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Comb-shaped, temperature-tunable and water-soluble porphyrin-based thermoresponsive copolymer for enhanced photodynamic therapy

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ABSTRACT

A novel *comb*-shaped porphyrin end-functionalized poly(N-isopropylacrylamide)-*b*-poly[oligo (ethylene glycol methyl ether methacrylate)] (Por-PNIPAM-*b*-POEGMA) was synthesized *via* reversible addition-fragmentation chain transfer (RAFT) polymerization. Due to the incorporation of hydrophilic POEGMA contents, the copolymer shows the lower critical solution temperatures (LCST) of 37–41.8 °C higher than PNIPAM. Moreover, this copolymer showed efficient singlet oxygen under light irradiation at 650 nm, and the productivity of singlet oxygen was 0.59, which could be used for photodynamic therapy. In addition, the *in vitro* study indicated that this copolymer showed no significant dark cytotoxicity, while showed apparent photo-toxicity toward HeLa cancer cells under red light irradiation at 650 nm. MTT results indicated that this copolymer with appropriate LCST could be accumulated on locally tumor tissues and killing of cancer cells (Hela), which may be a promising photosensitizer in photodynamic therapy for cancer treatment.

1. Introduction

Photodynamic therapy (PDT) as one of the most promising non-invasive and safe treatment protocols for cancer therapy, which arised from the combined use of photosensitizers (PSs), molecular oxygen and light irradiation [1–4]. During light irradiation, the excited photosensitizer transfers energy to ground-state triplet oxygen generating ($^3O_2$) to generate reactive oxygen species, which induces oxidative damage to the cancer cells [5–7].

Porphyrin and its derivatives as well-known photosensitizers have been extensively investigated in the field of photodynamic therapy due to their superior photochemical properties [8]. However, its hydrophobicity and non-selectivity induce molecular complexity, self-quenching, and photo-toxicity to the skin [9,10]. To overcome these obstacles, many researchers have devoted to synthesize the biomimetic polymer-porphyrin conjugates [11–18]. For example, Na et al. prepared a series of polymeric PSs using hydrophilic polymers such as hyaluronic acid, chondroitin sulfate, pluronic F127, polyethylenimine, which could effectively improve the solubility of the PSs and enhance cellular internalization [19–22]. The hydrophobicity of porphyrin could be solved obviously by introduction of hydrophilic polymers to it. However, non-selectivity greatly limited its further application. Now stimuli-responsive porphyrin-based polymer with passive targeting properties was used to increase tumor-selective accumulation [23].

Poly(*N*-isopropylacrylamide (PNIPAM) is a well-known responsive polymer due to its lower critical solution temperature (LCST) of 32 °C, which is close to the temperature of the human body. To date, there were some reports on the porphyrin functionalized PNIPAM [24–29]. For example, Xu et al. [30] reported that synthesis of porphyrin-containing amphiphilic copolymers for PDT. And our group [31–32] designed the thermo-sensitive poly (N-isopropylacrylamide) with porphyrin material. However, LCST of those porphyrin-based PNIPAM copolymer are less than 37 °C, which can cause the porphyrin-based PNIPAM copolymer to aggregate around the healthy tissues and cells, thus damaging healthy tissues. The temperature of tumor tissue is higher than the physiological body temperature around 37 °C, and low than 42 °C [33]. Therefore, it is necessary to design the porphyrin-based PNIPAM copolymer with the appropriate LCST, which could be recyclable when injected *in vivo* and insoluble to accumulate on a locally heated tumor tissues [34].

