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A Self-assembled Nanoparticle Platform Based on Amphiphilic Oleanolic Acid Polyprodrug for Cancer Therapy

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Abstract Oleanolic acid (OA) is a pentacyclic triterpenoid compound with extensive biological effects, such as anti-inflammatory and anticancer activities. However, the application of OA in chemotherapy is hampered by its poor solubility and severe adverse effects. To solve the problems, we developed a self-assembled nanoparticle platform based on amphiphilic oleanolic acid polyprodrug, poly[oligo(ethylene glycol) methyl ether methacrylate]-*b*-poly(oleanolic acid methacrylate) (POEGMA-*b*-POAMA), encapsulating 10-hydroxycamptothecin (HCPT) to achieve efficient cancer therapy. The polyprodrug was prepared *via* reversible addition-fragmentation chain transfer polymerization (RAFT), and could self-assemble to prepare POEGMA-*b*-POAMA/HCPT nanoparticles (NPs). The obtained nanoparticles exhibited appropriate particle size, excellent drug stability, good drug loading capacity, and high drug loading efficiency. *In vitro* drug release indicated that the drug release was prolonged to 132 h. The POEGMA-*b*-POAMA/HCPT NPs enhanced cell cytotoxicity in 4T1 cells and MCF-7 cells and could be efficiently uptaken by 4T1 cells. Furthermore, *in vivo* antitumor efficiency showed that the POEGMA-*b*-POAMA/HCPT NPs had great antitumor efficiency with considerably low adverse effects in the treatment of the 4T1 mouse breast tumor xenograft tumor. Therefore, POEGMA-*b*-POAMA/HCPT NPs provide great potential as a platform for drug delivery applications.

Keywords Self-assemble; Nanoparticle; Oleanolic acid; Amphiphilic polyprodrug; Drug delivery

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INTRODUCTION

Polymeric drug delivery systems for cancer therapy have received remarkable attention and are under development to overcome the drawbacks of traditional cancer therapy, such as severe side effects, poor water solubility, and low antitumor activities.^[1–4] Furthermore, polymeric drug delivery systems have advantages in diverse chemical structures, flexible synthesis methods, and easy functionalization.^[5–7] Numerous polymeric drug deliveries such as nanogels, nanoparticles, and nanospheres have played a significant role in medical applications.^[8–10] Therefore, polymeric drug delivery systems are considered as a potential strategy in the drug delivery field.

Prodrug-based polymeric nanoparticles, as one type of polymeric drug delivery systems, are advantageous owing to the combined desirable advantages of both prodrugs and nanocarriers.^[11,12] Traditional polymeric nanoparticles usually introduce a great number of inert hydrophobic materials which lead to the drawbacks of low drug payloads and undesired side effects. Prodrug-based polymeric nanoparticles could overcome the drawbacks of traditional polymeric nanoparticles and exhibit extra benefits such as improving drug

solubility, prolonging drug circulation, reducing the drug resistance, and enhancing drug accumulation in tumor sites because of the enhanced permeability and retention (EPR) effect.^[13–16]

Typical methods for the synthesis of polymeric prodrug nanoparticles are conjugation of the drug with amphiphilic or hydrophilic polymers and drug-initiated polymerization.^[17,18] A few polymers were conjugated with doxorubicin (DOX), betulinic acid (BA), paclitaxel (PTX), or camptothecin (CPT),^[19–22] which prolonged the drug release and decreased side effects. As for drug-initiated polymerization, reactive controlled radical polymerization is usually used to produce amphiphilic polymers, such as radical ring-opening polymerization (ROP), reversible addition-fragmentation chain transfer polymerization (RAFT), and atom transfer radical polymerization (ATRP).^[23–25] Thanks to these methods, polymeric prodrug nanoparticles were successfully fabricated. However, the potential of polymeric prodrug nanoparticles has not been well developed due to the low drug loading content and suboptimal therapeutic effects.

To overcome these drawbacks, an efficient polymeric drug delivery system is satisfactory with a high drug loading content and low side effects.^[26] Polyprodrug nanoparticles, which have been firstly proposed and developed since 2013,^[27] are desirable strategies by polymerizing therapeutic drugs with

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repeating prodrug units and possess tremendous advantages such as high drug dosage, controlled drug loading, self-assembling morphologies, and flexible structure design.^[28–32] In addition, combination therapy also plays a significant role to increase the efficiency of the nanoparticles and reduce drug resistance which cancer therapy often suffers from.^[33–36] Herein, polyprodrug nanoparticles which could co-deliver diverse therapeutic agents are of great potential to be developed.

Oleanolic acid (OA) is a ubiquitous triterpenoid in many natural plants which possesses a broad series of biological effects including anti-inflammatory, antioxidant, and antitumor activities.^[37–39] It has been confirmed that OA exhibits antitumor activity in the treatment of various cancers, such as hepatocellular carcinoma, thymic carcinoma, and breast carcinoma.^[40–42] However, the clinical use of OA is limited by the poor aqueous solubility ($< 1 \mu\text{g}\cdot\text{mL}^{-1}$) and low bioavailability.^[43] At present, there are several methods for enhancing the physicochemical properties of OA.^[44–46] Among them, polyprodrug nanoparticles are considered as a highly efficient approach to overcome the drawbacks.

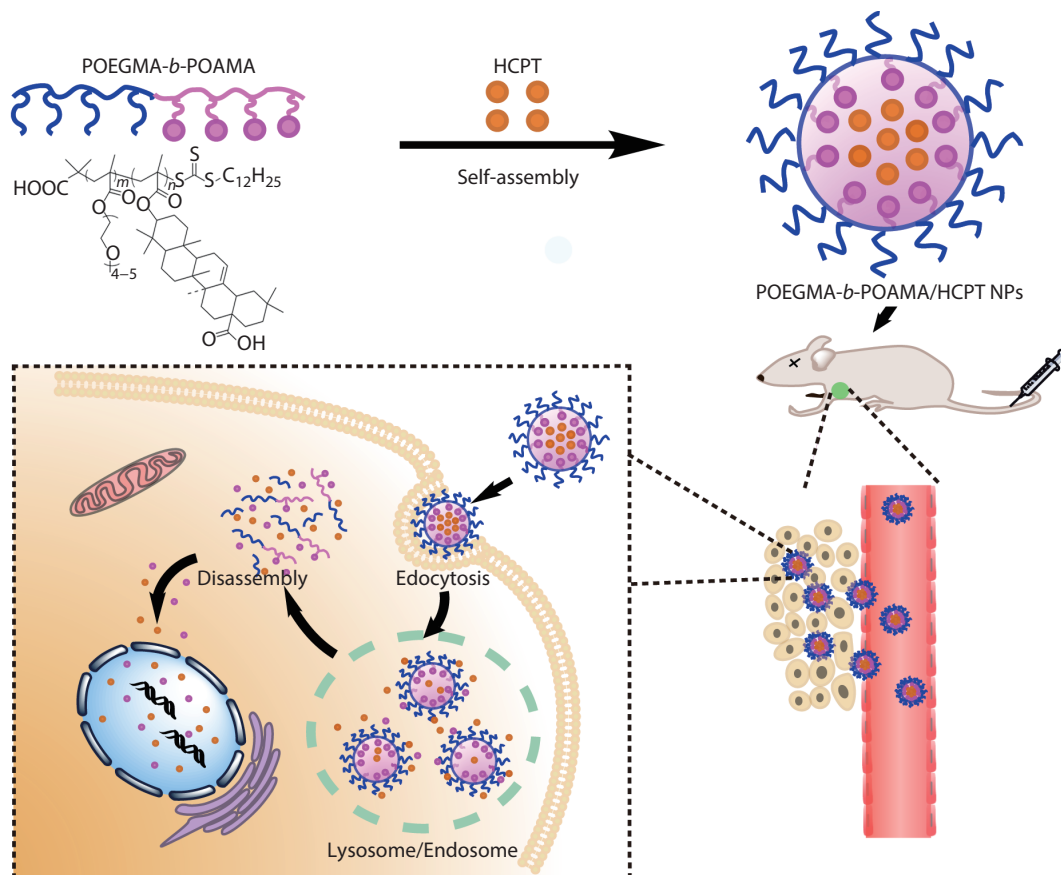
In the present work, a novel amphiphilic polyprodrug, poly[oligo(ethylene glycol) methyl ether methacrylate]-*b*-poly(oleanolic acid methacrylate) (POEGMA-*b*-POAMA), was synthesized via RAFT method, consisting of hydrophobic blocks of OA prodrug monomer and hydrophilic poly[oligo(ethylene glycol) methyl ether methacrylate]. Then, 10-hydroxycamptothecin (HCPT), another natural anticancer hydro-

phobic drug, was encapsulated into the self-assembled POEGMA-*b*-POAMA nanoparticles (NPs), which formed the POEGMA-*b*-POAMA/HCPT NPs. The nanoparticles could be accumulated at the tumor site via EPR effect and endocytosed into cancer cells. Meanwhile, the nanoparticles were disassembled with the release of anticancer drugs owing to the hydrolysis of ester linkage for cancer treatment (Scheme 1). The characteristics of the POEGMA-*b*-POAMA/HCPT NPs including particle size, zeta-potential, particle stability, and morphology were investigated. The drug release was confirmed by *in vitro* drug release study. *In vitro* cell cytotoxicity and cell uptake were studied in 4T1 cells and MCF-7 cells. Moreover, *in vivo* anticancer efficacy and the adverse effects of the POEGMA-*b*-POAMA/HCPT NPs were systematically evaluated. The results indicated that the self-assembled nanoparticle platform based on amphiphilic oleanolic acid polyprodrug encapsulating 10-hydroxycamptothecin was a promising drug carrier for enhanced cancer therapy.

