Porphyne functionalized nanoparticles of star-shaped poly(e-caprolactone)-b-D-α-tocopheryl polyethylene glycol 1000 succinate biodegradable copolymer for chemophotodynamic therapy on cervical cancer

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1. Introduction

Cancer is one of the leading causes of death. In the past 50 years, cancer death rate has remained to be around 1/4 of that from all diseases, which is 23.2% in the US in 2010, 22.17% in China and 30.1% in Singapore in 2012. The World Health Organization (WHO) predicted that the cancer rate could increase by 50% to 15 million cases in the world and by 60% to 7.1 million in Asia by 2020. We are indeed in crisis against cancer. The standard treatments have been surgery followed by radiotherapy and/or chemotherapy. If they fail, the patients can have only less than 10% chance of survival with other treatments such as immunotherapy, gene therapy, thermal therapy, photodynamic therapy, and by chance, some mysterious miracles.

The traditional chemotherapy usually has serious side effects. Also, its clinical application is confined by the multidrug resistance (MDR) \cite{1,2}. There are at least two molecular pumps in tumor cell membranes, multidrug resistance-associated protein (MRP) and P-glycoprotein (P-gp), to expel the anti-tumor drugs out of the cancer cells and attenuate the drug effect. A great deal of efforts have been made to solve the problem over the past decades, which may include the development of new drugs that could escape from MDR, the MDR modulators or chemosensitizers, and the RNA interference (RNAi) \cite{3}. To overcome these problems, various nanomedicine strategies have been developed for sustained,
controlled and targeted delivery of anticancer drugs. The common inorganic core nanoparticles include semiconductor quantum dots (2–8 nm), noble metal colloids (2–20 nm) and magnetic nanoparticles (5–50 nm). Liposomes (50–500 nm), micelles (<50 nm) and biodegradable polymeric capsules or spheres (10–1000 nm) are typical representatives of the organic core nanoparticles. Especially, biodegradable polymeric nanoparticles are mainly used as drug carriers, and often synergize thermo-, photo- or pH-sensitive functions, which make these drug-loaded nanoparticles more effective, selective and drug-release controllable [4–9].

Photodynamic therapy (PDT) has been under development as a promising treatment of cancer. PDT utilizes specific wavelength light to activate photosensitizers (PSs) and generate reactive oxygen species (ROS) which can destroy biomacromolecules and organelles, and induce cancer cell death [10,11]. Compared with other therapies, PDT has advantages in targeting accuracy because of the activation requiring selective wavelength light, repeatable at the same site and less invasive than surgery [5,11]. Porphyrin derivatives are a class of photosensitizers which specifically enrich in cancer cells and are widely used in PDT and sonodynamic therapy (SDT) (i.e., ultrasound activated PDT) [12]. In recent years, porphyrin has been under intensive investigation due to its great potential as a new-generation photosensitizer for photodynamic therapy [13]. Porphyrin and its derivatives are effective generators of singlet oxygen with favorable properties such as selectivity for particular diseased tissues and fast elimination from the body. However, porphyrin and its derivatives are hydrophobic and thus undispersed and unstable in an aqueous medium, which greatly limits their clinical applications [14]. Also, most photosensitizers tend to aggregate in an aqueous solution, affecting their phototoxicity in vivo due to decreased bioavailability and capacity to absorb light [15].

Previous studies have also demonstrated the advantages of the various nanocarriers in drug delivery, which may include high drug loading and drug encapsulation efficiency, desired drug release profile, efficient cellular uptake due to the endocytosis mechanism and the surface chemistry to escape from the multidrug resistance, and the high cytotoxicity and desired biodistribution [14–16]. It is reasonable to apply the various nanocarriers to co-deliver the chemotherapeutics and the photosensitizer to realize a combinatory chemophotodynamic therapy, which can thus combine the advantages for synergistic effects as well as to overcome the disadvantages for antagonistic effects of the two single modality therapy. Indeed, it is believed that although photodynamic therapy has limited therapeutic effect and chemotherapy has confined dose and multidrug resistance with serious side effects, the combinatory chemophotodynamic therapy has great potential to promote a synergistic effect of the two therapies. Indeed the drug may augment the cytotoxic effect of PDT as well as the PDT can greatly magnify the cytotoxicity of the chemotherapeutics [17].

Biodegradable copolymers are widely applied in nanocarriers for drug delivery [18–20]. Poly(ε-caprolactone) (PCL) is a biodegradable biomaterial of desired biocompatibility and has been approved by the US Food and Drug Administration (FDA) [18,19]. PCL-based NPs have been used in the formulation of a variety of drugs for controlled and targeted drug delivery [18,19]. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), e-caprolactone (CL), 1,3-diisopropylcarbodiimide (DCC), acetonitrile (HPLC grade), coumarin-6 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Docetaxel (DTX) was provided by Shanghai Jinhe Bio-tech Co., Ltd. (Shanghai, PR China). Commercial Taxotere® was from Sanofi-Aventis Pharma Dagenham, U.K. (Surrey, UK). All other chemicals of the highest quality were commercially available and used as received. Anti-α-tubulin was purchased from Abcam (Cambridge, UK) and A FITC conjugated goat anti-rabbit IgG antibody was purchased from Santa Cruz (Texas, USA).

2. Materials and methods

2.1. Materials

5,10,15,20-Tetrakis(4-aminophenyl)porphyrin (TAPP, C₄₄H₃₄N₈) was purchased from J&K Scientific Ltd. (Beijing, PR China). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS, C₃₂H₃₄O₄₆) and e-caprolactone (CL), 1,3-diisopropylcarbodiimide (DCC), acetonitrile (HPLC grade), coumarin-6 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Docetaxel (DTX) was provided by Shanghai Jinhe Bio-tech Co., Ltd. (Shanghai, PR China). Commercial Taxotere® was from Sanofi-Aventis Pharma Dagenham, U.K. (Surrey, UK). All other chemicals of the highest quality were commercially available and used as received. Anti-α-tubulin was purchased from Abcam (Cambridge, UK) and A FITC conjugated goat anti-rabbit IgG antibody was purchased from Santa Cruz (Texas, USA).

2.2. Synthesis and characterization of linear copolymer PCL-b-TPGS and star-shaped copolymer TAPP-PCL-b-TPGS

The star-shaped block copolymer TAPP-PCL-b-TPGS was prepared as described previously [32]. The synthesis scheme for the star-shaped copolymer TAPP-PCL-b-TPGS is shown in Fig. 1.