Oligo(ethylene glycol)methyl ether methacrylate (OEGMA) polymerization with NIPAM can provide external properties of the polymers, including improved solubility, biocompatibility, nontoxicity, and protein-resistance [35–36]. In this study, we used POEGMA to enhance water soluble and provide amphiphilic copolymer as an opportunity to from micelles [37]. When the particle size of the micelle is less than

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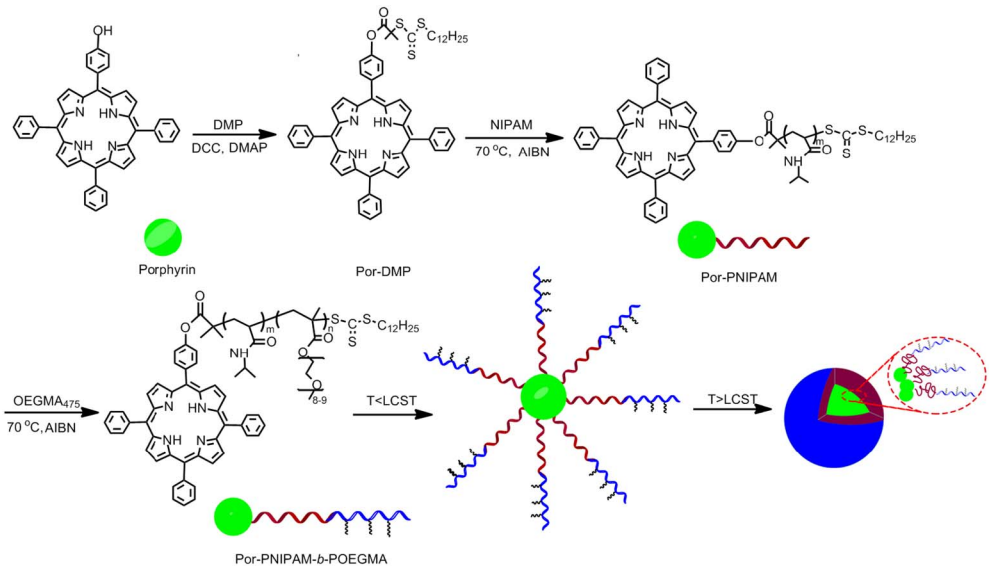

Fig. 1. Synthetic procedure of Por-PNIPAM-*b*-POEGMA copolymer and illustration of the thermally induced phase transitions of copolymer.

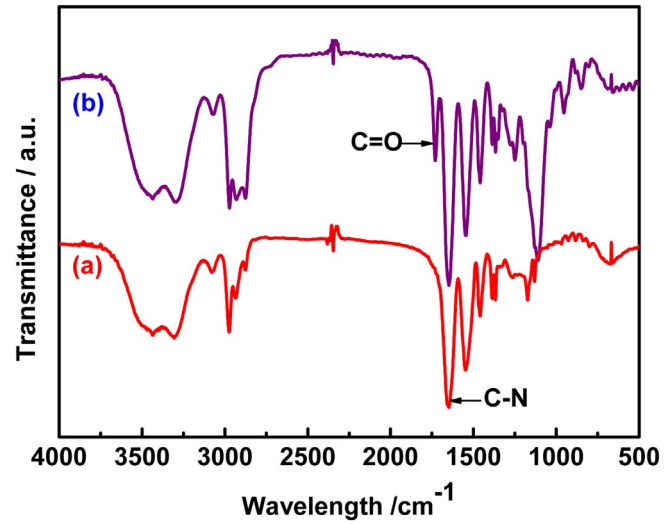

Fig. 2. FTIR spectra of (a)Por-PNIPAM ($M_{n,GPC}$ = 6100) and (b)Pro-PNIPAM-*b*- POEGMA ($M_{n,GPC}$ = 12,000).

100 nm, the micelles will aggregate around the tumor cells through the passive target. And the end-functionalized *comb*-shaped porphyrin copolymer with poly(N-isopropyl-acrylamide)-*b*-poly[oligo (ethylene glycol methyl ether methacrylate)] (Por-PNIPAM-*b*-POEGMA) which was synthesized *via* reversible addition-fragmentation chain transfer (RAFT) polymerization, and which had a potential application in PDT.