EXPERIMENTAL

Materials

Oleanolic acid (OA) and 10-hydroxycamptothecin (HCPT) were supplied by Chengdu Preferred Biotechnology Co., Ltd. (Sichuan, China). Oligo(ethylene glycol) methyl ether methacrylate (OEGMA, $M_n = 300 \text{ g}\cdot\text{mol}^{-1}$) and azobis(isobutyronitrile) (AIBN) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Methacryloyl chloride (MAC) was



Scheme 1 Synthesis of POEGMA-*b*-POAMA/HCPT NPs and intracellular drug release for cancer therapy.

purchased from Aladdin (Shanghai, China). *N,N*-dimethylformamide (DMF) and dichloromethane (DCM) were obtained from J&K Chemical Reagent Co., Ltd. (Beijing, China). 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT), 4-dimethylaminopyridin (DMAP), triethylamine (TEA), and ethyl ether were supplied by Sigma-Aldrich (Shanghai, China). Fetal bovine serum (FBS), Penicillin and streptomycin (PS), and Roswell Park Memorial Institute (RPMI-1640) were obtained from Hyclone. Dialysis bags (cut off $M_w = 3500$) were obtained from Fisher (Ottawa, Canada).

4T1 cell line and MCF-7 cell line were supplied by the Peking University Health Science Center (Beijing, China). The cell lines were both incubated in full RPMI-1640 culture medium (RPMI-1640 supplement with 1% PS and 10% FBS) in 5% CO₂ and at 37 °C. Female BALB/C mice (6–8 weeks) were supplied by Peking University Health Science Center (Beijing, China) and used as the hosts for tumor xenografts. All animal experiments were complied with the guideline which was built by National Institutes of Health and ratified by the Beijing administration office of laboratory animal.

Synthesis of Oleanolic Acid Methacrylate (OAMA) Monomers

The OAMA monomers were synthesized according to Scheme 2(a). OA (1.0 g, 2.2 mmol), DMAP (0.1 g, 1.1 mmol), and TEA (0.5 g, 5.2 mmol) were added to DCM (5 mL) at 0 °C, and MAC (0.5 g, 4.8 mmol) was added dropwise for 20 min. The solution was stirred for 5 h under the room temperature. Subsequently, the resulting mixture was quenched with MeOH and rinsed with NaHCO₃ and brine. Then, the product was purified by column chromatography with hexane/DMC/ethyl acetate (6/1/1, V/V/V) to obtain OAMA monomers (0.6 g, 52 %).

Synthesis of MacroRAFT Agent POEGMA

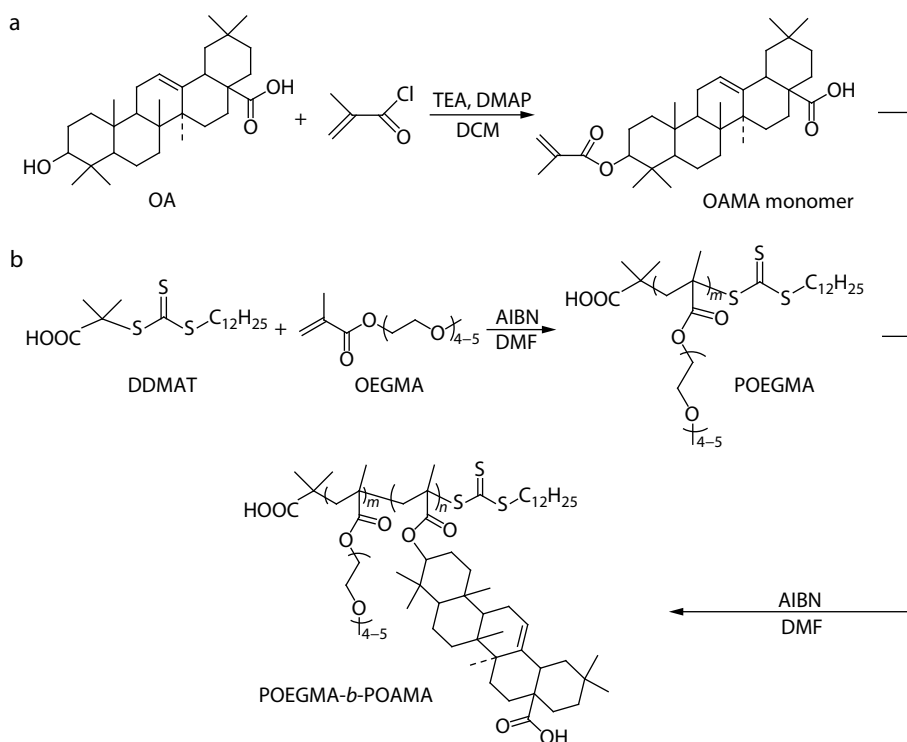
The macroRAFT agent POEGMA was prepared by using DDMAT as the chain transfer agent (Scheme 2b). OEGMA (1.8 g, 6.0 mmol), AIBN (2.63 mg, 0.016 mmol), and DDMAT (47.4 mg, 0.13 mmol) were dissolved in 6 mL of DMF inside a Schlenk flask. After degassed with nitrogen for 20 min, the Schlenk flask was immersed into the oil bath at 70 °C under nitrogen protection. The polymerization was carried out for 9 h and stopped by exposing to the air and cooling the flask with an ice bath. The polymer was purified with excess diethyl ether for twice and dried under vacuum overnight.

Synthesis of POEGMA-*b*-POAMA Polyprodrugs

The POEGMA-*b*-POAMA polyprodrugs were synthesized using POEGMA as the chain transfer agent (Scheme 2b). Monomer OAMA (50 mg, 0.06 mmol), DMF (4 mL), AIBN (1.9 mg, 0.012 mmol), and POEGMA (140 mg, 0.01 mmol) were added into a Schlenk flask. After degassed with nitrogen for 20 min, the reaction was allowed to proceed in nitrogen at 90 °C for 11 h. Then, the solution was quenched by cooling the flask with an ice bath and exposing to the air, and the mixture was purified by co-solvent precipitation. The crude products were dissolved in DMF and precipitated into diethyl ether for three times. The resulting polymer was dried under vacuum overnight and obtained as POEGMA-*b*-POAMA polyprodrugs.

Characterization of the POEGMA-*b*-POAMA Polyprodrugs

¹H-NMR spectroscopy of monomers and the polyprodrugs was performed in deuterated chloroform (CDCl₃-d₆) as the solvent by a Bruker AV400-MHz spectrometer (Bruker, Germany). Gel permeation chromatography (GPC) measurements were conducted on a GPC Shimadzu Rid-20A apparatus (Kyoto,



Scheme 2 Synthesis of (a) OAMA Monomer and (b) amphiphilic POEGMA-*b*-POAMA polyprodrugs.

Japan) to analyze the absolute molecular weights using DMF as the eluent solvent.

Synthesis of POEGMA-*b*-POAMA/HCPT NPs

The POEGMA-*b*-POAMA/HCPT NPs were prepared using the methods combining nanoprecipitation and self-assembly.^[47] Briefly, POEGMA-*b*-POAMA polyprodrugs (20 mg) and HCPT (5, 10, and 15 mg) were dissolved in DMSO (1 mL). The solution was added dropwise into 3.5 mL phosphate buffered saline (PBS) under constant stirring. Then, the mixture solution was dialyzed against PBS in dialysis bags (MWCO = 3500) for 24 h and filtered using a 1 µm filter. Finally, the POEGMA-*b*-POAMA/HCPT NPs were received after lyophilization. The POEGMA-*b*-POAMA NPs were also prepared by a similar method without encapsulated HCPT.

Characterization of POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCPT NPs

Transmission electron microscopy (TEM) observations were carried out on a JEM-1010 microscope (100 kV) to determine the morphology of the nanoparticles. Dynamic light scattering (DLS) was analyzed on a Zetasizer Nano-ZS instrument (Malvern, UK). The stability of the nanoparticles was evaluated by particle size and PDI measurements in pH 7.2 for 72 h of storage at 4 °C. Drug loading capacity (DC) and drug loading efficiency (DE) of the drug in nanoparticles were calculated using high performance liquid chromatography (HPLC). The mobile solvent was acetonitrile/water, and the volume ratios for OA and HCPT were 95/5 and 70/30, respectively. DC and DE were quantitated as follows: DC (%) = (the weight of free drug in the samples/the weight of total samples) × 100, DE (%) = (the weight of free drug in the nanoparticles/the weight of feeding drug) × 100.

In Vitro Drug Release

The *in vitro* drug release of POEGMA-*b*-POAMA/HCPT NPs was examined by a dialysis method.^[48] Briefly, POEGMA-*b*-POAMA/HCPT NPs (10 mg) were dissolved in PBS (10 mL) and then removed to the dialysis bag. The dialysis bag was placed into a beaker filled with 100 mL of PBS (pH 5.0, 6.0 or 7.2, respectively) under gentle shaking (100 r) at 37 °C. Subsequently, the external medium (200 µL) was collected and replaced with PBS at the specific time. The samples were analyzed to determine the release of OA and HCPT *via* the HPLC method. The mobile solvent was acetonitrile/water at a flow rate of 1 mL·min⁻¹ with the C18 column, and the volume ratios for OA and HCPT were 95/5 and 70/30 respectively. The UV detectors were 210 nm for OA and 254 nm for HCPT.