2.2.1. Synthesis of star-shaped TAPP-PCL using TAPP as initiator

e-Caprolactone (CL, 3.21 g, 28.2 mmol), initiator TAPP (0.05 g, 0.075 mmol), and catalyst Sn(Oct)₂ (0.011 g, 0.1 mol% of monomer) were added in a glass tube, which was connected to a vacuum system. An exhausting–refilling with argon process was then repeated three times. The tube was sealed and heated to 140 °C in oil bath for 16 h. After the reaction, the tube was cooled to room temperature. The product was dissolved in dichloromethane (DCM) and then precipitated in excess cold anhydrous ether, washed with mixture solvent ether and methanol. The product denoted TAPP-PCL was collected and dried in vacuo at 40 °C for 24 h. Additionally, the linear copolymer denoted TAPP-PCL-b-TPGS (Mn = 36,410) was prepared in the same way except that the initiator TAPP was substituted by TPGS.

2.2.2. Synthesis of star-shaped copolymer TAPP-PCL-b-TPGS

The star-shaped copolymer TAPP-PCL-b-TPGS was prepared following published procedures with mild modification [32]. Carboxyl-terminated TPGS (CTPGS) was synthesized as described previously [13,33]. In brief, TAPP-PCL (200 mg, 0.006 mmol) and CTPGS (48 mg, 0.030 mmol) were placed into a flask, then the flask
was evacuated at 90 °C for 4 h. After the mixture was cooled to room temperature, the solution of 4-(dimethylamino)pyridine 4-toluenesulfonate (DTPS) (6 mg, 0.02 mmol) in 15 mL of DCM and the DCC (16 mg, 0.08 mmol) in 2 mL of dry DCM was added to the reaction mixture under Argon. The reaction solution was stirred at room temperature for 2 days. The resulting product was filtered and precipitated in excess cold diethyl ether. The precipitate was collected by filtration and washed several times with diethyl ether, then dried under vacuum for 24 h. The dried solid was dissolved in acetone, and then dropped into distilled water. Thereafter, the mixture solution was transferred into a dialysis membrane bag (MWCO: 10,000 Da) and dialyzed against deionized water for 48 h to remove unreacted CTPGS and other impurities. The obtained product denoted TAPP-PCL-b-TPGS was collected by freeze-drying.

2.2.3. Characterization of copolymers

1H NMR (Bruker AMX 500) was used to confirm the structures of copolymers TAPP-PCL and TAPP-PCL-b-TPGS dissolved in CDCl3. Molecular weights of the copolymers were determined by GPC (Waters GPC analysis system with RI-G1362A refractive index detector, Waters Corp., Milford, MA, USA) with THF as the eluent at the flow rate of 1 mL/min. Molecular weight and polydispersity index (PDI) were evaluated using standard polystyrene samples.

2.3. Cell culture

Human cervical cancer cell line HeLa was obtained from the American Type Culture Collection (ATCC). Human adriamycin resistant (ADR) breast cancer cell line MCF7/ADR was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. MCF7/ADR cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. Additionally, 10 μM adriamycin was added into MCF7/ADR medium. All the cells were cultured in a humidified atmosphere with 5% CO2 at 37 °C. MCF-7/ADR cells were subcultured for 2 weeks in adriamycin-free medium prior to use [34].

2.4. Preparation of DTX- and coumarin-6-loaded NPs

DTX-loaded TAPP-PCL, PCL-b-TPGS and TAPP-PCL-b-TPGS NPs were prepared by a nanoprecipitation method as reported previously using an acetone/water system [27]. Briefly, 100 mg of TAPP-PCL-b-TPGS (TAPP-PCL or PCL-b-TPGS) and 10 mg DTX powder were dissolved in 8 mL acetone by mild blending and the DTX-nanoparticle mixture were slowly added into 100 mL of 0.03% TPGS solution (w/v) using 1 mL injector under stirring...
(800 rpm). When the acetone was removed completely overnight at room temperature, the mixture containing DTX-loaded NPs was centrifuged at 20,000 rpm for 15 min at 4 °C and the precipitates were washed by deionized water for 3 times to remove the TPGS emulsifier and unencapsulated drug. The precipitation was resuspended in 10 mL deionized water and placed in –80 °C refrigerator overnight. The frozen mixture was then freeze-dried for 2 days to obtain the DTX-loaded nanoparticle powder. The fluorescent coumarin-6-loaded TAPP-PCL-TPGS (TAPP-PCL or PCL-TPGS) was formulated the same way as above except DTX for 1 mg of coumarin-6 for encapsulation.

2.5. Characterization of TAPP-PCL-TPGS (TAPP-PCL and PCL-TPGS) NPs

2.5.1. Size and zeta potential

Measurements of mean size and size distribution of DTX-loaded TAPP-PCL-TPGS (TAPP-PCL and PCL-TPGS) NPs were conducted by a Malvern Mastersizer 2000 (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK). Zeta potential of the NPs was measured by Laser Doppler Anemometry (LDA; Zetasizer Nano ZS90, Malvern Instruments Ltd., Malvern, UK). All measurements were done at room temperature after equilibration for 10 min and the data were obtained in triplicate.

2.5.2. Morphological analysis of NPs

The morphology of DTX-loaded NPs was examined by field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) [8,28]. For FESEM imaging, the NPs were first fixed on a double-sided sticky tape on a sample stub. Thereafter, we sprayed platinum for 45 s onto the pretreated samples using JFC-1300 automatic fine platinum coater (JEOL, Tokyo, Japan) and observed the samples by JEOL JSM-6301F (JEOL, Tokyo, Japan). For TEM imaging, samples were dropped onto a copper grid coated with a carbon membrane. The grid was dried at room temperature and the samples were characterized by TEM (Tecnai G2 20, FEI Company, Hillsboro, Oregon, USA).

