Renal-Clearable PEGylated Porphyrin Nanoparticles for Image-Guided Photodynamic Cancer Therapy

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Nanomaterials with renal clearance from the body within a reasonable timescale have shown great promises in the area of nanomedicine recently. However, the integration of theranostic and renal clearance properties into a single ultrasmall nanostructure remains a great challenge. Herein, meso-tetra(4-carboxyphenyl)porphyrin (TCPP) structure is utilized as a model, for the first time using noninvasive dynamic positron emission tomography (PET) imaging to investigate the balance of the renal clearance and tumor uptake behaviors of polyethylene glycol (PEG)-modified porphyrin nanoparticles (TCPP-PEG) with various molecular weights. This study finds that TCPP-PEG nanoparticles with larger molecular weight show higher tumor uptake due to the enhanced permeability and retention effect, while the lower ones tend to be better for renal clearance. Based on dynamic PET and fluorescence dual-modal imaging modalities, the TCPP-PEG10k nanoparticles seem to be an excellent choice for the balance of renal clearance and tumor retention. In vitro and in vivo photodynamic therapy confirms an excellent therapeutic efficacy. Therefore, this work presents a simplified approach to fabricate and select biocompatible multifunctional TCPP-PEG-based theranostic agents with renal clearance behavior, which highlights the clinical application potential of TCPP-PEG nanoparticles as theranostic probes for imaging-guided cancer therapy.

1. Introduction

Cancer has overtaken heart disease to become the leading cause of death worldwide.[1] Fortunately, with the continued development of nanomaterials and nanotechnology, new types of effective cancer treatment, such as photothermal therapy (PTT) and photodynamic therapy (PDT), have emerged as powerful cancer therapy techniques.[2] Various inorganic/organic nanomaterials including carbon-based nanomaterials, gold-based nanomaterials, metallic nanoparticles, transition metal dichalcogenides, organic polymers, and albumin carriers have shown great potential for cancer treatment due to the unique physiochemical properties.[2a,b,3] However, most of these nanoparticles suffer from high uptake by the reticuloendothelial system (RES), resulting in slow and inefficient clearance. According to the U.S. Food and Drug Administration principle, any diagnostic or therapeutic agent should be completely cleared from the body within a reasonable period of time.[4] Considering the unique structure of the glomerular capillary wall, only ultrasmall nanoparticles with hydrodynamic diameters (HDs) less than 5.5 nm can pass through the kidneys and rapidly undergo renal excretion. Previous studies have shown that nanoparticles ranging from 15 to 50 nm are readily sequestered by the RES, while those nanoparticles larger than 50 nm undergo partial hepatobiliary clearance.[5] In general, renal clearance is much faster than hepatobiliary clearance. Both nanoparticle size and surface coating will affect the HDs and the way in which the nanoparticle is cleared from the body,[5a,6] and it is a great challenge to fabricate renal-clearable nanomaterials.

In clinical applications, increased circulation time and exposure of nanomaterials to healthy tissues have strongly correlated...
with increased toxicities and adverse effects. However, the rapid removal of nanoparticles by renal clearance results in lower tumor accumulation, weaker imaging sensitivity, and poorer therapeutic effects. Therefore, it is difficult to design nanomaterials that satisfy the balance between the imaging and therapeutic desires and the requirement for renal clearance within a reasonable timescale. Recently, glutathione-coated ultrasmall gold nanoparticles (2.3 ± 0.3 nm) showed rapid renal clearance. However, the ultrasmall gold nanoparticles displayed slow clearance and gradual passive tumor retention after coating with PEG. Liu et al. also developed iron coordination polymer nanodots with renal clearance properties for cancer theranostic applications. Ultrasmall polymer nanodots showed excellent renal clearance and effectively accumulated in the tumor via the enhanced permeability and retention (EPR) effect for magnetic resonance imaging-guided PTT. However, fabricating renal-clearable nanomaterials with desirable multiple functionalities remains a great challenge.