In Vitro Cell Cytotoxicity

The *in vitro* cell cytotoxicity of POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCPT NPs was determined on both 4T1 cells and MCF-7 cells with free OA and free HCPT used as a control. Firstly, 4T1 cells and MCF-7 cells (2 × 10⁴ cell·mL⁻¹, 180 µL per well) were separately plated in 96-well plates and incubated in 5% CO₂ for 24 h. Then, the cells were treated with free OA, free HCPT, POEGMA-*b*-POAMA NPs, or POEGMA-*b*-POAMA/HCPT NPs at different drug concentrations for different time. The cells were added with 20 µL CCK-8 reagent per well and incubated for another 2 h. Finally, the absorbance of the cells at 450 nm was detected on a Tecan infinite M200 microplate spectrophotometer. The IC₅₀ values were calculated which meant the sample concentration that inhibited 50% cell

growth compared with the control group. Combination index (CI) was also calculated to evaluate the synergistic effect between OA and HCPT, by the following equation: CI = (A/I₁) + (B/I₂), where A and B are the IC₅₀ values when OA and HCPT were administered in co-treatment, while I₁ and I₂ are the IC₅₀ values when OA and HCPT were administered in single treatment.

Cellular Uptake Study

Cellular uptake study of POEGMA-*b*-POAMA/HCPT NPs was assessed using 4T1 cells. In the flow cytometric (FCM) study, 4T1 cells (2 × 10⁵ cells, 1.5 mL media per well) were separately plated in 6-well plates and incubated overnight. Then, the cells were treated with 1 mL of fresh culture media containing POEGMA-*b*-POAMA/HCPT NPs and free HCPT with equivalent concentration of HCPT (2 µg·mL⁻¹). After a certain time, the cells were rinsed with PBS for three times to remove the supernatant and trypsinized from the plates. Finally, the cells were measured by a flow cytometer (Becton Dickinson, USA).

In Vivo Antitumor Efficiency

The *in vivo* antitumor efficiency of POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCPT NPs was evaluated with the 4T1 mouse breast tumor xenografts as the animal model. 5 × 10⁴ 4T1 cells were inoculated into the mice *via* tail-vein injection. The mice bearing 4T1 tumors (100–150 mm³) were divided into 4 groups (*n* = 4) randomly and injected with normal saline, free HCPT, POEGMA-*b*-POAMA NPs, or POEGMA-*b*-POAMA/HCPT NPs at a dose of equivalent HCPT (10 mg·mL⁻¹) every two days for three times. Mouse body weight and tumor sizes were evaluated every other day during the entire treatment. The tumor sizes (*V*) were determined using the equation: $V = (m \times n^2)/2$, where *m* and *n* are the longest and shortest of the tumor diameter. Relative tumor volume (RTV) was determined using the equation: $RTV = V_i/V_0$, where *V_i* and *V₀* are the tumor size at the specific time and the initial tumor size. Tumor growth inhibition (TGI) was measured based on the equation: $TGI (\%) = [(a - b)/a] \times 100$, where *a* and *b* are RTV of the control group and the test group, respectively. On day 12, all mice were sacrificed after the treatment and tumors were harvested and weighted.

Evaluation of Adverse Effects

The mice bearing 4T1 tumors were injected with PBS, free HCPT, POEGMA-*b*-POAMA NPs or POEGMA-*b*-POAMA NPs at the equivalent HCPT dose of 10 mg·mL⁻¹ *via* tail-vein injection. The mice blood was separated and centrifuged at 10 day. The samples were analyzed using the mouse IgE ELISA procedure to evaluate the type I hypersensitivity reactions, which is one of the most common hypersensitivity reactions.

Statistical Analysis

All the experiments were performed at least three times. The results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Synthesis and Characterization of POEGMA-*b*-POAMA Polyprodrugs

We have designed and synthesized a novel drug carrier based on an amphiphilic POEGMA-*b*-POAMA polyprodrug for cancer therapy with good drug loading efficiency and biocompatibility. The amphiphilic POEGMA-*b*-POAMA polyprodrug prepared

by a multiple synthetic approach consisted of the hydrophilic POEGMA and hydrophobic OAMA (Scheme 1). The structures and compositions of the monomers and the intermediates were calculated by $^1\text{H-NMR}$ and GPC. The characteristic resonance signals of OA were observed at 0.76–2.01 ppm (Fig. 1a). After the reaction between OA and MAC, new signals appeared at 6.74 ppm (b) and 6.89 ppm (c) and the chemical shift δ 3.22 (1H, t) of OA moved to 3.27 (1H, t), indicating the successful formation of OAMA monomers (Fig. 1A). As shown in Fig. 1(B), the signals at 3.46–3.93 ppm are ascribed to the PEG ethylene protons. The GPC analysis indicated that the number-average molecular weight (M_n) and polydispersity index (PDI) of POEGMA were $1.32 \times 10^4 \text{ g}\cdot\text{mol}^{-1}$ and 1.12, respectively (Table 1).

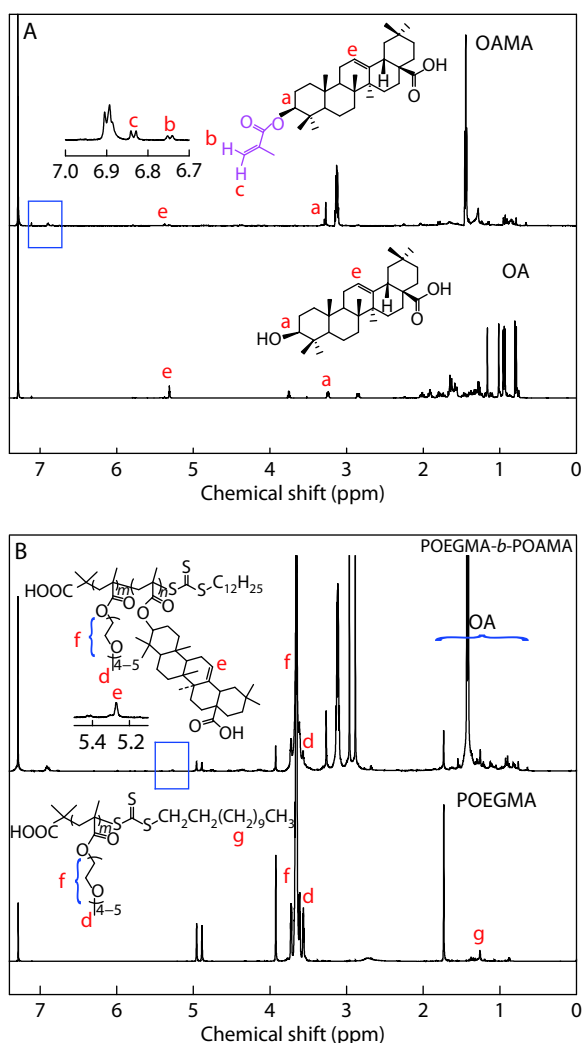


Fig. 1 $^1\text{H-NMR}$ spectra of (A) OAMA and OA, (B) POEGMA-*b*-POAMA and POEGMA.

Table 1 Characterizations of POEGMA and POEGMA-*b*-POAMA block copolymer.

Block copolymer	M_n^a (NMR)	M_n^b (GPC)	M_w^b (GPC)	PDI (GPC)
POEGMA	1.39×10^4	1.32×10^4	1.48×10^4	1.12
POEGMA- <i>b</i> -POAMA	1.70×10^4	1.52×10^4	1.81×10^4	1.19

^a Calculated by $^1\text{H-NMR}$ results; ^b Measured by GPC with THF as the eluent.

Fig. 1(B) shows that POEGMA-*b*-POAMA polyprodrug displayed the characteristic signals of both hydrophilic POEGMA and hydrophobic OAMA segments, suggesting that the copolymers were synthesized successfully. The GPC results demonstrated that the POEGMA-*b*-POAMA copolymer had a M_n of $1.52 \times 10^4 \text{ g}\cdot\text{mol}^{-1}$ and a PDI of 1.19. The degree of polymerization (m) of the POEGMA block was calculated by comparing the integral areas of the characteristic signal of POEGMA at 3.46 ppm (d) to that at 1.24 ppm (g). The repeating segments of POEGMA and OAMA were calculated by comparing the integral areas of the characteristic signal of POEGMA-*b*-POAMA at 3.46 ppm (d) to that at 5.3 ppm (e) (Fig. 1B). The POEGMA-*b*-POAMA copolymer contained 6 units of OAMA and 45 units of OEGMA. The POEGMA-*b*-POAMA copolymer in the study was POEGMA₄₅-*b*-POAMA₆. The GPC results agreed well with the M_n calculated by $^1\text{H-NMR}$ and the feeding ratio, showing a well-controlled polymerization reaction.

