeting ability of DIHPm was assessed
through cellular uptake against A549, U87MG, and 4T1 cells and quantified by flow cytometry. As shown in Figure 4b, the fluorescence measured in homotypic 4T1 cells was far stronger than that of other cell lines, indicating the highly specific association between DIHPm and 4T1 cells. Such an excellent result was more directly demonstrated by the quantification of flow cytometry results (Figure 4c). Cell uptake of DIHPm was evaluated against 4T1 cells, with DIHP employed as a control (without cell membrane coating). As expected, the respective green and red fluorescence of DOX and ICG observed in 4T1 cells treated with DIHPm was significantly stronger than in those treated with DIHP (Figure 4a). This cellular uptake, promoted by homotypic targeting ability, was supposed to enhance the effectiveness of tumor treatment and reduce the side effects in normal tissues. Except for homotypic binding, the tumor-associated antigens and immunological adjuvants present on the CC membrane were also proved to account for the immune evasive properties of CCs.\textsuperscript{35−37} Inspired by previous studies, the immunocompatibility of DIHPm was assessed using RAW 264.7 murine macrophages. As expected, much weaker red and green fluorescence, respectively, was observed for ICG and DOX in RAW 264.7 cells after the DIHPm treatment relative to those treated with DIHP (Figure 4d). This implied that CC membrane cloaking on the DIHP NPs could realize enhanced macrophage engulfment inhibition and immune evasive capability.

3.6. In Vitro Synergistic Therapeutic Efficacy. The efficient cellular internalization and intrinsic photodynamic features, coupled with excellent photothermal effects and the photothermal-promoted DOX release, suggest that DIHPm could have outstanding \textit{in vitro} anticancer performance. The synergistic therapeutic effect of DIHPm was evaluated on 4T1 cells by the standard MTT assay. NIR light (808 nm or/and 660 nm lasers) was used to irradiate 4T1 cells following incubation with varying concentrations of DIHPm for 24 h. As indicated in Figure 5a, the inhibition of CCs gradually enhanced with the dosage of DIHPm increasing from 0.125 to 4 \( \mu \)g mL\(^{-1}\). The IC\textsubscript{50} of DIHPm + 808 nm + 660 nm is 0.11 \( \mu \)g mL\(^{-1}\), smaller than that of DIHPm in dark (3.86 \( \mu \)g mL\(^{-1}\)), DIHPm + 808 nm (0.41 \( \mu \)g mL\(^{-1}\)), and DIHP + 660 nm (0.21...
μg mL⁻¹), indicating a superior synergistic therapeutic performance and a predominant contribution of H-PMOF-based PDT in the synergistic therapy. Notably, excellent CC killing was achieved by the trimodal therapy even at a very low dose of 2 μg mL⁻¹, with only 8% of cell viability at this point. Prominent cytotoxicity with ultralow doses indicated the superiority of DIHPm for combined therapy in vitro. To visualize the in vitro anticancer efficacy of DIHPm, live/dead cell staining was applied to 4T1 cells incubated with 4 μg mL⁻¹ of DIHPm. It was demonstrated that 4T1 cells were dramatically killed after dual laser irradiation and there were almost no living cells observed (Figure 5b), which is consistent with the results of the corresponding MTT assay. Over 37% of 4T1 cells treated with DIHPm under both 808 and 660 nm laser irradiation were at the stage of apoptosis (Figure 5c). This is notably higher than the group without or with just single source irradiation, indicating an outstanding synergistic effect in CC killing.