2.5.3. Drug loading and encapsulation efficiency

The drug loading content (LC) and drug encapsulation efficiency (EE) of the DTX-loaded NPs were determined by high performance liquid chromatography (HPLC) (LC 1200, Agilent Technologies, Inc., Highland Park, Winooski, USA), and the cell death rate was calculated using the MTT assay. The absorbance was measured at 570 nm by Epoch Multi-Volume Spectrophotometer System (BioTek Instruments Inc., Highland Park, Winooski, USA), and the cell death rate was calculated as follows:

\[ \text{Cell death} \% = \frac{\text{average } A_{570} \text{ of the experimental group} - \text{average } A_{570} \text{ of the control group}}{\text{average } A_{570} \text{ of the control group}} \times 100\% \]

For investigation into coumarin-6-loaded-nanoparticle LC and EE, we used a 96-well microplate reader (Infinite M1000 PRO, Tecan, Switzerland) with the excitation wavelength of 430 nm and the emission wavelength of 485 nm to quantify the coumarin-6 encapsulated into the NPs. Briefly, 5 mg of coumarin-6-loaded NPs were dissolved in 1 mL of DCM by vigorous vortex and the fluorescence intensity was determined by the microplate reader. The coumarin-6-loaded-nanoparticle LC and EE were calculated by the following formulas:

\[ \text{LC (\%)}_{\text{coumarin-6}} = \frac{\text{Weight of coumarin-6 in the NPs}}{\text{Weight of the NPs}} \times 100\% \]

\[ \text{EE (\%)}_{\text{coumarin-6}} = \frac{\text{Weight of the feeding coumarin-6}}{\text{Weight of the NPs}} \times 100\% \]

2.5.4. Differential scanning calorimetry (DSC) analysis

To examine the physical status of DTX in TAPP-PCL-TPGS (TAPP-PCL and PCL-TPGS) NPs, samples were analyzed by DSC using Q2000 thermogravimetric analyzer (USA). The NPs were purged with dry nitrogen at a flow rate of 20 mL/min with heating from room temperature to 200 °C at a rate of 10 °C/min [28].

2.6. In vitro drug release

To investigate the release profile of DTX of NPs, 5 mg of lyophilized DTX-loaded TAPP-PCL-TPGS (TAPP-PCL and PCL-TPGS) NPs was dispersed in 1 mL of release buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, 0.1% Tween 80, pH = 7.4) and transferred into a semipermeable membrane pouch (Spectra Por, MWCO = 3,500, Spectrum, Houston, TX, USA). Then the pouches were immersed into 15 mL of release buffer and incubated at 37 °C with 200 rpm shaking in water. The 15 mL of release buffer was removed for analysis and replenished with fresh release buffer every day for 12 days. The collected release buffer was extracted with DCM followed by vacuum evaporation and the DTX precipitation was dissolved in methanol (HPLC grade) and analyzed by HPLC as described above. Therefore, we can obtain the cumulative DTX amount released at different time points [25,28].

2.7. Effect of blank NPs on P-gp efflux

2.7.1. MTT assay

To explore the effect of blank NPs on P-gp efflux, MCF7/ADR, a DOX/DTX-resistant and P-glycoprotein (P-gp) over-expression cancer cell line, were seeded in 96-well plate at a concentration of 1 × 10^4 cells/well and allowed to adhere overnight. Then the medium was replaced for fresh medium containing 20 μg/mL blank NPs (TAPP-PCL-TPGS, TAPP-PCL or PCL-TPGS). After 30 min treatment, cells were treated with 2 μg/mL DOX or DTX for another 24 h or 48 h. Cytotoxicity was determined using the MTT assay. The absorbance was measured at 570 nm by Epoch Multi-Volume Spectrophotometer System (BioTek Instruments Inc., Highland Park, Winooski, USA), and the cell death rate was calculated as follows:

\[ \text{Cell death} \% = \frac{\text{average } A_{570} \text{ of the experimental group} - \text{average } A_{570} \text{ of the control group}}{\text{average } A_{570} \text{ of the control group}} \times 100\% \]
2.7.2. P-gp ATPase assay

To further investigate the blank NPs' effect on P-gp, P-gp-GloTM Assay Systems (Promega, Madison, USA) were used to conduct the assay following the manufacturer's instruction. Briefly, 20 μL of DTX-free TAPP-PCL-b-TPGS NPs at different concentrations (0, 0.05, 0.5, 5, 50 μg/ml) for test, 20 μL of P-gp-GloTM Assay Buffer for blank or 20 μL of Na3VO4 (0.5 mM, sodium orthovanadate, a selective inhibitor of P-gp) for negative control was mixed with 20 μL of P-gp membranes at 37 °C for 5 min. To start the reaction, 10 μL of MgATP (25 mM) was added into each well and incubated at 37 °C for 40 min. Then 50 μL of ATP Detection Regent was added into each well to develop luminescence and the luminescence value was read by a 96-well microplate reader (Infinite M1000 PRO, Tecan, Switzerland). The difference in value between the value was calculated and presented as difference in luminescence intensity (ΔRLU).

2.8. Cellular effects of NPs

2.8.1. Cellular uptake of fluorescent NPs

To assess the efficiency of cellular uptake, coumarin-6 was encapsulated into TAPP-PCL-b-TPGS, TAPP-PCL or PCL-b-TPGS for its innate fluorescence. 1 × 10⁵/well HeLa cells were seeded in 12-well plates covered with 18 mm² glass and after 12 h of cell attachment, coumarin-6-loaded NPs were added into cells at the equivalent concentration of 100 μg/mL. After 30 min, cells were washed with PBS for two times and fixed in 4% paraformaldehyde for 15 min. Then the cell nucleus were stained with 5 μg/mL DAPI and observed under laser scanning confocal microscope as above.

To quantify the nanoparticle cellular uptake, 3 × 10⁵/well HeLa cells were seeded into black 96-well plates. After 12 h of cell attachment, cells were washed with PBS twice and incubated with HBSS (137 mM NaCl, 5.4 mM KCl, 0.25 mM Na₂HPO₄, 0.1 g glucose, 0.44 mM KH₂PO₄, 1.3 mM CaCl₂, 1.0 mM MgSO₄, 4.2 mM NaHCO₃) at 37 °C for 1 h following the addition of coumarin-6-loaded NPs at a concentration of 20, 100 and 200 μg/mL, respectively. After 2 h, the medium was discarded and washed with PBS for three times. Cells were lysed with 50 μL of lysis buffer (0.5% Triton X-100, 0.2 M NaOH) and the fluorescence value was read with a microplate reader (Infinite M1000 PRO, Tecan, Switzerland) with excitation wavelength at 430 nm and emission wavelength at 485 nm.

2.8.2. DTX-loaded NPs' effect on microtubules

DTX can reversibly bind to microtubules with high affinity and stabilize microtubule assembly [30,31], so we would examine the effect of DTX-loaded NPs on microtubules. 1 × 10⁵ HeLa cells were seeded in 12-well plates. After 12 h, 100 ng/mL DTX loaded in the NPs was added to the cells and incubated for 30 min for cellular uptake. The nanoparticle-containing medium was discarded and changed for nanoparticle-free medium followed by another 6 h of culture. Cells were fixed and stained with a primary antibody against α-tubulin at a dilution of 1:100 at 4 °C overnight. A FITC-conjugated goat anti-rabbit IgG was used for the secondary antibody. Cell nucleus was stained with 5 μg/mL DAPI and observed under laser scanning confocal microscope as above.