Herein, we investigate the clearance and tumor uptake behavior of nanoparticles using meso-tetra(4-carboxyphenyl)porphyrin (mTcP) fabricated with polyethylene glycol (PEG) as a model. TCPP and their derivatives are widely employed for cancer diagnosis and PDT, as they have shown enhanced affinity to cancer cells. However, the hydrophobic nature of most porphyrins has limited their potential use in clinic. We hypothesize that the addition of PEG to TCPP would enhance the aqueous solubility and increase the molecular volume, resulting in enhanced tumor localization. Thus, different molecular weights of PEG molecule (2, 5, 10, and 30 K) are conjugated to TCPP, and the resulting TCPP-PEG nanoparticles are tested for clearance and tumor uptake behaviors. Moreover, TCPP is a good chelating agent for the isotope $^{64}$Cu, a useful positron emission tomography (PET) radionuclide with a 12.7 h half-life, for PET imaging. As a noninvasive analytical technique, we first investigate the dynamic PET imaging of TCPP-PEG nanoparticles with various molecule weights after intravenous (i.v.) injection. It was found that larger sizes of the nanoparticles are better for tumor uptake, while the smaller ones are more amenable to renal clearance. Under the guidance of PET and fluorescence dual-modality imaging, TCPP-PEG nanoparticles with a HD size of ~10 nm would be a good candidate for balancing of clearance and tumor uptake behaviors. In vitro and in vivo PDT is carried out which gave excellent therapeutic efficacy for the selected nanoparticles. Our study presents a simplified approach to fabricate and indicate biocompatible multifunctional TCPP-PEG-based theranostic agents with clearance behavior, which highlights the clinical application potential of TCPP-PEG nanoparticles as theranostic probes for image-guided cancer therapy.

2. Results and Discussion

TCPP porphyrin nanoparticles with various molecular weights of PEG chains were synthesized using a simple amide coupling reaction (Figure 1a). Unlike the previous porphyrin-PEG mesh structure, only a single NH$_2$ terminal of PEG polymer was used. Carboxyl groups on TCPP were first activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and then PEG was added into the reaction with a ratio of 2:1 (TCPP:PEG) to ensure only one carboxyl group of TCPP molecule was conjugated with a single polymer chain of PEG. After the reaction, several suitable filters were used to remove excess free TCPP molecules and coupling reagents. Matrix-assisted desorption/ionization time-of-flight (MALDI-TOF) spectra were used to confirm the molecular weight of the various synthesized TCPP-PEG (2, 5, 10, and 30 K) polymers. From the spectra, we found that mostly single PEG polymers were linked to the surface of TCPP molecules to form TCPP-PEG nanoparticles with various molecular weights based on the PEG used for synthesis (Figure S1, Supporting Information). However, when the ratios of TCPP-PEG changed to 1:1 or lower, mixtures of TCPP-PEG polymers were found in the system and were too complicated to purify (Figure S2, Supporting Information). $^1$H nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectra also confirmed that various molecular weight of PEG molecules were conjugated on TCPP (Figures S3 and S4, Supporting Information). Transmission electron microscopy (TEM) images revealed that all these synthesized porphyrin polymers existed in a micelle structure with sizes of 3.6 ± 1.4, 5.4 ± 2.3, 8.8 ± 1.6, and 14.2 ± 2.8 nm for TCPP-PEG$_{2k}$, TCPP-PEG$_{5k}$, TCPP-PEG$_{10k}$, and TCPP-PEG$_{30k}$, respectively (Figure 1b). By increasing the molecular weight of PEG conjugates, the HDs also increased. The final HDs were 4.6 ± 1.4, 7.5 ± 2.2, 10.1 ± 2.8, and 17.3 ± 3.2 nm for TCPP-PEG$_{2k}$, TCPP-PEG$_{5k}$, TCPP-PEG$_{10k}$, and TCPP-PEG$_{30k}$ nanoparticles, respectively, agreed with the above TEM results (Figure 1c). All these TCPP-PEG nanoparticles were highly stable in various physiological solutions without aggregation for 3 months (Figures S6 and S7, Supporting Information). Zeta potential of all TCPP-PEG nanoparticles was slightly negative (Figure S8, Supporting Information), confirming that parts of TCPP-PEG nanoparticles satisfy the size and surface charge threshold requirements for renal clearance. In addition, there was no fluorescence quenching after PEG conjugation (Figure S9, Supporting Information), indicating that our synthesized TCPP-PEG nanoparticles were viable fluorescence imaging agents.