3.7. In Vivo Tumor Imaging and Pharmacokinetics. Given the highly encouraging results outlined above, we further explored the tumor-targeting ability of DIHPm in the animal tumor model. DIHPm or DIHP were intravenously administered into 4T1 tumor-bearing mice at a low dosage of 0.4 mg kg⁻¹. NIR fluorescence images of the mice at the predetermined time are shown in Figure 6a. For mice injected with DIHPm, obvious fluorescence signals of ICG were observed at the tumor site from 2 to 24 h post-injection, and the tumor fluorescence signal was strongest at 4 h, indicating the highest tumor accumulation time for DIHPm. Therefore, 4 h post-injection was selected as the optimal time for the follow-up phototherapy. In stark contrast, a much weaker fluorescence signal was found at the tumor site of mice injected with DIHP,
while most of the fluorescence signal appeared in the liver, indicating an insufficient tumor accumulation of DIHP via the electron paramagnetic resonance (EPR) effect at such a low dose. In addition, tumors and major organs of mice were collected from 24 h post-injection for a semi-quantitative biodistribution study based on ex vivo NIR fluorescence imaging (Figure 6a). As shown by mean fluorescence intensity (MFI) analysis (Figure 6b), for mice treated with DIHPm, the NIR fluorescence signal intensity observed at the tumor site was much higher than that of normal organs. In contrast, the fluorescence signal in mice treated with DIHP only occurred in normal organs and mostly in the liver, consistent with previous fluorescence imaging in vivo. The great difference observed in tumor accumulation between DIHP and DIHPm suggests that

Figure 7. In vivo evaluation of DIHPm-based synergistic PDT/PTT/chemotherapy. (a) Tumor growth curves after 10 different treatments. The tumor volume was relative to their initial sizes. (b) Body weight changes of mice after different treatments. (c) Tumor weight in different treatments obtained at day 17 post treatment. (d) Representative photographs and (e) H&E-stained sections of 4T1 tumors subjected to different treatments, respectively: (1) PBS, (2) PBS + 808/660 nm, (3) H-PMOF@mem, (4) H-PMOF@mem + 808/660 nm, (5) free DOX and ICG (D + I), (6) D + I + 808/660 nm, (7) DIHPm, (8) DIHPm + 660 nm, (9) DIHPm + 808 nm, and (10) DIHPm + 808/660 nm. Scale bar: 50 μm. (f) Blood biochemical analysis of various treatment groups after 17 days. (g) Lung and (h) liver metastases after PBS or synergistic treatments for 17 days. Data are presented as means ± sd (n = 4 mice per group) (**p < 0.001).
this membrane-coating strategy had remarkable advantages in terms of blood circulation time and tumor targeting. It is worth noting that certain proteins on the CC membrane, including Rho family proteins and focal adhesion protein, could avoid the immune recognition of DIHPm and prolong blood circulation time, the result being a further improvement in tumor accumulation of DIHPm.\textsuperscript{46–48} In vivo photothermal imaging of the tumors was performed with a NIR thermal imaging camera at 4 h post intravenous injection of DIHPm or DIHP with a dose of 0.81 mg kg\textsuperscript{-1}. In the case of DIHPm treatment, the temperature of the tumor site rapidly increased from 32.6 to 51.3 °C upon 808 nm laser irradiation for 2 min and then the highest temperature of 54.3 °C which was sufficient to kill tumor cells.\textsuperscript{38} In contrast, there was no significant temperature increase at the tumor sites of the PBS or DIHP treatment group (Figure 6c,d). These results further proved that DIHPm had favorable tumor accumulation ability and provided NIR fluorescence imaging and photothermal imaging guidance for in vivo therapy.