Synthesis and Characterization of the Nanoparticles

POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCTP NPs were synthesized via the nanoprecipitation method. The POEGMA-*b*-POAMA copolymer was an amphiphilic polymer which could self-assemble into nanoparticles and encapsulate HCTP in aqueous solution with hydrophilic POEGMA corona and hydrophobic drug core.^[49,50] The morphology of the POEGMA-*b*-POAMA NPs and the POEGMA-*b*-POAMA/HCTP NPs was investigated by TEM. Particle size, zeta potential, and size distribution of the nanoparticles were measured by DLS. As shown in Table 2, the average particle size of the POEGMA-*b*-POAMA NPs was $164.23 \pm 4.21 \text{ nm}$ with narrow PDI of 0.18, and the zeta potential of $9.03 \pm 0.24 \text{ mV}$. The OA content in POEGMA-*b*-POAMA NPs was calculated as $17.35\% \pm 0.21\%$. The POEGMA-*b*-POAMA/HCTP NPs displayed average particle sizes of 267.84 ± 5.82 , 190.12 ± 6.15 , and $283.68 \pm 7.64 \text{ nm}$, and the zeta potentials of 7.96 ± 0.53 , 8.05 ± 0.36 , and $8.19 \pm 0.36 \text{ mV}$ at the carrier/drug mass ratios of 20/5, 20/10, and 20/15, respectively. The POEGMA-*b*-POAMA/HCTP NPs displayed a prominent encapsulation performance with the highest DC_{HCTP} of $32.64\% \pm 0.82\%$ and DE_{HCTP} of $92.34\% \pm 1.12\%$. The carrier/drug mass ratio reached as low as 20/15. DC_{HCTP} in the POEGMA-*b*-POAMA NPs was improved with the decrease of carrier/drug weight ratio, which might be due to enhanced interaction between the carrier and drug.

In order to deliver the nanoparticles with an appropriate size below 200 nm, which is essential for EPR effect, the POEGMA-*b*-POAMA/HCTP NPs at the carrier/drug mass ratio of 20/10 were chosen for the subsequent study. Both of the nanoparticles exhibited spherical shapes (Figs. 2a and 2b) and equally distributed (Figs. 2c and 2d). To determine the colloidal stability of the nanoparticles, the particle size and the PDI were detected in pH 7.2 for 72 h at 4 °C. As shown in Figs. 2(e) and 2(f), there was no distinct change in particle size and PDI, indicating that the nanoparticles exhibited high redispersion stability. Therefore, these results demonstrated that the POEGMA-*b*-POAMA/HCTP NPs could be effective for the delivery of the two antitumor drugs.

In Vitro Drug Release

The pH (< 6.8) value in the tumor site is lower than that in normal cells (~ 7.4). The pH value is more acidic in the lysosomal

Table 2 Characterizations of POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCPT NPs.

Sample	OA content (%)	Mass ratio (mg/mg)	Size (nm)	Zeta potential (mV)	PDI	DC _{HCPT} (wt%)	DE _{HCPT} (wt%)
POEGMA- <i>b</i> -POAMA NPs	17.35 ± 0.21	—	164.23 ± 4.21	9.03 ± 0.24	0.18	—	—
POEGMA- <i>b</i> -POAMA/HCPT NPs	—	20/5	267.84 ± 5.82	7.96 ± 0.53	0.22	17.85 ± 1.19	92.34 ± 1.12
	—	20/10	190.12 ± 6.15	8.05 ± 0.36	0.23	28.33 ± 0.67	83.42 ± 0.98
	—	20/15	283.68 ± 7.64	8.19 ± 0.36	0.19	32.64 ± 0.82	75.16 ± 1.05

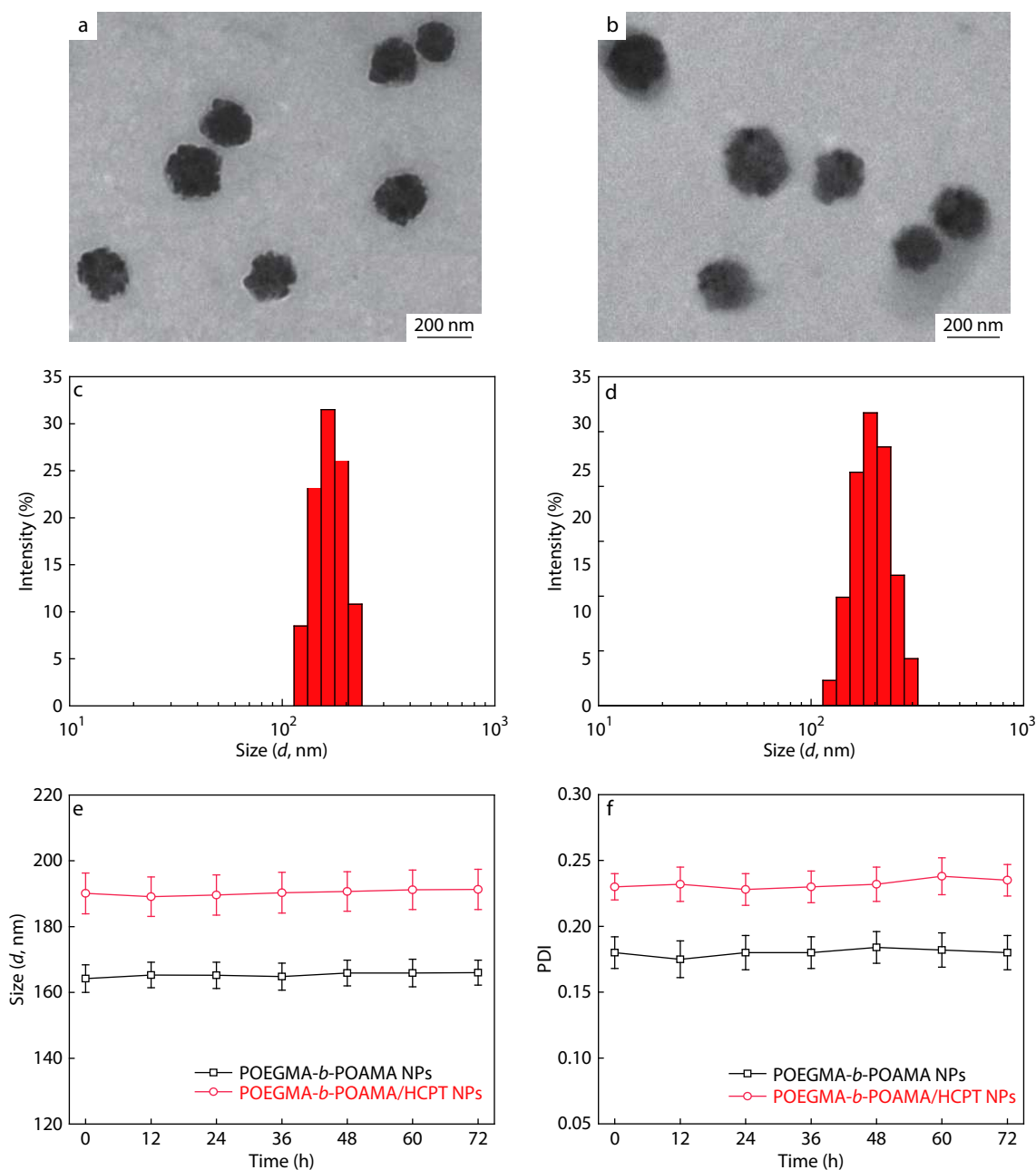


Fig. 2 TEM images of (a) the POEGMA-*b*-POAMA NPs and (b) the POEGMA-*b*-POAMA/HCPT NPs. The size distribution of (c) the POEGMA-*b*-POAMA NPs and (d) the POEGMA-*b*-POAMA/HCPT NPs. The stability of the POEGMA-*b*-POAMA NPs and the POEGMA-*b*-POAMA/HCPT NPs by (e) the particle size and (f) the PDI measurements in pH 7.2 for 72 h of storage at 4 °C ($n = 3$).

compartment (pH 4.5–5.0) and the endosomal (pH 5.0–6.5). Therefore, *in vitro* release performances of OA and HCPT from the nanoparticles were investigated in PBS at pH 5.0, 6.0, and 7.2 (Fig. 3). There were two stages that could be distinguished for

all drug release profiles. The POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCPT NPs showed an initial burst release due to the concentration gradient between the medium and the nanoparticles. After 12 h, the cumulative release of OA from