2.8.3. Cytotoxicity of drug-free and DTX-loaded nanoparticle mediated PDT

To explore the nanoparticle mediated PDT effect, 3 × 10⁴/well HeLa cells were seeded into 96-well plates and after cell attachment for 12 h, changed with fresh medium at various concentrations of TAPP-PCL-b-TPGS or DTX-loaded TAPP-PCL-b-TPGS for 6 h at 37 °C. The treated cells were washed 3 times with PBS followed by culture in phenol red-free medium for another 24 h. Thereafter, cells were exposed to the illuminant at various doses of 0.7 J/cm², 1.4 J/cm² and 2.1 J/cm² and cultured for another 4 h. When the photosensitizing NPs were added into the medium, all the protocols were conducted in subdued light. The light emitting diodes (LEDs) emitted at wavelengths from 650 nm to 670 nm with the highest intensity at 660 nm. The cytotoxicity of the drug-free and DTX-loaded nanoparticle was determined using the MTT assay [32].

2.8.4. Reactive oxygen species (ROS) assay

Following the manufacturer’s procedure, ROS induced by NPs was measured using a Reactive Oxygen Species Assay Kit from Beyotime Institute of Biotechnology (Jiangsu, P.R. China).DCFH-DA is a nonfluorescent penetrant and can pass through the cell membrane followed by hydrolyzed by esterases to form DCFH, while DCFH can not pass through the cell membrane and consequently be retained in the cytoplasm. Then DCFH is oxidized by ROS in the cytoplasm and is converted to DCF which has fluorescence. HeLa cells were treated with NPs and incubated with 10 μM DCFH-DA at 37 °C for 20 min. Cells were washed with PBS 3 times and fixed in 4% paraformaldehyde. The nuclei were stained with 5 μg/mL DAPI and observed under laser scanning confocal microscope as above.

2.9. In vivo antitumor efficacy of the DTX-loaded TAPP-PCL-b-TPGS

40 female nude immunodeficient mice (nu/nu), 6 weeks old, were purchased from Guangdong Province Medical Animal Center, and fed and monitored in a specific pathogen-free environment at Tsinghua University Shenzhen Graduate School. All animal studies were carried out according to protocols approved by the Tsinghua University Animal Care and Use Committee, complying with the rules of REGULATIONS FOR THE ADMINISTRATION OF AFFAIRS CONCERNING EX-PERIMENTAL ANIMALS (Approved by the State Council of China). To establish xenograft human cervical cancer model, HeLa cells were harvested and resuspended in serum-free DMEM medium. 2 × 10⁶ HeLa cells in 100 μL medium were subcutaneously injected into the right flank of each mouse [33,34]. When the tumor grew to palpable size, the mice were randomly divided into four groups (n = 10) and treated with saline, Taxotere® (10 mg/kg), DTX-loaded TAPP-PCL-b-TPGS (equivalent to 10 mg/kg of DTX) without PDT and DTX-loaded TAPP-PCL-b-TPGS (equivalent to 10 mg/kg of DTX) with PDT. For the PDT combination treatment group, after administration of the DTX-loaded TAPP-PCL-b-TPGS, mice were exposed to light with ~660 nm wave length at a light dose of 30 J/cm²/treatment. The longest perpendicular tumor diameters were recorded to estimate the tumor volume, using the following formula: 4π/3 × (width/2)² × (length/2), representing the 3-dimensional volume of an ellipsoid tumor tissue. When tumors reached 2 cm or if the mice suffered from moribund, mice would be sacrificed. After the death of the mice, tumors were excised and imaged.

To further investigate the nanoparticle toxicity on other tissues during treatment, tissues including heart, liver, spleen, lung and kidney from different treatment groups were isolated followed by fixation in 4% paraformaldehyde for at least 24 h. Then the tissues were embedded in paraffin followed by sectioning into ~4 μm slices, and stained with hematoxylin and eosin (H&E) and observed under optical microscope.

2.10. Statistical analysis

All experiments were repeated at least three times and the data were presented as mean ± SD unless noted otherwise. Differences between data groups were evaluated for significance using Student’s t-test of unpaired data or one-way analysis of variance and Bonferroni post-test. A value of p < 0.05 indicates statistical significance.
3. Results

3.1. Synthesis and characterization of star-shaped copolymers

The synthetic scheme of star-shaped copolymer TAPP-PCL-b-TPGS is shown in Fig. 1. Firstly, TAPP-PCL was synthesized by the ring-opening polymerization of CL using TAPP as the initiator and Sn(Oct)$_2$ as the catalyst under an Argon atmosphere at 140 °C for 16 h. Then, the resultant copolymer TAPP-PCL was conjugated with carboxylated TPGS through an esterification reaction that generated a 4-armed star-shaped block copolymer TAPP-PCL-b-TPGS.

Fig. 2A shows the $^1$H NMR spectrum of synthesized TAPP-PCL: (a) ($\delta$ = 4.05 ppm, CL repeating unit: $\text{CH}2\text{CH2CH2CH2O}$), (b) ($\delta$ = 1.63 ppm, CL repeating unit: $\text{CH2COCH2CH2CH2O}$), (c) ($\delta$ = 1.38 ppm CL repeating unit: $\text{CH2CH2CH2CH2O}$), and (d) ($\delta$ = 2.31 ppm, CL

![Fig. 2. Typical $^1$H NMR spectra of copolymers (A) TAPP-PCL and (B) H40-PLA-COOH.](image)

Fig. 3. (A) Schematic diagram of the preparation of the DTX-loaded TAPP-PCL-b-TPGS NPs and its multi-effects on cancer cells. (B and C) Characterization of TAPP-PCL-b-TPGS via FESEM image (B) and TEM image (D) of the NPs. (D) DLS size distribution of the NPs.
repeating unit: –CO–CH₂–CH₂–CH₂–CH₂–O–). The characteristic peak signals of TAPP moieties (ε = 7.94 ppm, f = 8.18 ppm, g = 9.16 ppm) could also be observed. As shown in Fig. 2B, the successful coupling of star-shaped TAPP-PCL and TPGS was confirmed by the appearance of peak h (δ = 3.65 ppm, methylene protons of TPGS: –CH₂–CH₂–O–). The 1H NMR spectrum indicated that the star-shaped copolymers TAPP-PCL and TAPP-PCL-b-TPGS with a well-defined structure were synthesized successfully. The results are in accordance with those reported by Zhang et al. [35] and Zeng et al. [8].