2. Experiment

2.1. Materials

4-dimethylaminopyridine (DMAP), *N, N′*-dicyclohexylcarbodiimide (DCC) and all monomers were purchased from Aldrich. NIPAM was recrystallized twice from benzene/hexane (v/v, 1/10). Oligo(ethylenglyco)-methylether methacrylate (OEGMA, 475 g/mol) was purified through a short basic alumina column before using. S-1-Dodecyl-S′-(α, α'-dimethyl-α′′-acetic acid) trithiocarbonate (DMP) was synthesized according to the literature [38]. 2,2′-Azobis(2-methylpropionitrile) (AIBN, 99%, Aldrich) was purified by recrystallization from methanol. Dichloromethane (CH_2Cl_2) was purified with CaH_2 by distillation. Other chemical reagents were analytic grade and used without further purification.

2.2. Characterization

^{1}HNMR spectra were obtained on a Bruker Avance-400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent at ambient temperature, TMS for ^{1}H calibration. Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu-8400S spectrometer. The spectra were collected at 40 scans with a resolution of 4 cm^{-1}. Molecular weights ($M_{n,GPC}$) and (M_w/M_n) were determined on a gel permeation chromatograph (GPC) equipped with a Waters-e2695 separations module and a Waters differential refractometer. Ultraviolet-visible (UV–vis) spectra were measured on a Shimadzu-1240 spectrophotometer using a quartz cell of 1 cm path length. The cloud point (CP) or LCST of the thermo-responsive polymers were obtained on a Shimadzu-1240 Ultraviolet-visible (UV–vis) spectrophotometer with a thermo-regulator. The size of nanoparticles was determined by dynamic light scattering (DLS) using a Malvern Nano S instrument (Malvern, UK) consisting of an Autosizer-4700 spectrometer, a pump/filter unit, a model 2013 air-cooler argon ion laser. The measurement angle was 90°.

2.3. Synthesis of 5-(4-hydroxyphenyl)-10,15,20-phenyl-21H, 23H–porphyrin

5-(4-hydroxyphenyl)-10,15,20-phenyl-21H,23H–porphyrin (MHTPP) was synthetized according to the literature [39]. Yield: 5%. FTIR (KBr, cm^{-1}): 3319 cm^{-1}, 964 cm^{-1} (ν_{N-H}), 725 cm^{-1}, 800 cm^{-1}, 1008 cm^{-1}($\nu_{C=C}$) (Fig.S1a). ^{1}H NMR (400 MHz, DMSO), δ ppm: 9.98 (s, 1H, O*H*), 8.89 (8H, s, pyrrole-*H*), 8.2 (d, 6H, Ar-O*H*), 7.91 (m, 9H, Ar-*H*), 7.23 (m, 4H, Ar-*H*), − 2.76 (s, 2H, N—*H*) (Fig.S2).

2.4. Synthesis of S-1-dodecyl-S′-(α,α'-dimethyl-α′′-acetic acid) trithiocarbonate with porphyrin derivate (Por-DMP)

A mixture of *S*-1-dodecyl-*S′*-(α,α'-dimethyl-α′′-acetic acid)trithiocarbonate (DMP, 605 mg, 1.66 mmol), 5-(4-hydroxyphenyl)-10,15,20-phenyl-porphine (1 g, 1.6 mmol) and 4-dimethylaminopyridine (DMAP, 21 mg, 0.17 mmol) in dichloromethane (25 mL) was stirred at ice-bath under nitrogen atmosphere. *N,N′*-dicyclohexylcarbodiimide (DCC, 350 mg, 1.69 mmol) dissolved in dichloromethane (5 mL) was added in portions. The resulting mixture was stirred at room temperature for 24 h. The product was added to water (20 mL) and then extracted into dichloromethane (30 mL × 3). All of the organic phase was dried over Mg_2SO_4 and removed under reduced pressure. The crude product was