3.8. In Vivo Antitumor and Anti-metastasis Performance. On the basis of these exciting specific tumor targeting and bioimaging results, we further explored the synergistic therapeutic effect of DIHPm in vivo with the mice bearing 4T1 breast tumors. A total of 40 mice were divided into ten groups randomly (4 mice per group): (1) PBS, (2) PBS + 808/660 nm, (3) H-PMOF@mem, (4) H-PMOF@mem + 808/660 nm, (5) free DOX and ICG (D + I), (6) D + I + 808/660 nm, (7) DIHPm, (8) DIHPm + 660 nm, (9) DIHPm + 808 nm, and (10) DIHPm + 808/660 nm. The mice were intravenously injected with PBS, free DOX and ICG (D + I), and H-PMOF@mem or DIHPm solution at low doses; these were 0.11 mg kg\textsuperscript{-1} for H-PMOF, 0.29 mg kg\textsuperscript{-1} for ICG, 0.41 mg kg\textsuperscript{-1} for DOX, and 0.81 mg kg\textsuperscript{-1} for DIHPm. Laser irradiation (660 or 808 nm or combined 808 nm/660 nm) was administrated at 4 h post-injection, and the tumor volumes in each group were recorded and normalized against their volumes of the first day (Figure 7a). Compared with DIHPm, the single H-PMOF@mem or free DOX and ICG with dual laser irradiation only showed partial tumor growth inhibition effect. The combination of the PTT and chemotherapy of photothermal-promoted DOX release using DIHPm exhibited a better tumor-growth inhibition effect than 660 nm excited PDT or the dual-modal chemotherapy and PDT. As expected, owing to the sufficient tumor retention of DIHPm even at such low doses, the PDT/PTT/chemotherapy trimodal treatment significantly suppressed tumor growth compared with other treatment groups. Three mice in this group had completely eliminated tumors without recurrence. There was no significant change in the body weight of all mice in the 10 treatment groups before they were sacrificed at 17 d post-injection (Figure 7b).

The excised tumors of all mice were weighed separately and representative tumors from each group were photographed. As shown in Figure 7c,d, the mice injected with DIHPm under trimodal treatment demonstrated the lightest tumor weight and the smallest tumor size, which is in line with the above experimental results. To further illustrate the antitumor effect of DIHPm from the perspective of histological analysis, H&E staining of typical mice was performed. Obvious destruction and death of tumor tissues were observed after the mice were treated with DIHPm under dual laser irradiation. In contrast, part or most of the tumor tissues in the other treatment groups retained the normal cell morphology (Figure 7e). These results clearly show that DIHPm possesses excellent antitumor performance. To evaluate the biosafety of DIHPm in vivo, blood samples from representative groups (groups 1, 3, 7, and 10) were collected for further analysis. The physiological and biochemical blood indexes of the four groups demonstrated favorable biocompatibility and low systemic toxicity of the employed membrane-coating strategy (Figure 7f and Table S2, Supporting Information).

Since lethal hepatic and pulmonary metastasis is the main cause of mortality in breast cancer patients,\textsuperscript{45,49,50} the histological analysis of liver and lung of the PBS control group and DIHPm group with dual laser irradiation was performed to observe tumor metastasis in vivo. The mice treated with DIHPm + 808/660 nm displayed no visible lung metastatic lesion, while obvious pulmonary metastasis of the PBS control group was observed (Figure S17). According to the quantitative analysis, the number of metastatic lung nodules was significantly reduced after the combined PDT/PTT/chemotherapy treatment (Figure 7g). Clear hepatic metastases were also observed in the liver of the PBS control group. However, the hepatic micro metastases were remarkably reduced following DIHPm treatment (Figure 7h). The results demonstrated that DIHPm-based trimodal treatment could effectively inhibit distant metastasis of 4T1 cells. It is inferred that the extended blood circulation time of DIHPm in vivo and high accumulation in the tumor could inhibit metastases. In addition, the combination of PDT and PTT might also stimulate the immune system against tumor growth and metastasis. Based on therapeutic evaluations we conclude that because of the homologous targeting strategy of cell membrane camouflage and highly efficient synergistic treatment of the tumors, DIHPm in vivo could not only significantly ablate primary tumors but also remarkably suppress tumor metastasis to other organs.