The number-average molecular weights (Mn) of the copolymers were determined by GPC. The Mn of star-shaped copolymers TAPP-PCL, TAPP-PCL-b-TPGS was 33,652 and 38,741, respectively. Furthermore, the polydispersity index (PDI) of the copolymers is narrow, the PDI calculated from Mn/Mn, is 1.31 and 1.26, respectively.

3.2. Preparation and characterization of DTX-loaded NPs

In the present study, clinically well-used chemotherapeutic medication DTX was encapsulated into the NPs of TAPP-PCL-b-TPGS star-shaped copolymers for chemophotodynamic therapy. The TAPP-PCL-b-TPGS NPs can also inhibit the multi-drug efflux mediated by P-gp and consequently have the potential in overcoming the MDR in cancer treatment (Fig. 3A). Three DTX-loaded NPs were prepared as demonstrated above, which were referred to as DTX-loaded TAPP-PCL-b-TPGS NPs, TAPP-PCL NPs and PCL-b-TPGS NPs. The physical properties of those resulted NPs are principal factors to assess the drug delivery carrier, which include the nanoparticle size and size distribution, surface morphology, physical state of DTX in NPs, drug loading content (LC), drug encapsulation efficiency (EE) and in vitro drug release profile.

As shown in Table 1, the average size of the DTX-loaded TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs is 139.3 ± 5.4, 157.1 ± 4.3 and 162.7 ± 3.1 nm, respectively. To further observe the morphology of the TAPP-PCL-b-TPGS NPs, FESEM and TEM were carried out. The FESEM image (Fig. 3B and C) and TEM image (Fig. 3D) showed that all the NPs had a spherical shape and the average size was about 100 nm. The DTX-loaded TAPP-PCL-b-TPGS NPs’ size distribution obtained from DLS is shown in Fig. 3E.

Zeta potential is an important factor for nanoparticle stability. A reasonable zeta potential can prevent the NPs from aggregation [36]. We examined the zeta potential of the DTX-loaded TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs and the values were −5.9 ± 1.7, −14.2 ± 1.0 and −12.5 ± 0.9 mV, respectively. The loading content of the drug encapsulated in the TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs were 9.72%, 8.81% and 8.67%, respectively. The drug encapsulation efficiency of the TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs was quite high, namely 95.31%, 82.73% and 85.05%, respectively (Table 1). These data suggested that the drug and the star-shaped copolymer had strong affinity and the TAPP-PCL-b-TPGS NPs can thus have exhibited satisfying characters for drug delivery.

### Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Size (nm)</th>
<th>ZP (mV)</th>
<th>PDI (%)</th>
<th>LC (%)</th>
<th>EE (%)</th>
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<tbody>
<tr>
<td>TAPP-PCL</td>
<td>157.1 ± 4.3</td>
<td>−14.2 ± 1.0</td>
<td>1.43</td>
<td>8.81 ± 1.08</td>
<td>82.73 ± 2.16</td>
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<tr>
<td>PCL-b-TPGS</td>
<td>162.7 ± 3.1</td>
<td>−12.5 ± 0.9</td>
<td>1.26</td>
<td>8.67 ± 1.02</td>
<td>85.05 ± 1.52</td>
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<tr>
<td>TAPP-PCL-b-TPGS</td>
<td>139.3 ± 5.4</td>
<td>−5.9 ± 1.7</td>
<td>1.31</td>
<td>9.72 ± 1.27</td>
<td>95.31 ± 2.29</td>
</tr>
</tbody>
</table>

ZP = zeta potential, PDI = polydispersity index, LC = loading content, EE = encapsulation efficiency, Standard deviation (SD) for n = 3.

3.3. In vitro drug release

The physical status of DTX in the NPs, pure DTX, DTX-loaded PCL-b-TPGS, TAPP-PCL, and TAPP-PCL-b-TPGS NPs was investigated by the DSC analysis. As shown in Fig. 4, the endothermic peak of pure DTX appeared at 169.2 °C, which, however, disappeared after being encapsulated in the NPs. Therefore, the anticancer drug DTX in the NPs’ formulations was in essentially amorphous or solid solution state after being loaded into NPs, which may thus improve its solubility.

Fig. 4. DSC analysis thermograms of the pure DTX, the DTX-loaded TAPP-PCL NPs, the PCL-b-TPGS NPs and the TAPP-PCL-b-TPGS NPs.

In vitro drug release profile of DTX-loaded NPs.

The DTX sustained release profile from the DTX-loaded TAPP-PCL-b-TPGS NPs was investigated in vitro and compared with that from the DTX-loaded TAPP-PCL NPs and PCL-b-TPGS NPs. As shown in Fig. 5, the accumulative drug release from the TAPP-PCL-b-TPGS NPs reached 47.78% in the first 6 days and 60.22% after 12 days, which were much higher than that released from the TAPP-PCL NPs, i.e., 22.30% and 32.30%, and that from the PCL-b-TPGS NPs, i.e., 27.00% and 44.70% respectively, in the same period. These can be explained by two reasons, i.e., TPGS causes porous structure of the NPs and thus promote drug release, and TAPP may drag the drug diffusion within the hydrophobic matrix.

Fig. 5. In vitro drug release profile of DTX-loaded NPs.
3.4. Effect of blank NPs on P-gp efflux

Previous studies have suggested that TPGS reversibly inhibited P-gp mediated antitumor drug efflux such as DOX, vinblastine, DTX [37,38]. In the present study, we also assessed the possibility of P-gp inhibition by the TAPP-PCL-PCL-TPGS and PCL-TPGS NPs vs the blank NPs.

A significant amount of TPGS is contained in the TAPP-PCL-PCL-TPGS and PCL-TPGS copolymers, but not in the TAPP-PCL copolymer. We used 1 mg/mL TPGS as positive control and fresh nanoparticle-free medium as negative control. Since both DTX and DOX are substrates of P-gp, we tested their cytotoxicity on the MCF-7/ADR cells, respectively. As shown in Fig. 6A and B, MCF-7/ADR cells were treated with DOX (2 mg/mL) or DTX (2 mg/mL) combined with the TAPP-PCL, PCL-TPGS or TAPP-PCL-PCL-TPGS for 24 h or 48 h and we found that at each time point, PCL-TPGS or TAPP-PCL-PCL-TPGS combined with DOX or DTX induced greater cytotoxicity, but not in the TAPP-PCL treatment. To further confirm the blank TAPP-PCL-PCL-TPGS NPs’ inhibition effect on P-gp, we conducted P-gp ATPase assay, in which cell membrane P-gp were treated with the NPs. As shown in Fig. 6C, the blank TAPP-PCL-PCL-TPGS NPs showed the direct inhibition of
P-gp ATPase. These data suggested that the TPGS-containing NPs have the ability of inhibition of P-gp efflux effect and increase drug effectiveness.