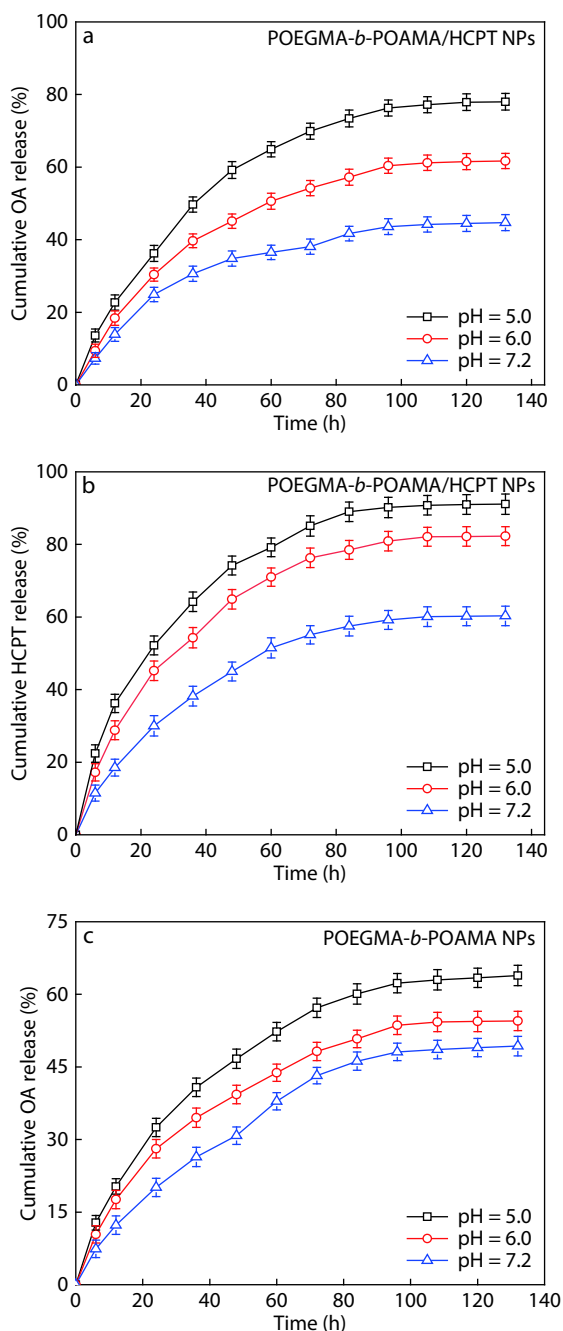


Fig. 3 (a) OA release kinetics and (b) HCPT release kinetics from the POEGMA-*b*-POAMA/HCTP NPs under different pH at 37 °C, (c) OA release kinetics from the POEGMA-*b*-POAMA NPs under different pH at 37 °C via HPLC analysis. Data are displayed as mean $\pm$ SD ($n = 3$).

POEGMA-*b*-POAMA NPs was 20.3% (pH 5.0), 17.6% (pH 6.0), and 12.3% (pH 7.2) and that from the POEGMA-*b*-POAMA/HCTP NPs was 22.7% (pH 5.0), 18.4% (pH 6.0), and 13.9% (pH 7.2) (Figs. 3a and 3c), while the cumulative release of HCPT from the POEGMA-*b*-POAMA/HCTP NPs was about 36.2% (pH 5.0), 28.8% (pH 6.0), and 18.5% (pH 7.2) (Fig. 3b). Then, the nanoparticles degraded and released slowly at a low level, which indicated that the POEGMA-*b*-POAMA NPs and the POEGMA-*b*-POAMA/HCTP NPs significantly prolonged the release of OA and

HCPT. After 132 h, the cumulative release of OA from the POEGMA-*b*-POAMA NPs reached up to 63.9% (pH 5.0), 54.5% (pH 6.0), and 49.3% (pH 7.2) and that from the POEGMA-*b*-POAMA/HCTP NPs reached 78.0% (pH 5.0), 61.7% (pH 6.0), and 44.7% (pH 7.2) (Figs. 3a and 3c). Meanwhile, the drug OA from the POEGMA-*b*-POAMA NPs and POEGMA-*b*-POAMA/HCTP NPs was slowly hydrolyzed and released at weakly acidic or neutral pH without the burst release phenomenon. The OA release rate in acidic solution (pH 5.0) was higher than that in slightly acidic solution (pH 6.0) or neutral solution (pH 7.2) (Figs. 3a and 3c), which was due to the re-protonation of the hydroxyl group of OA.^[51,52] The HCPT encapsulated by POEGMA-*b*-POAMA/HCTP NPs displayed a similar release profile at the acid condition (Fig. 3b). This was attributed to the disassembly of nanoparticles in the acid environment. These results indicated that the prepared POEGMA-*b*-POAMA NPs and the POEGMA-*b*-POAMA/HCTP NPs significantly prolonged the drug release under the weakly acid condition of the tumor microenvironment.

In Vitro Cell Cytotoxicity

In vitro cell cytotoxicity of the POEGMA-*b*-POAMA NPs and the POEGMA-*b*-POAMA/HCTP NPs was measured on the 4T1 cell lines and MCF-7 cell lines using free OA and free HCPT as the control. Figs. 4(a) and 4(b) show that the cell viability was significantly reduced with the increasing sample concentration.

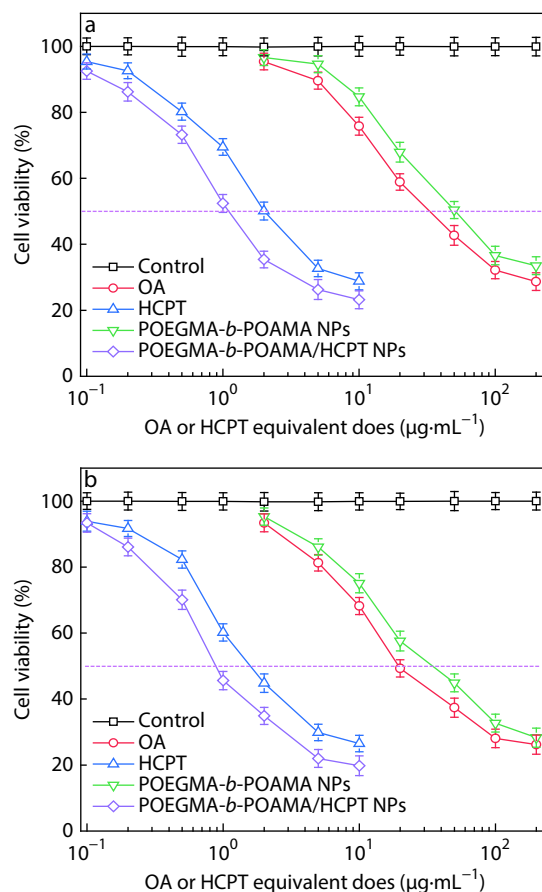


Fig. 4 CCK-8 assay of OA, HCPT, POEGMA-*b*-POAMA NPs, and POEGMA-*b*-POAMA/HCTP NPs at different concentrations in the (a) 4T1 cell lines and (b) MCF-7 cell lines. Data are displayed as mean $\pm$ SD ($n = 3$).

In comparison to free OA, the POEGMA-*b*-POAMA NPs showed low cytotoxicity against 4T1 cells and MCF-7 cells, which might be attributed to the limited OA cleavage from the POEGMA-*b*-POAMA polyprodrug. The IC_{50} values of different samples toward 4T1 cells and MCF-7 cells are displayed in Table 3. The POEGMA-*b*-POAMA/HCPN NPs exhibited the highest cytotoxicity against 4T1 cells and MCF-7 cells compared with free HCPN, free OA, and the POEGMA-*b*-POAMA NPs, which might be due to the enhanced cytotoxicity depending on the release of OA, and co-delivered HCPN. Furthermore, the CI values were 0.74 for 4T1 cells and 0.69 for MCF-7 cells, both of which were < 1, suggesting the synergistic effect between OA and HCPN in 4T1 cells and MCF-7 cells.^[53,54] In summary, the POEGMA-*b*-POAMA/HCPN NPs displayed high therapeutic efficacy to 4T1 cells and MCF-7 cells.

Table 3 *In vitro* cytotoxicity analysis (IC_{50} , $\mu\text{g}\cdot\text{mL}^{-1}$).

Compound	4T1 cells	MCF-7 cells
POEGMA- <i>b</i> -POAMA NPs	77.1	35.4
OA	44.6	19.4
HCPN	1.58	1.61
POEGMA- <i>b</i> -POAMA/HCPN NPs	1.14	0.88

In Vitro Cellular Uptake Study

The amount of cellular internalization was investigated by FCM (Figs. 5a and 5b). After incubation for 4 h, the HCPN fluorescence intensity became stronger, which might be due to the increasing nanoparticle concentration. The mean fluorescence intensity (MFI) of the POEGMA-*b*-POAMA/HCPN NPs was also increased in 4T1 cells with prolonged incubation time, which indicated that the POEGMA-*b*-POAMA/HCPN NPs could be internalized into the 4T1 cells successfully. Therefore, it could be concluded that the POEGMA-*b*-POAMA/HCPN NPs were good drug carriers across the cell membranes of the 4T1 cells.

In Vivo Antitumor Efficiency

The *in vivo* antitumor efficiencies of the POEGMA-*b*-POAMA/HCPN NPs were evaluated using the 4T1 cell tumor model. As shown in Fig. 6(a), the curves of relative tumor volume in the POEGMA-*b*-POAMA/HCPN NPs, the POEGMA-*b*-POAMA NPs, and free HCPN were increased more slowly than the saline control group. The mean tumor volume of the POEGMA-*b*-POAMA/HCPN NPs group was $1004.6 \pm 527.4 \text{ mm}^3$, which was smaller than that of POEGMA-*b*-POAMA NPs ($2458.4 \pm 1124.3 \text{ mm}^3$) and free HCPN ($1852.1 \pm 1022.4 \text{ mm}^3$) at the end of the study (Table 4). As shown in Fig. 6(b), the body weights in POEGMA-*b*-POAMA/HCPN NPs and POEGMA-*b*-POAMA NPs displayed no significant change, indicating that the nanoparticles were safe without systemic toxicity. Table 4 shows that the average tumor weights in POEGMA-*b*-POAMA/HCPN NPs, free HCPN, POEGMA-*b*-POAMA NPs, and the control group were 0.63 ± 0.08 , 1.18 ± 0.12 , 1.89 ± 0.09 , and $2.23 \pm 0.10 \text{ g}$ on day 12. Tumors of the POEGMA-*b*-POAMA/HCPN NPs group were the smallest (Figs. 6c and 6d), which was in agreement with the results of the tumor volume, suggesting that POEGMA-*b*-POAMA/HCPN NPs showed the highest antitumor efficiency among all the treatment groups. Overall, the POEGMA-*b*-POAMA/HCPN NPs with excellent antitumor efficiency were promising for future clinical treatment.