Porphyrins and their derivatives can be readily radiolabeled with copper-64 ($^{64}$Cu) for PET imaging, and dynamic PET imaging provides a highly useful tool for studying renal clearance behavior. By simply mixing $^{64}$Cu$^{2+}$ with TCPP-PEG nanoparticles at 37°C for 1 h under constant shaking, we found that $^{64}$Cu$^{2+}$ was immediately chelated by TCPP-PEG nanoparticles with more than 80% labeling yields for all TCPP-PEG nanoparticles (Figure 2a–h). Moreover, the $^{64}$Cu-labeled TCPP-PEG nanoparticles ($^{64}$Cu-TCPP-PEG) were also found to be highly stable in serum for 48 h, even in a 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) competitive situation (Figure 2i–l). Such highly efficient and stable intrinsic radiolabeling of nanoparticles would be suitable for in vivo PET imaging. In order to investigate both clearance and tumor uptake behaviors, we used BALB/c mice bearing murine breast cancer 4T1 tumors. Mice were i.v. injected with ~10 MBq of various $^{64}$Cu-TCPP-PEG nanoparticles (2, 5, 10, and 30 K) and imaged by a microPET/microCT Inveon rodent.
model scanner. Figure 3a shows the representative maximum intensity projections of coronal PET images depicting the in vivo biodistribution of 64Cu-TCPP-PEG nanoparticles at 10 s, 1 min, 5 min, 15 min, 30 min, 2 h, 4 h, 6 h, 12 h, and 24 h postinjection (p.i.). During the initial 30 min, a dynamic scan was performed (Figures S10–S13, Supporting Information). Static PET imaging of the same mice was performed to provide more detailed information regarding the clearance of TCPP-PEG nanoparticles at later time points. It was found that all the TCPP-PEG nanoparticles showed high accumulation in the blood pool directly after injection, yet TCPP-PEG nanoparticles were rapidly filtered through kidneys and accrued in the bladder. Interestingly, the blood circulation and metabolic rate showed significant differences among TCPP-PEG nanoparticles with various molecular weights. Figure 3b shows the detailed blood circulation curves of 64Cu-TCPP-PEG nanoparticles during the initial 4 h. Classical two-compartment pharmacokinetics were observed for all the TCPP-PEG nanoparticles (Figure S14a–d, Supporting Information), with distribution half-life ($t_{1/2}^\alpha$) and elimination half-life ($t_{1/2}^\beta$) values of 4.26 ± 1.95 min and 10.67 ± 2.13 min for TCPP-PEG$_{2K}$, 7.5 ± 2.2 nm for TCPP-PEG$_{5K}$, 10.1 ± 2.8 nm for TCPP-PEG$_{10K}$, and 17.3 ± 3.2 nm for TCPP-PEG$_{30K}$ nanoparticles.

$$t_{1/2}^\alpha = 4.26 \pm 1.95 \text{ min}$$
$$t_{1/2}^\beta = 10.67 \pm 2.13 \text{ min}$$

The $t_{1/2}^\beta$ of TCPP-PEG 30K nanoparticles was 8.1 times longer than that of TCPP-PEG 2K nanoparticles. In addition, the area under the curve for TCPP-PEG 30K (1082.3%ID h g$^{-1}$) was 2.6 times larger than that of TCPP-PEG$_{2K}$ (412.2%ID h g$^{-1}$) at 4 h p.i. (Figure S14e, Supporting Information). The elevated tumor uptake for TCPP-PEG$_{30K}$ was attributed to the enhanced blood circulation. The tumor-targeting efficiencies of the TCPP-PEG$_{30K}$ were determined to be 5.6 ± 1.5 and 6.9 ± 1.1%ID g$^{-1}$ at 4 and 24 h p.i., respectively, slightly higher than TCPP-PEG$_{10K}$ (4.2 ± 0.7 and 4.7 ± 0.35%ID g$^{-1}$), but much higher than TCPP-PEG$_{3K}$ (2.3 ± 0.57 and 2.2 ± 0.50%ID g$^{-1}$) and TCPP-PEG$_{5K}$ (2.4 ± 0.57 and 2.8 ± 0.48%ID g$^{-1}$) nanoparticles (Figure 3c). Such an efficient passive tumor homing of the nanoparticles could be attributed to the EPR effect of 4T1 tumors through the long blood circulation.[12]