3.5. Cellular uptake of coumarin-6-loaded NPs in HeLa cells

To explore the cellular uptake efficiency of NPs, coumarin-6-loaded TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs were prepared and incubated with HeLa cells at NP concentrations of 20 µg/mL, 100 µg/mL and 200 µg/mL respectively for 30 min. As shown in Fig. 6D, the cellular uptake efficiency, which is defined as the percentage of the internalized NPs to the NPs provided in the culture, decreased with the increase in nanoparticle concentration. The cellular uptake efficiency of the TAPP-PCL-b-TPGS NPs is much higher than that of the TAPP-PCL and PCL-b-TPGS NPs at the designated three concentrations. Meanwhile, HeLa cells exhibited almost equal ratios of cellular uptake of the TAPP-PCL and PCL-b-TPGS NPs at all three nanoparticle concentrations.

To directly observe the cellular uptake of the three types of coumarin-6-loaded NPs, 100 µg/mL coumarin-6-loaded TAPP-PCL-b-TPGS, TAPP-PCL and PCL-b-TPGS NPs were incubated with HeLa cells, respectively. After 30 min, the treated cells were washed with PBS for three times followed by fixation in 4% paraformaldehyde for 15 min. The nuclei were stained with 5 µg/mL DAPI. Then the treated cells were observed under CLSM images. As shown in Fig. 6E, HeLa cells efficiently took in the coumarin-6-loaded TAPP-PCL-b-TPGS NPs in comparison with that of pure DTX at the same docetaxel dose for 24 h (C) and 48 h (D) with 2.1 J/cm² irradiation; (E and F) Cell inhibition rate of HeLa cells incubated with DTX-loaded TAPP-PCL-b-TPGS NPs for 24 h (E) and 48 h (F) at different doses of irradiation.

Fig. 7. Inhibition rate of blank NPs or DTX-loaded NPs with or without irradiation as indicated. (A) HeLa cells were treated with TAPP-PCL, PCL-b-TPGS and TAPP-PCL-b-TPGS at different doses of irradiation, and the cell inhibition of the NPs were detected using the MTT assay; (B) HeLa cells were treated with 5 mg/mL, 10 mg/mL, 20 mg/mL and 30 mg/mL blank TAPP-PCL-b-TPGS NPs at different doses of irradiation. The cell inhibition of the NPs were detected using the MTT assay; (C and D) Cell inhibition rate of HeLa cells incubated with DTX-loaded TAPP-PCL-b-TPGS NPs in comparison with that of pure DTX at the same docetaxel dose for 24 h (C) and 48 h (D) with 2.1 J/cm² irradiation; (E and F) Cell inhibition rate of HeLa cells incubated with DTX-loaded TAPP-PCL-b-TPGS NPs for 24 h (E) and 48 h (F) at different doses of irradiation.
that of the TAPP-PCL-b-TPGS NPs. Also, the coumarin-6-loaded TAPP-PCL and PCL-TPGS NPs were spotted mainly in the cytoplasm and much less in the nucleus.

3.6. DTX-loaded NPs’ effect on microtubules

Previous studies have demonstrated that DTX bound to and stabilized microtubules by the inhibition of microtubule depolymerization [30] and consequently induced cell apoptosis [39,40]. To further explore the DTX-loaded nanoparticles’ effects on microtubules, we used anti-α-tubulin primary antibody and FITC-conjugated secondary antibody to label microtubules, which were then observed by CLSM. Since the previous result showed that HeLa cells took in NPs in 30 min, we treated the cells for 30 min in order to ensure that the effect on microtubules was from the NPs internalized by the cells rather than by the drug released from NPs. Additionally, we added 100 ng/mL equivalent DTX, a relatively low concentration, to intuitively observe the microtubules’ variation since DTX can take effect at a very low concentration [28,40]. As shown in Fig. 6F, HeLa cells treated with the DTX-free TAPP-PCL-b-TPGS NPs had a well-organized microtubule network, but cells treated with the DTX-loaded TAPP-PCL-b-TPGS NPs and the pure DTX showed more condensed microtubules. With the confocal microscopy result, we further confirmed that DTX-loaded TAPP-PCL-b-TPGS NPs could be efficiently taken in by cancer cells, enhancing drug therapeutic effects.

3.7. Cytotoxicity and phototoxicity of TAPP-PCL-b-TPGS NPs

TAPP is a photosensitizer and can be easily and specifically internalized by cancer cells. When it is activated and exposed to light and oxygen, ROS and other reactive cytotoxic species can be generated to destroy cancer cells [28,41]. We first examined the

![Image: Generation of ROS by irradiation with TAPP-PCL, PCL-b-TPGS, TAPP-PCL-b-TPGS and DTX-loaded TAPP-PCL-b-TPGS treatment. Green: DCF (representing ROS); Blue: nucleus.]

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>IC50 (µg/mL)</th>
<th>Taxotere®</th>
<th>DTX-loaded TAPP-PCL-b-TPGS</th>
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<tr>
<td>Dark 0.7 J/cm² 2.1 J/cm²</td>
<td></td>
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<tr>
<td>24 17.13 ± 1.72 17.94 ± 1.06 8.90 ± 1.07 1.88 ± 0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 13.57 ± 0.86 10.17 ± 1.39 1.51 ± 0.79 0.24 ± 0.03</td>
<td></td>
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</tbody>
</table>
cytotoxicity of the blank (drug-free) TAPP-PCL-b-TPGS NPs, TAPP-PCL NPs and PCL-b-TPGS NPs on the HeLa cells and found that these NPs all showed very little cytotoxicity without irradiation, which indicated the blank NPs were almost nontoxic and possessed excellent bio-compatibility. Similar results were reported by Jiang’s group and Lai’s group [11,24].

However, the TAPP-PCL-b-TPGS NPs and TAPP-PCL NPs, but not the PCL-b-TPGS NPs, showed elevated cytotoxicity at different light dose irradiations, i.e., 32.1% and 27.4% inhibition, respectively (at 2.1 J/cm² irradiation) (Fig. 7A). As for the blank TAPP-PCL-b-TPGS NPs, the cell inhibition rate increased with the elevation of the NPs’ concentration and the irradiation dose (Fig. 7B). These results suggested that the TAPP-PCL-b-TPGS NPs could be activated by irradiation and showed potential for cancer treatment by PDT.