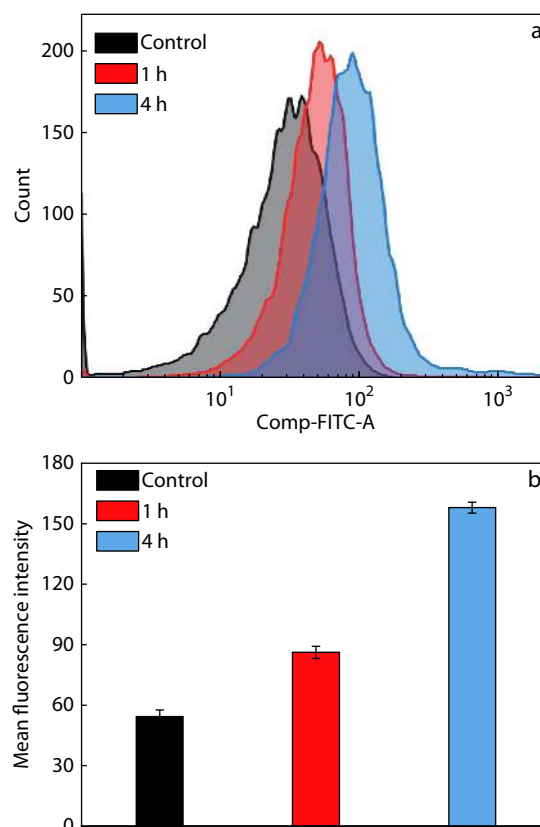


Fig. 5 *In vitro* cellular uptake study. (a) FCM analysis of the cellular uptake of the POEGMA-*b*-POAMA/HCPN NPs at different time points; (b) MFI of the POEGMA-*b*-POAMA/HCPN NPs at different time points. Data are displayed as mean $\pm$ SD ($n = 3$).

Table 4 4T1 xenograft model: efficacy comparison.

Compound	Mean TV $\pm$ SD ^a (mm^3)	RTV ^a	Tumor weight (g)
Control	2897.2 ± 986.7	19.3 ± 11.3	2.23 ± 0.10
POEGMA- <i>b</i> -POAMA NPs	2458.4 ± 1124.3	16.5 ± 8.2	1.89 ± 0.09
HCPN	1852.1 ± 1022.4	12.3 ± 7.1	1.18 ± 0.12
POEGMA- <i>b</i> -POAMA/HCPN NPs	1004.6 ± 527.4	6.9 ± 3.8	0.63 ± 0.08

^a Mean tumor volume (TV) and RTV were taken on day 12.

Evaluation of Adverse Effects

To further investigate the adverse effects, the IgE levels and the WBC count of the mice blood were evaluated on the 10th day. Fig. 7(a) presents that compared to the control group, the IgE levels of the POEGMA-*b*-POAMA/HCPN NPs and the POEGMA-*b*-POAMA NPs displayed no significant difference, while the IgE levels of free HCPN had a great increase, which might be due to the low blood solubility. The results demonstrated that the nanoparticles displayed no obvious adverse effects on mice. To further assess the safety of the nanoparticles, the WBC counts of the mice blood were also examined after treatment with the samples (Fig. 7b). No significant change was observed in the POEGMA-*b*-POAMA/HCPN NPs and the POEGMA-*b*-POAMA NPs group in comparison to the control group, while the apparent decrease occurred in free HCPN group. In conclusion, the nanoparticles could avoid adverse effects to confirm prominent for drug delivery.

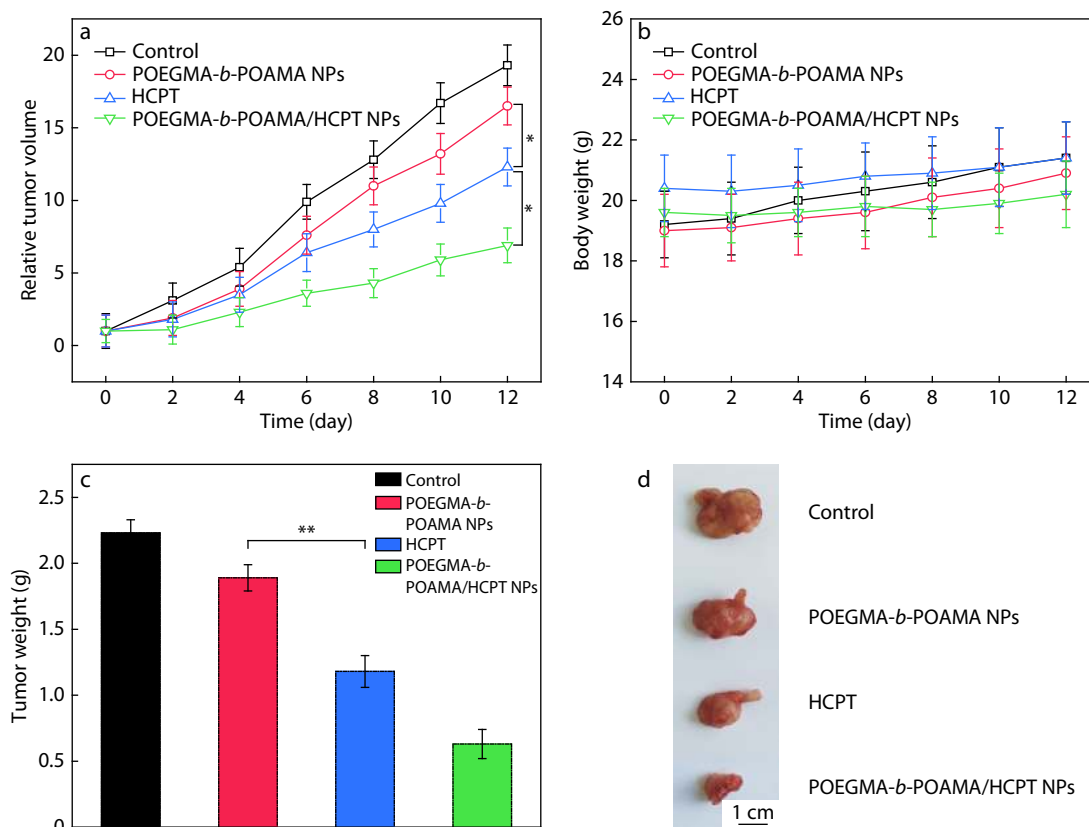


Fig. 6 *In vivo* antitumor efficiency of POEGMA-*b*-POAMA NPs, HCPT, and POEGMA-*b*-POAMA/HCPT NPs in the 4T1 cell tumor model. (a) Relative tumor volume, (b) body weight, (c) tumor weight, and (d) tumor weight images after treatment of HCPT, POEGMA-*b*-POAMA NPs, and POEGMA-*b*-POAMA/HCPT NPs. Data are displayed as mean $\pm$ SD ($n = 4$). * $P < 0.05$, ** $P < 0.01$.

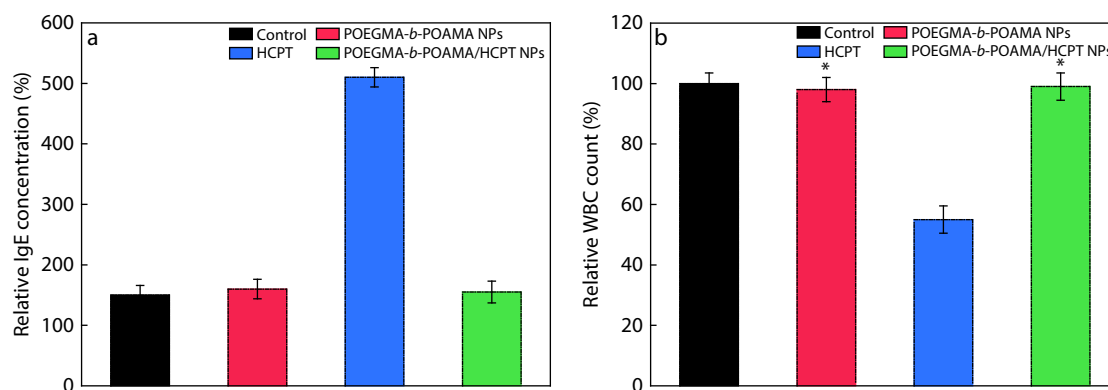


Fig. 7 Evaluation of adverse effects. (a) Relative IgE concentration and (b) relative WBC count of the mice blood after treatment of POEGMA-*b*-POAMA NPs, HCPT, and POEGMA-*b*-POAMA/HCPT NPs for 10 days. * $P < 0.05$, ** $P < 0.01$.