Next, we carefully investigated the clearance behaviors of these various TCPP-PEG nanoparticles. For TCPP-PEG$_{30K}$...
nanoparticles, only radioactive signal in the bladder in the first 30 min was observed. However, strong PET signals were found in the kidneys and bladder in the initial 6 h time point for the lower molecular weight of TCPP-PEG nanoparticles (2, 5, and 10 K) after i.v. injection (Figure 3a and Figure S15a (Supporting Information)), proving that the majority of them underwent clearance. However, their rate of kidney filtration varied between the nanoparticles. Detailed biodistribution of $^{64}$Cu-TCPP-PEG nanoparticles in the left kidney and bladder was summarized in Figure S15b,c (Supporting Information). In the initial 4 h, all the TCPP-PEG nanoparticles showed high signal in bladder with up to $172.1 \pm 32.4$%ID g$^{-1}$, except TCPP-PEG$_{30K}$ nanoparticles ($9.7 \pm 1.4$%ID g$^{-1}$). All the samples showed high signal in the kidneys in the initial time points, indicating that our synthesized TCPP-PEG nanoparticles with small HD sizes can pass through the kidneys for rapid clearance. As expected, the radioactive signal of $^{64}$Cu-TCPP-PEG in the kidneys with lower molecular weights (2 and 5 K) was stronger than that with larger molecular weights (for example, 10 and 30 K) at all time points. In order to investigate the renal-clearable TCPP-PEG nanoparticles, a unilateral ureteral obstruction (UUO) mouse model was used, which was generated by complete ligation of the left ureter of the mouse while the right ureter was kept intact\(^{[13]}\) (Figure S16, Supporting Information). After i.v. injection of the $^{64}$Cu-TCPP-PEG$_{2K}$ nanoparticles, the obstructed left kidney exhibited dramatically reduced radioactivity signals compared to the contralateral right kidney (RK) in UUO mice in the first half an hour, but much higher than the RK at 1 and 2 h time points p.i. Such a diminished accumulation of contrast agent in the UUO kidney indicated that the blood perfusion had been dramatically reduced upon obstruction, which was consistent with
previous findings obtained by SPECT imaging of the UUO mice. Importantly, these findings suggested that glomerular filtration was likely a major clearance route for the small size of nanoparticles, such as TCPP-PEG2K nanoparticles.

The mouse liver also showed the same phenomenon that signal of radioactivity after i.v. injection of TCPP-PEG nanoparticles with the molecular weight less than 10 K decreased with the increasing time (Figure 3d and Figure S17 (Supporting Information)). Uptakes in the liver were 7.2 ± 1.4%ID g⁻¹ for TCPP-PEG2K, 7.3 ± 1.1%ID g⁻¹ for TCPP-PEG5K, and 8.8 ± 1.98%ID g⁻¹ for TCPP-PEG10K at 24-h time point p.i. Despite high tumor uptake for TCPP-PEG30K nanoparticles, most of them were still deposited in the liver for a long time (up to 17.8 ± 1.6%ID g⁻¹), which was 2 times higher than any other smaller molecular weight of TCPP-PEG nanoparticles. Finally, the ⁶⁴Cu radionuclide dose left in the body was found to be 20.5, 24.2, 28.5, and 50.8%ID for TCPP-PEG2K, TCPP-PEG5K, TCPP-PEG10K, and TCPP-PEG30K nanoparticles 24 h after i.v. injection of ⁶⁴Cu-TCPP-PEG nanoparticles (Figure S18, Supporting Information). Considering the balance