In porphyrin-based nanomedicines, the liposomal nanostructures with hollow cores have been extensively used for photosensitizer delivery in human clinical trials. However, they suffer from a relatively low loading capacity of porphyrins intercalated in the enclosed lipid bilayer shell or the self-quenching of the photosensitizer when trapped in a highly stacked nanostructure; these greatly hinder PDT efficacy.\textsuperscript{51} Compared to liposomal porphyrin nanostructures, the hollow NPs of porphyrinic MOFs not only retain the hollow cavity to ensure ultrahigh-capacity drug delivery but also realize high LC of porphyrinic photosensitizers in the MOF shell, wherein the self-quenching from photosensitizer aggregation can be prevented/overcome. In addition, the mesoporous shell allows for the enhanced diffusion of ROS and controlled release of encapsulated drugs. The H-PMOF nanoplatform also has multimodal imaging capacities. Except for the intrinsic fluorescence imaging of porphyrins, it can be further endowed with copious imaging functionalities such as magnetic resonance imaging, positron emission tomography, or single-photon emission computed tomography through metal chelation on the constituent porphyrins. These merits collectively make H-PMOF nanaostructures an intriguing multifunctional theranostic platform with excellent PDT efficacy and concomitant superior drug delivery/loading properties for combined cancer therapy. In the case of PDT and combined therapy, the amount of drug-loaded nanoparticles (DIHPm) internalized into targeted tumor cells is critical for the efficacy of synergistic treatment. Passive targeting via the EPR effect may not result in sufficient cellular
internalization and tumor accumulation. As has been extensively demonstrated, active homologous tumor targeting through CC membrane camouflage can efficiently increase selective cellular internalization and specific tumor accumulation of DIHPm, which not only improves the tumor-specific combined therapy efficacy but also minimizes nonspecific side effects in normal tissues.

4. CONCLUSIONS

In summary, we have developed a facile and efficient one-pot synthetic strategy to construct H-PMOF NPs via a self-sacrificial ZIF-8 NPs template. This delicate NPs engineering endows the PMOF nanoplates with greatly enhanced PDT efficacy and extremely high drug-loading capacities. Based on these advantages, we fabricated a multifunctional biomimetic nanoplatform (DIHPm) by encapsulating DOX and ICG into H-PMOF NPs with ultrahigh loading capacities, followed by the coating of CC membranes. The DIHPm realizes outstanding homologous tumor targeting due to the cell membrane camouflage and shows imaging-guided synergistic PDT/PTT/chemotherapy with excellent efficiency for killing tumor cells in vitro and inhibiting tumor growth and metastasis in vivo at very low doses. The high-performance H-PMOF nanoplatform represents a new class of multifunctional phototheranostic vectors with ultrahigh drug-loading capacity and good biocompatibility. Work continues with a view to advancing the development of therapeutic hollow MOF nanostructures as powerful theranostic platforms, the results of which will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c20617.

Additional experimental details; thermogravimetric analysis of fresh H-PMOF NPs; schematic illustration of the proposed mechanism for the fabrication of H-PMOF; TEM and scanning electron microscopy images of the different PMOF samples, UV–vis spectra and fluorescence emission spectra, colloidal stability of DIHPm in H2O, PBS, and HEPES solutions, cell viabilities of different NPs in dark, fluorescence stability of DIHPm, and blood biochemistry data (PDF).

AUTHOR INFORMATION

Corresponding Authors

Ying-Wei Yang — State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, International Joint Research Laboratory of Nano–Micro Architecture Chemistry (NNAC), College of Chemistry, Jilin University, Changchun 130012, P. R. China; orcid.org/0000-0001-8839-8161; Email: ywyang@jlu.edu.cn
Jian Tian — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China; orcid.org/0000-0003-1032-6410; Email: jian.tian@whu.edu.cn

Authors

Xin Sun — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Guihua He — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Chuxiao Xiong — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Chenyuan Wang — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Xiang Lian — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Liefeng Hu — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Zhike Li — Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China
Scott J. Dalgarno — Institute of Chemical Sciences, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh EH14 4AS, U.K.; orcid.org/0000-0001-7831-012X

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (grant nos. NSFC 21601140, 21871214); the Fundamental Research Funds for the Central Universities (2042017kf0186); and Open Research Fund of State Key Laboratory of Bioelectronics, Southeast University.

REFERENCES

Photodynamic Therapy.

Applications.