CONCLUSIONS

In conclusion, a novel nanoparticle platform based on amphiphilic oleanolic acid polyprodrug, poly[oligo(ethylene glycol) methyl ether methacrylate]-*b*-poly[oleanolic acid methacrylate] (POEGMA-*b*-POAMA), encapsulating 10-hydroxycamptothecin (HCPT) for tumor therapy was prepared. The POEGMA-*b*-POAMA/HCPT NPs displayed spherical morphology with a narrow size distribution, excellent stability in PBS, high drug loading capacity, and rapid cellular internalization. The drug

release from the POEGMA-*b*-POAMA/HCPT NPs was sustained for 132 h. *In vitro* cell cytotoxicity results indicated that the nanoparticles revealed notable toxicity against 4T1 cells and MCF-7 cells. Furthermore, the POEGMA-*b*-POAMA/HCPT NPs exhibited strong antitumor efficacy in the 4T1 xenograft tumor murine model and minimal adverse effects *in vivo* study, which made the POEGMA-*b*-POAMA/HCPT NPs an efficient carrier for clinical practice. All the results suggest that the POEGMA-*b*-POAMA/HCPT NPs have paved a new way of drug delivery for tumor therapy.

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REFERENCES

- Tao, X. G.; Gou, J. X.; Zhang, Q. Y.; Tan, X. Y.; Ren, T. Y.; Yao, Q.; Tian, B.; Kou, L. F.; Zhang, L.; Tang, X. Synergistic breast tumor cell killing achieved by intracellular co-delivery of doxorubicin and disulfiram via core-shell-corona nanoparticles. *Biomater. Sci.* **2018**, *6*, 1869–1881.
- Gao, S. T.; Tang, G. S.; Hua, D. W.; Xiong, R. H.; Han, J. Q.; Jiang, S. H.; Zhang, Q. L.; Huang, C. B. Stimuli-responsive bio-based polymeric systems and their applications. *J. Mater. Chem. B* **2019**, *7*, 709–729.
- Pei, P.; Sun, C. Y.; Tao, W.; Li, J.; Yang, X. Z.; Wang, J. ROS-sensitive thioketal-linked polyphosphoester-doxorubicin conjugate for precise phototriggered locoregional chemotherapy. *Biomaterials* **2019**, *188*, 74–82.
- Gupta, P. K.; Pappuru, S.; Gupta, S.; Patra, B.; Chakraborty, D.; Verma, R. S. Self-assembled dual-drug loaded core-shell nanoparticles based on metal-free fully alternating polyester for cancer theranostics. *Mater. Sci. Eng. C-Mater. Biol. Appl.* **2019**, *101*, 448–463.
- Kumari, A.; Yadav, S. K.; Yadav, S. C. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloid Surf. B-Biointerfaces* **2010**, *75*, 1–18.
- Oh, J. K. Disassembly and tumor-targeting drug delivery of reduction-responsive degradable block copolymer nanoassemblies. *Polym. Chem.* **2019**, *10*, 1554–1568.
- Dong, S. X.; Sun, Y.; Liu, J.; Li, L.; He, J. L.; Zhang, M. Z.; Ni, P. H. Multifunctional polymeric prodrug with simultaneous conjugating camptothecin and doxorubicin for pH/reduction dual-responsive drug delivery. *ACS Appl. Mater. Interfaces* **2019**, *11*, 8740–8748.
- Wang, X. M.; Xu, K. Y.; Yao, H. C.; Chang, L. M.; Wang, Y.; Li, W. J.; Zhao, Y. L.; Qin, J. L. Temperature-regulated aggregation-induced emissive self-healable hydrogels for controlled drug delivery. *Polym. Chem.* **2018**, *9*, 5002–5013.
- Pound-Lana, G. E. N.; Garcia, G. M.; Trindade, I. C.; Capelari-Oliveira, P.; Pontifice, T. G.; Vilela, J. M. C.; Andrade, M. S.; Nottelet, B.; Postacchini, B. B.; Mosqueira, V. C. F. Phthalocyanine photosensitizer in polyethylene glycol-block-poly(lactide-co-benzyl glycidyl ether) nanocarriers: probing the contribution of aromatic donor-acceptor interactions in polymeric nanospheres. *Mater. Sci. Eng. C-Mater. Biol. Appl.* **2019**, *94*, 220–233.
- Zabihi, F.; Koeppe, H.; Achazi, K.; Hedtrich, S.; Haag, R. One-pot synthesis of poly(glycerol-co-succinic acid) nanogels for dermal delivery. *Biomacromolecules* **2019**, *20*, 1867–1875.
- Liang, K.; Chung, J. E.; Gao, S. J.; Yongvongsoontorn, N.; Kurisawa, M. Highly augmented drug loading and stability of micellar nanocomplexes composed of doxorubicin and poly(ethylene glycol)-green tea catechin conjugate for cancer therapy. *Adv. Mater.* **2018**, *30*, 8.
- You, C. Q.; Wu, H. S.; Gao, Z. G.; Sun, K.; Chen, F. H.; Tao, W. A.; Sun, B. W. Subcellular co-delivery of two different site-oriented payloads based on multistage targeted polymeric nanoparticles for enhanced cancer therapy. *J. Mater. Chem. B* **2018**, *6*, 6752–6766.
- Shao, L. H.; Wan, K. W.; Wang, H.; Cui, Y. K.; Zhao, C. Y.; Lu, J. Q.; Li, X. L.; Chen, L.; Cui, X. Y.; Wang, X.; Deng, X. W.; Shi, X. H.; Wu, Y. A non-conjugated polyethylenimine copolymer-based unorthodox nanoprobe for bioimaging and related mechanism exploration. *Biomater. Sci.* **2019**, *7*, 3016–3024.
- Yao, J. K.; Kang, S. S.; Zhang, J.; Du, J.; Zhang, Z.; Li, M. Amphiphilic near-infrared conjugated polymer for photothermal and chemo combination therapy. *ACS Biomater. Sci. Eng.* **2017**, *3*, 2230–2234.
- Matsumura, Y.; Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **1986**, *46*, 6387–6392.
- Zhou, L. Y.; Qiu, T.; Lv, F. T.; Liu, L. B.; Ying, J. M.; Wang, S. Self-assembled nanomedicines for anticancer and antibacterial applications. *Adv. Healthc. Mater.* **2018**, *7*, 29.
- Pierini, F.; Nakielski, P.; Urbanek, O.; Pawlowska, S.; Lanzi, M.; De Sio, L.; Kowalewski, T. A. Polymer-based nanomaterials for photothermal therapy: from light-responsive to multifunctional nanoplatforms for synergistically combined technologies. *Biomacromolecules* **2018**, *19*, 4147–4167.
- Tao, R.; Gao, M.; Liu, F.; Guo, X. L.; Fan, A. P.; Ding, D.; Kong, D. L.; Wang, Z.; Zhao, Y. J. Alleviating the liver toxicity of chemotherapy via pH-responsive hepatoprotective prodrug micelles. *ACS Appl. Mater. Interfaces* **2018**, *10*, 21836–21846.
- Ma, X. Q.; Shi, X. X.; Bai, S.; Gao, Y. E.; Hou, M. L.; Han, M. Y.; Xu, Z. G. Acid-activatable doxorubicin prodrug micelles with folate-targeted and ultra-high drug loading features for efficient antitumor drug delivery. *J. Mater. Sci.* **2018**, *53*, 892–907.
- Dai, L.; Cao, X.; Liu, K. F.; Li, C. X.; Zhang, G. F.; Deng, L. H.; Si, C. L.; He, J.; Lei, J. D. Self-assembled targeted folate-conjugated eight-arm-polyethylene glycol-betulinic acid nanoparticles for co-delivery of anticancer drugs. *J. Mater. Chem. B* **2015**, *3*, 3754–3766.
- Palvai, S.; Anandi, L.; Sarkar, S.; Augustus, M.; Roy, S.; Lahiri, M.; Basu, S. Drug-triggered self-assembly of linear polymer into nanoparticles for simultaneous delivery of hydrophobic and hydrophilic drugs in breast cancer cells. *ACS Omega* **2017**, *2*, 8730–8740.
- Zhou, Z.; Ma, X.; Jin, E.; Tang, J.; Sui, M.; Shen, Y.; van Kirk, E. A.; Murdoch, W. J.; Radosz, M. Linear-dendritic drug conjugates forming long-circulating nanorods for cancer-drug delivery. *Biomaterials* **2013**, *34*, 5722–5735.
- Yar, Y.; Khodadust, R.; Akkoc, Y.; Utkur, M.; Saritas, E. U.; Gozuacik, D.; Acar, H. Y. Development of tailored SPION-PNIPAM nanoparticles by ATRP for dually responsive doxorubicin delivery and mr imaging. *J. Mater. Chem. B* **2018**, *6*, 289–300.
- Cheng, G. Q.; Xu, D.; Lu, Z. Y.; Liu, K. Chiral self-assembly of nanoparticles induced by polymers synthesized via reversible addition-fragmentation chain transfer polymerization. *ACS Nano* **2019**, *13*, 1479–1489.
- Guegain, E.; Tran, J.; Deguettes, Q.; Nicolas, J. Degradable polymer prodrugs with adjustable activity from drug-initiated radical ring-opening copolymerization. *Chem. Sci.* **2018**, *9*, 8291–8306.
- Lomkova, E. A.; Chytil, P.; Janouskova, O.; Mueller, T.; Lucas, H.; Filippov, S. K.; Trhlikova, O.; Aleshunin, P. A.; Skorik, Y. A.; Ulbrich, K.; Etrych, T. Biodegradable micellar HPMA-based polymer-drug conjugates with betulinic acid for passive tumor targeting. *Biomacromolecules* **2016**, *17*, 3493–3507.
- Hu, X.; Hu, J.; Tian, J.; Ge, Z.; Zhang, G.; Luo, K.; Liu, S. Polyprodrug amphiphiles: hierarchical assemblies for shape-regulated cellular internalization, trafficking, and drug delivery. *J. Am. Chem. Soc.* **2013**, *135*, 17617–17629.
- Hu, X.; Liu, G.; Li, Y.; Wang, X.; Liu, S. Cell-penetrating hyperbranched polyprodrug amphiphiles for synergistic reductive milieu-triggered drug release and enhanced magnetic resonance signals. *J. Am. Chem. Soc.* **2015**, *137*, 362–368.
- Hu, X. L.; Zhai, S. D.; Liu, G. H.; Xing, D.; Liang, H. J.; Liu, S. Y. Concurrent drug unplugging and permeabilization of