Figure 3. In vivo PET imaging: a) PET images of 4T1 tumor-bearing mice taken at various time points (10 s, 1 min, 5 min, 15 min, 30 min, 2 h, 4 h, 6 h, 12 h, and 24 h) postinjection of ⁶⁴Cu-TCPP-PEG2K, ⁶⁴Cu-TCPP-PEG5K, ⁶⁴Cu-TCPP-PEG10K, and ⁶⁴Cu-TCPP-PEG30K nanoparticles. Liver (L), kidneys (K), heart, and bladder are indicated in each figure. Tumor (T) is indicated by yellow arrowheads. b–d) Time-activity curves of ⁶⁴Cu-TCPP-PEG (2, 5, 10, and 30 K) in different major organs of (b) blood, (c) tumor, and (d) liver.
of clearance and tumor uptake, TCPP-PEG10K nanoparticles were chosen for the following investigation.

In vivo fluorescence imaging conducted at different time points after i.v. injection of TCPP-PEG10K nanoparticles (1 mg mL^{-1}, 200 µL) allowed us to further investigate the behaviors of clearance and tumor uptake (Figure 4a, supine position). The TCPP-PEG10K nanoparticles were rapidly excreted into the bladder, and the fluorescence intensity of the bladder area reached its maximum in the initial half hour p.i. In the remaining time points, we still found strong signal in the bladder, further confirming that TCPP-PEG10K nanoparticles were clearable after i.v. injection. The fluorescence signal from the TCPP-PEG10K nanoparticles also increased in the tumor over time, with the prominent uptake at 24 h p.i. (Figure 4a, prone position; Figure S19, Supporting Information), which was consistent with the above PET imaging results. Ex vivo imaging at 24 h p.i. revealed that TCPP primarily accumulated in the tumor, liver, spleen, and kidneys (Figure 4b and Figure S20 (Supporting Information)). To further confirm the tumor accumulation of TCPP-PEG10K nanoparticles, tumor tissues were sectioned and imaged by confocal microscopy. As shown in Figure 4c, strong red fluorescence was clearly visualized in tumor slices, verifying the prominent uptake of TCPP-PEG10K nanoparticles in the tumor, while no fluorescence signal was detected from the tumor tissues devoid of TCPP-PEG10K nanoparticles when using the same imaging condition. The results clearly indicated the efficient tumor passive uptake of our TCPP-PEG10K nanoparticles. Next, we examined the potential toxicities associated with the renal clearance of our nanoparticles. The kidneys were sectioned and investigated for the nanoparticle uptake and cellular toxicity. Strong fluorescence signal was observed from the kidney tissues 24 h p.i. (Figure 4c), indicating that the nanoparticles passed through the kidneys for clearance. Moreover, the tissues were devoid of any noticeable abnormalities or lesions, confirming the nontoxicity of our TCPP-PEG10K nanoparticles with clearance behavior (Figure S21, Supporting Information).

TCPP porphyrins have been widely used as a photosensitizer for photodynamic cancer therapy and could effectively generate singlet oxygen under light irradiation.^{[15]} It motivated us to investigate the PDT effect of TCPP-PEG10K nanoparticles. Compared with free TCPP, TCPP-PEG10K nanoparticles showed a slightly higher efficiency of light-triggered singlet oxygen production at the same TCPP concentrations (Figure S22, Supporting Information), which was attributed to the better solubility after modification, indicating that the synthesized TCPP-PEG10K nanoparticles could be a better agent for PDT. No significant cytotoxicity was observed for all TCPP-PEG nanoparticles, even at high concentrations of up to $20 \times 10^{-6}$ M (Figure S23, Supporting Information). The intracellular uptake of our TCPP-PEG10K nanoparticles was also time-dependent (Figure S24, Supporting Information). Next, we used TCPP-PEG10K nanoparticles as a photodynamic agent for in vitro cancer therapy under laser irradiation. The majority of 4T1 cells were destroyed after incubation with $5 \times 10^{-6}$ M of TCPP-PEG10K nanoparticles and exposure to the 658 nm laser (Figure S25a, Supporting Information). The PDT effects of TCPP-PEG10K nanoparticles on 4T1 cells were further investigated using Trypan blue staining. Upon laser irradiation, most cells incubated with TCPP-PEG10K nanoparticles were damaged, as
indicated by the intense homogeneous blue color (Figure S25b, Supporting Information). The amount of cell death gradually increased upon increasing the concentrations of TCPP-PEG10K nanoparticles. These findings altogether revealed that TCPP-PEG10K nanoparticles hold great promise as an effective photodynamic agent for in vivo tumor therapy.