The cytotoxicity of Taxotere® and the DTX-loaded TAPP-PCL-b-TPGS NPs on HeLa cells with irradiation was also investigated. We found that after 2.1 J/cm² irradiation treatment for 24 h (Fig. 7C) and 48 h (Fig. 7D), we found that the DTX-loaded TAPP-PCL-b-TPGS NPs showed much more significant cell inhibition than that of Taxotere® after longer durations of treatment due to the more efficient internalization and PDT effect of longer durations.

We further determined whether the cytotoxicity of the DTX-loaded TAPP-PCL-b-TPGS NPs is concentration- and irradiation dose-dependent. Fig. 7E and F shows the inhibition rate of the HeLa cells incubated with the DTX-loaded TAPP-PCL-b-TPGS NPs at the irradiation dose of 0, 0.7, 2.1 J/cm² for 24 h and 48 h respectively. It can be seen that the cell inhibition rate in the dark case was 53.1% with the DTX-loaded TAPP-PCL-b-TPGS NPs. However, the inhibition rate was increased to approximately 80.3% under 2.1 J/cm² irradiation at a concentration of 25 μg/mL equivalent DTX incubated for 24 h (Fig. 7E). The increase in the inhibition rate was found to be even higher up to 90% in the case of longer incubation for 48 h (Fig. 7F). The results of Fig. 7E and F can be quantitatively summarized by IC₅₀, i.e., the 50% inhibition concentration, the drug concentration needed to kill 50% of cancer cells in a designated time period. It was found to be 17.13 ± 1.72, 17.94 ± 1.06, 8.90 ± 1.07 and 1.88 ± 0.30 μg/mL for 24 h treatment, and 13.57 ± 0.86, 10.17 ± 1.39, 1.51 ± 0.79, and 0.24 ± 0.03 μg/mL for 48 h treatment with Taxotere® and the DTX-loaded TAPP-PCL-b-TPGS NPs formulation after 0, 0.7 and 2.1 J/cm² irradiation, respectively (Table 2). This means that the DTX-loaded TAPP-PCL-b-TPGS NPs’ formulation can be 1.92 and 9.36-fold efficient for 24 h treatment, and 8.95-fold and 56.5-fold efficient for 48 h treatment by the combinatory chemophotodynamic treatment in comparison with the corresponding chemotherapy. There may be three reasons for such advantages of the proposed chemophotodynamic therapy by the DTX-loaded TAPP-PCL-b-TPGS NPs. Firstly, the TPGS component inhibits P-gp and thus overcomes the multidrug resistance [37]; secondly, the photochemical internalization (PCI) effect may also contribute, which can breakdown the endosomal or/and lysosomal membranes to release drug molecules and entrap them in the endosome or/and lysosome [5,11,32]. Thirdly, the TAPP may react with oxygen in the water microenvironment by irradiation to generate ROS to induce cell death [10,42].

In order to examine the ROS generated by irradiation, HeLa cells were treated with the NPs with or without irradiation and analyzed with Reactive Oxygen Species Assay Kit (Beyotime) by CLSM. As shown in Fig. 8, there were no differences in the ROS level between the PCL-b-TPGS treated HeLa cells with and without irradiation. However, the ROS level was elevated in the TAPP-PCL NPs, the TAPP-PCL-b-TPGS NPs and the DTX-loaded TAPP-PCL-b-TPGS NPs with or without PDT.
NPs-treated cells with irradiation compared with the case with no irradiation treatment. All the results demonstrate that the drug loaded with TAPP-PCL-b-TPGS NPs could have great advantages for combinatory chemophotodynamic therapy.

3.8. Antitumor effect on cervical cancer xenograft tumor model

To evaluate the in vivo anti-tumor effect of the chemophotodynamic therapy by the DTX-loaded TAPP-PCL-b-TPGS NPs, we subcutaneously injected \(2.0 \times 10^6\) HeLa cells in 100 \(\mu\)L of DMEM medium without serum into the right flank of the nude mice. After 5 days, the tumors grew to a palpable size, and the nude mice were randomly divided into 4 groups (\(n = 10\)). The control group was administrated with saline, and the experimental groups were administrated with Taxotere\(^\text{\textregistered}\) (10 mg/kg), and the DTX-loaded TAPP-PCL-b-TPGS NPs (equivalent to 10 mg/kg of DTX) without/with PDT, respectively. We ended the experiment when the tumors grew to 2 cm in diameter or paralysis or major compromise in their quality of life occurred. In accordance with our expectation, both Taxotere\(^\text{\textregistered}\) and the DTX-loaded TAPP-PCL-b-TPGS NP formulation efficiently repressed tumor growth compared with saline treatment (Fig. 9A). Interestingly, the DTX-loaded TAPP-PCL-b-TPGS combined with PDT therapy greatly reduced tumor volume compared with the saline treatment (\(p < 0.001\)) and the Taxotere\(^\text{\textregistered}\) treatment (\(p < 0.01\)).

We also weighed the mice in the course of the treatment and found that the treatment with the DTX-loaded TAPP-PCL-b-TPGS without/with PDT did not reduce body weight of the mice, which suggested that the chemophotodynamic therapy had no apparent side effects (Fig. 9B).

At the end of the treatment, all the mice were euthanized and tumors were isolated, imaged and weighed (Fig. 9C and D). Consistent with the tumor volume, tumor weights were also significantly reduced by the DTX-loaded TAPP-PCL-b-TPGS NPs which was further enhanced by combination with PDT therapy. To investigate the potential toxicity of the nanomedicine with PDT, tissues, including the heart, liver, spleen, lung and kidney, from the four groups of mice with different treatments were isolated and stained with H&E. As shown in Fig. 10, all the tested tissue samples did not cause any significant lesion to the treatment, which suggested that the DTX-loaded TAPP-PCL-b-TPGS nanomedicine with PDT treatment had no obvious side effects on mice.

Our research suggests that the chemophotodynamic therapy by the DTX-loaded TAPP-PCL-b-TPGS NPs could be much more efficient than the Taxotere\(^\text{\textregistered}\) chemotherapy alone and thus may have great potential for clinical application in cancer treatment.