- polyprodrug-gated crosslinked vesicles for cancer combination chemotherapy. *Adv. Mater.* **2018**, *30*, 7.
- 30 Zhu, K. N.; Liu, G. H.; Hu, J. M.; Liu, S. Y. Near-infrared light-activated photochemical internalization of reduction-responsive polyprodrug vesicles for synergistic photodynamic therapy and chemotherapy. *Biomacromolecules* **2017**, *18*, 2571–2582.
 - 31 Tan, J. J.; Deng, Z. Y.; Liu, G. H.; Hu, J. M.; Liu, S. Y. Anti-inflammatory polymersomes of redox-responsive polyprodrug amphiphiles with inflammation-triggered indomethacin release characteristics. *Biomaterials* **2018**, *178*, 608–619.
 - 32 Zhang, W. J.; Hu, X. L.; Shen, Q.; Xing, D. Mitochondria-specific drug release and reactive oxygen species burst induced by polyprodrug nanoreactors can enhance chemotherapy. *Nat. Commun.* **2019**, *10*, 14.
 - 33 Pusuluri, A.; Krishnan, V.; Lensch, V.; Sarode, A.; Bunyan, E.; Vogus, D. R.; Menegatti, S.; Soh, H. T.; Mitragotri, S. Treating tumors at low drug doses using an aptamer-peptide synergistic drug conjugate. *Angew. Chem. Int. Ed.* **2019**, *58*, 1437–1441.
 - 34 Duan, X. P.; Xiao, J. S.; Yin, Q.; Zhang, Z. W.; Yu, H. J.; Mao, S. R.; Li, Y. P. Smart pH-sensitive and temporal-controlled polymeric micelles for effective combination therapy of doxorubicin and disulfiram. *ACS Nano* **2013**, *7*, 5858–5869.
 - 35 He, Y.; Li, X. L.; Ma, J. K.; Ni, G. L.; Yang, G.; Zhou, S. B. Programmable codelivery of doxorubicin and apatinib using an implantable hierarchical-structured fiber device for overcoming cancer multidrug resistance. *Small* **2019**, *15*, 14.
 - 36 Qin, J. W.; Wei, X. J.; Chen, H. Y.; Lv, F.; Nan, W. B.; Wang, Y. X.; Zhang, Q. Q.; Chen, H. L. mPEG-g-CS-modified PLGA nanoparticle carrier for the codelivery of paclitaxel and epirubicin for breast cancer synergistic therapy. *ACS Biomater. Sci. Eng.* **2018**, *4*, 1651–1660.
 - 37 Lee, W.; Yang, E. J.; Ku, S. K.; Song, K. S.; Bae, J. S. Anti-inflammatory effects of oleanolic acid on LPS-induced inflammation *in vitro* and *in vivo*. *Inflammation* **2013**, *36*, 94–102.
 - 38 Wang, X.; Ye, X. L.; Liu, R.; Chen, H. L.; Bai, H.; Liang, X.; Zhang, X. D.; Wang, Z.; Li, W. L.; Hai, C. X. Antioxidant activities of oleanolic acid *in vitro*: possible role of Nrf2 and MAP kinases. *Chem. Biol. Interact.* **2010**, *184*, 328–337.
 - 39 Yu, Z. J.; Sun, W. Z.; Peng, W. B.; Yu, R. L.; Li, G. Q.; Jiang, T. Pharmacokinetics *in vitro* and *in vivo* of two novel prodrugs of oleanolic acid in rats and its hepatoprotective effects against liver injury induced by CCl₄. *Mol. Pharm.* **2016**, *13*, 1699–1710.
 - 40 Niu, S. W.; Williams, G. R.; Wu, J. R.; Wu, J. Z.; Zhang, X. J.; Zheng, H.; Li, S. D.; Zhu, L. M. A novel chitosan-based nanomedicine for multi-drug resistant breast cancer therapy. *Chem. Eng. J.* **2019**, *369*, 134–149.
 - 41 Fontana, G.; Bruno, M.; Notarbartolo, M.; Labbozzetta, M.; Poma, P.; Spinella, A.; Rosselli, S. Cytotoxicity of oleanolic and ursolic acid derivatives toward hepatocellular carcinoma and evaluation of NF-κB involvement. *Bioorg. Chem.* **2019**, *90*, 103054.
 - 42 Pollier, J.; Goossens, A. Oleanolic acid. *Phytochemistry* **2012**, *77*, 10–15.
 - 43 Jeong, D. W.; Kim, Y. H.; Kim, H. H.; Ji, H. Y.; Yoo, S. D.; Choi, W. R.; Lee, S. M.; Han, C. K.; Lee, H. S. Dose-linear pharmacokinetics of oleanolic acid after intravenous and oral administration in rats. *Biopharm. Drug Disposition* **2007**, *28*, 51–57.
 - 44 Jiang, Q. K.; Yang, X. X.; Du, P.; Zhang, H. F.; Zhang, T. H. Dual strategies to improve oral bioavailability of oleanolic acid: enhancing water-solubility, permeability and inhibiting cytochrome P450 isozymes. *Eur. J. Pharm. Biopharm.* **2016**, *99*, 65–72.
 - 45 Ghosh, S.; Kar, N.; Bera, T. Oleanolic acid loaded poly lactic co-glycolic acid-vitamin E TPGS nanoparticles for the treatment of leishmania donovani infected visceral leishmaniasis. *Int. J. Biol. Macromol.* **2016**, *93*, 961–970.
 - 46 Zhang, L.; Chen, Y.; Shi, R.; Rang, T. G.; Pang, G. S.; Wang, B. R.; Zhao, Y.; Zeng, X.; Zou, C. X.; Wu, P.; Li, J. Y. Synthesis of hollow nanocages MOF-5 as drug delivery vehicle to solve the load-bearing problem of insoluble antitumor drug oleanolic acid (OA). *Inorg. Chem. Commun.* **2018**, *96*, 20–23.
 - 47 Hornig, S.; Heinze, T.; Becer, C. R.; Schubert, U. S. Synthetic polymeric nanoparticles by nanoprecipitation. *J. Mater. Chem.* **2009**, *19*, 3838–3840.
 - 48 Modi, S.; Anderson, B. D. Determination of drug release kinetics from nanoparticles: overcoming pitfalls of the dynamic dialysis method. *Mol. Pharm.* **2013**, *10*, 3076–3089.
 - 49 Guo, Y. F.; Hao, C. Y.; Wang, X. K.; Zhao, Y. N.; Han, M. H.; Wang, M. C.; Wang, X. T. Well-defined podophyllotoxin polyprodrug brushes: preparation via raft polymerization and evaluation as drug carriers. *Polym. Chem.* **2017**, *8*, 901–909.
 - 50 Xu, M. Z.; Zhang, C. Y.; Wu, J. G.; Zhou, H. G.; Bai, R.; Shen, Z. Y.; Deng, F. L.; Liu, Y.; Liu, J. PEG-detachable polymeric micelles self-assembled from amphiphilic copolymers for tumor-acidity-triggered drug delivery and controlled release. *ACS Appl. Mater. Interfaces* **2019**, *11*, 5701–5713.
 - 51 Du, J. Z.; Du, X. J.; Mao, C. Q.; Wang, J. Tailor-made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery. *J. Am. Chem. Soc.* **2011**, *133*, 17560–17563.
 - 52 Ke, W. D.; Yin, W.; Zha, Z. S.; Mukerabigwi, J. F.; Chen, W. J.; Wang, Y. H.; He, C. X.; Ge, Z. S. A robust strategy for preparation of sequential stimuli-responsive block copolymer prodrugs via thiolactone chemistry to overcome multiple anticancer drug delivery barriers. *Biomaterials* **2018**, *154*, 261–274.
 - 53 Lin, J. T.; Ye, Q. B.; Yang, Q. J.; Wang, G. H. Hierarchical bioresponsive nanocarriers for codelivery of curcumin and doxorubicin. *Colloids Surfaces B* **2019**, *180*, 93–101.
 - 54 Yang, H.; Li, J.; Patel, S. K.; Palmer, K. E.; Devlin, B.; Rohan, L. C. Design of poly(lactic-co-glycolic acid) (PLGA) nanoparticles for vaginal co-delivery of griffithsin and dapivirine and their synergistic effect for HIV prophylaxis. *Pharmaceutics* **2019**, *11*, 184.