Encouraged by the high PDT efficiency in vitro and the efficient passive tumor targeting of TCPP-PEG10K nanoparticles, in vivo PDT studies were performed to evaluate the anticancer efficacy of TCPP-PEG10K nanoparticles. For the PDT group (n = 4), the mice were i.v. injected with TCPP-PEG10K nanoparticles (200 μL, 1 mg mL⁻¹) and then irradiated for 30 min with the 658 nm laser at 50 mW cm⁻² after 24 h p.i. As illustrated in Figure 5a, TCPP-PEG10K nanoparticle-mediated PDT can significantly inhibit tumor growth, whereas laser irradiation alone or PBS injection with laser irradiation did not affect the tumor growth. These results suggest that TCPP-PEG10K nanoparticles are a powerful agent for in vivo PDT of cancer (Figure 5b). To further understand the PDT effects after various treatments down to the cellular level, hematoxylin and eosin (H&E) staining was utilized to study the morphology and apoptosis of tumor cells after 2 d posttreatment. The results indicated that most cancer cells were completely destroyed in the TCPP-PEG10K nanoparticles with laser treatment group (Figure 5c), while cells in the other three control groups mainly retained their normal morphology, further confirming the efficacy of TCPP-PEG10K polymer for in vivo photodynamic ablation of cancer.

3. Conclusion

In summary, our study introduced a simple method to synthesize PEGylated TCPP porphyrin nanoparticles and employed noninvasive dynamic PET imaging to investigate the balance of the renal clearance and tumor uptake behaviors. We found that PEG with larger molecular weights was better for tumor uptake and the lower ones were suitable for renal clearance. Considering this balance, TCPP-PEG10K nanoparticles were the most suitable model to investigate clearance and tumor uptake. In vitro and in vivo PDT experiments were carried out which led to excellent therapeutic efficacy. Therefore, our work presented a simple fabrication method for selection of biocompatible multifunctional TCPP-PEG-based theranostic agents with renal clearance behavior, which highlights the clinical application potential of TCPP-PEG as theranostic probes for imaging-guided cancer therapy.

4. Experimental Section

Materials: mTCPP and dimethyl sulfoxide (DMSO) were obtained from Frontier Scientific, Inc. (Logan, UT, USA). PEG2k, PEG5k, and PEG10k were obtained from Biomatrik Co., Ltd. (Jiaxing, China) with the molecular weight of 2, 5, 10, and 30 kDa, respectively. EDC and N-hydroxysulfosuccinimide (Sulfo-NHS) were obtained from Sigma-Aldrich. Deionized water used in our experiments was obtained from a Milli-Q water system.
Synthesis of TCPP-PEG Nanoparticles: TCPP-PEG nanoparticles with various molecular weights were synthesized according to the protocol reported previously with slight modification.\(^1\) First, 10 μmol of TCPP was mixed with 5 μmol of EDC and 5 μmol of Sulfo-NHS in 100 μL anhydrous DMSO for 0.5 h at room temperature. Then, PEG with different molecular weight (2, 5, 10, and 30 K) in 2 mL water was slowly added to the above-activated TCPP molecules. The molar ratio of PEG-NH₂: TCPP:EDC:NHS was 1:2:1:1. After reacting for 24 h at room temperature, excess TCPP molecules, catalysts, and DMSO were removed with Millipore filters with different molecular weight cut off. Afterward, TCPP-PEG\(_{2k}\), TCPP-PEG\(_{5k}\), TCPP-PEG\(_{10k}\), and TCPP-PEG\(_{30k}\) nanoparticles in water were obtained and stored at −4 °C for future use. The concentration of TCPP-PEG nanoparticles was defined by TCPP content.