4. Discussion

The current treatment of cancer mainly includes surgery followed by radiotherapy and/or chemotherapy, and other emerging modalities such as immunotherapy, gene therapy, thermal therapy as well as photodynamic therapy. It is clear, however, that each of these modalities could hardly provide complete treatment due to dose limitation and the resistance of cancer cells to these individual therapies. It is thus reasonable to combine two or more of these...
modalities to take advantage and avoid disadvantages, and most likely, to achieve synergistic effects. Chemotherapy is efficient for certain types of cancer in practice; however, it has problems such as side effects, which are caused by a lack of drug targeting, the multidrug resistance of the cancer cells, and the problems of solubility, permeability and stability of anticancer drugs. Chemotherapy itself is confined by maximum tolerance of the drug, which can be enhanced in combination with other modalities such as thermal therapy and immunotherapy.

The recent development of nanomedicine has brought hope to realize sustained, controlled and targeted delivery of therapeutics by various nanocarriers including prodrugs, micelles, liposomes, solid lipid nanoparticles and nanoparticles of biodegradable polymers. Compared with other nanoparticles, most polymeric nanoparticles are about 100 nm which confers increased cellular uptake [43] and easy-access to tissues [44]. Polymeric nanoparticles can be modified to prolong circulation time and escape from clearance by monocytes and macrophages [45]. Certain pH-sensitive polymeric nanoparticles, such as polyketal nanoparticles and poly(ethylene oxide)-modified poly(betaamino ester) nanoparticles, and temperature sensitive polymeric nanoparticles, such as poly(ethylenimine) nanoparticles, could realize the loading and controlled release of specific drug molecules [46–49]. Imaging agents can be encapsulated or conjugated to polymeric nanoparticles for cancer diagnostic applications and precision therapy [50]. As most are fabricated with FDA approved biodegradable and biocompatible polymers, the biodegradable polymeric nanoparticles have excellent biocompatibility and low toxicity, whereas certain metal nanoparticles have heavy metal toxicity. Therefore, it is thus reasonable to assume that multimodality treatment of cancer by biodegradable polymeric nanomedicine strategies, i.e., co-formulation of the various single modality therapeutic agents in the same nanocarrier, could result in even higher synergistic effects. For example, our group has developed a system of nanoparticles of poly[lactide]-D-α-tocopheryl polyethylene glycol succinate (PLA-TPGS) and carboxyl group-terminated TPGS (TPGS-COOH) copolymer blend for multimodality treatment of cancer, which formulated DTX for chemotherapy, herceptin (Her) for biotherapy and targeting, and iron oxides (IOs) for hyperthermia therapy. We further developed a method by employing the concept of NPs IC50, the concentration of the agent-, or agents-loaded nanoparticles that are needed to kill 50% of the cancer cells, to quantitatively assess the synergistic effects of the multimodality treatment. It is shown by employing the SK-BR-3 cell line as an in vitro model of the HER2-positive breast cancer that the total NPs concentration was 2.34 mg/mL for the treatment of a physical mixture of the DTX-loaded NPs, Her-loaded NPs and IOs-loaded NPs at the 1:2:7 weight ratio, while it is only 0.0011 mg/mL for the multimodality NPs for 24 h, which is 2130-fold more efficient than the physical mixture of the corresponding single modality treatments [51,52]. Ma et al. tried to functionalize the hydrophobic porphyrin with the peptide dendrons to obtain a star-shaped copolymer, porphyrin-poly(lysine) dendrons (PP-PLLD), with a desired structure. Such a star-shaped structure could decrease the aggregation of porphyrin in an aqueous solution and thus result in a low critical micellar concentration. An anticancer drug doxorubicin (DOX) was employed to realize an efficient chemophotodynamic therapy. The in vitro toxicity assay showed no dark cytotoxicity but significant phototoxicity. Moreover, the DOX-loaded PP-PLLD micelles shows higher cytotoxicity under light conditions than the PP-PLLD or DOX alone, suggesting PP-PLLD has a potential application in combined photodynamic therapy and chemotherapy. It seems, however, that the drug loading is not as high (6.4%) and no drug encapsulation efficiency was assessed. Also, no quantitative analysis of the in vitro cytotoxicity, for example the IC50 of the DOX-loaded micelles with and without photodynamic treatment, was investigated. Moreover, no in vivo performance was assessed [53]. Thus, it is necessary to develop and identify novel photodynamic nanoparticles for anti-cancer drug delivery and synergy therapy.

5. Conclusions

We synthesized a novel system of nanoparticles of the 4-arm star-shaped porphyrin-centered PCL-b-TPGS copolymer (TAPP-PCL-b-TPGS NPs) to encapsulate hydrophobic anti-tumor drug DTX for a combinatorial chemophotodynamic therapy of cervical cancer. We found that the TPGS component in the biodegradable copolymer can help to not only enhance the size and size distribution, drug encapsulation efficiency and drug release profiles, and the cellular uptake of the nanoparticles, but also overcome multidrug resistance. The proposed chemophotodynamic therapy by the DTX-loaded TAPP-PCL-b-TPGS NPs has shown great advantages in the treatment of cervical cancer in vitro and in vivo, and thus may have great potential for clinical applications.

Conflict of interest

There is no conflict of interest.

Acknowledgments

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Appendix.

Abbreviations

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<tr>
<th>Acronym</th>
<th>Full Form</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1H NMR</td>
<td>proton nuclear magnetic resonance</td>
<td></td>
</tr>
<tr>
<td>ADR</td>
<td>adriamycin resistant</td>
<td></td>
</tr>
<tr>
<td>DAPI</td>
<td>4,6-diamidino-2-phenylindole dihydrochloride</td>
<td></td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
<td></td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's modified Eagle's medium</td>
<td></td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
<td></td>
</tr>
<tr>
<td>DTPS</td>
<td>4-((dimethylamino)pyridine 4-toluenesulfonate</td>
<td></td>
</tr>
<tr>
<td>EE</td>
<td>encapsulation efficiency</td>
<td></td>
</tr>
<tr>
<td>FESEM</td>
<td>field emission scanning electron microscopy</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>LC</td>
<td>loading content</td>
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<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoiium bromide</td>
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</tr>
<tr>
<td>PCL-b-TPGS</td>
<td>poly(e-caprolactone)-b-D-α-tocopheryl polyethylene glycol 1000 succinate</td>
<td></td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index</td>
<td></td>
</tr>
<tr>
<td>PDT</td>
<td>photodynamic therapy</td>
<td></td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<td>TAPP</td>
<td>5,10,15,20-tetrakis(4-aminophenyl)-21H,23H-chorophine</td>
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<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
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