Characterization: TEM images of the nanoparticles were obtained using a FEI Tecnai F30 transmission electron microscope at an acceleration voltage of 300 kV. UV–vis–near infrared absorbance (NIR) spectra were obtained with PerkinElmer Lambda 750 UV-Vis-NIR spectrophotometer. Fluorescence spectra of different samples were obtained on a FluoroMax 4 luminescence spectrometer (HORIBA Jobin Yvon). The HDs of TCPP-PEG nanoparticles were determined by a ZetaSizer Nano-ZS (Malvern Instruments, UK). MALDI-TOF spectra of TCPP-PEG nanoparticles, but NOTA were obtained from MDS SCIEX 4800 MALDI-TOF/TOF (Applied Biosystems, Foster City, CA, USA). \(^1\) H NMR spectra were recorded on a 400 MHz NMR spectrometer (INOVA-400) at 25 °C with DCDCl\(_3\) as the solvent and tetramethylsilane as the internal standard. FTIR absorption spectra of the nanoparticles were recorded by using a Nicolet 6700 spectrometer at 4 cm\(^{-1}\) resolution, over the wavenumber range 400–4000 cm\(^{-1}\).

Tumor Models: All animal studies were conducted under a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. The 4T1 subcutaneous xenografts were generated by mice. In order to investigate the serum stability of nanoparticles, not TCPP-PEG\(_{2k}\), TCPP-PEG\(_{5k}\), TCPP-PEG\(_{10k}\), and TCPP-PEG\(_{30k}\) nanoparticles were synthesized according to the protocol reported previously.\(^2\) The same concentration of free TCPP molecules dissolved in DMSO/water under laser irradiation was used as the control. The fluorescence intensity of SOSG was measured with an excitation wavelength of 494 nm.

Cell Culture Experiment: Murine breast cancer 4T1 cells were obtained from the American Type Culture Collection and cultured at 37 °C under 5% CO\(_2\). All cell culture-related reagents were purchased from Invitrogen. 4T1 cells were cultured in normal RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were seeded into 96-well plates at a density of 1 × 10\(^4\) cells per well and incubated with different concentrations of TCPP-PEG\(_{10k}\) nanoparticles for 24 h. Relative cell viabilities were determined by the standard methyl thiazolyl tetrazolium (MTT) assay. To examine the cellular uptake of TCPP-PEG\(_{10k}\) nanoparticles, 4T1 cells were plated in 35 mm dish with 14 mm bottom well (1 × 10\(^4\) cells per well) for 24 h. After adhesion, TCPP-PEG\(_{10k}\) nanoparticles were added into the wells at the concentration of 5 × 10\(^4\) μM (TCPP) and cultured for different time periods (1, 3, 6, and 24 h). After washing 3 times with PBS (pH = 7.4), cells were fixed by 4% paraformaldehyde and labeled with 4′, 6-diamidino-2-phenylindole before imaging with a
Nikon A1RS confocal microscope, and imaging analysis was performed using the NIS-ELEMENTS Ar with Deconvolution package.

**In Vitro PDT:** For in vitro PDT, 4T1 cells (1 \times 10^6) were seeded in 96-well plates and added with TCPP-PEG\(_{10K}\) nanoparticles at various concentrations. After incubation for 12 h, the experimental groups were exposed to 658 nm laser irradiation under a power density of 20 mW cm\(^{-2}\) for 15 min, while the control groups were still cultured in dark. Afterward, all samples were incubated in the dark for another 12 h. In order to determine relative cell viabilities after various treatments, the MTT assay was carried out following the standard protocol. After laser irradiation, cells were washed with PBS and stained with 0.4% Trypan blue (Sigma-Aldrich) before imaged by a Nikon Elipse Ti microscope.

**In Vivo PDT:** To develop the tumor model, 4T1 cells (1 \times 10^4) were seeded in 50 µL of PBS were subcutaneously injected into the back of each BALB/c mouse. After the tumor volume reached \(\sim 150 \text{ mm}^3\), mice were i.v. injected with the NIS-Elements Ar with Deconvolution package.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

dynamic PET imaging, fluorescence imaging, photodynamic therapy, renal clearance, TCPP-PEG nanoparticles

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