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Construction of reduction-responsive photosensitizers based on amphiphilic block copolymers and their application for photodynamic therapy

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ABSTRACT

A novel reduction-responsive porphyrin monomer bearing a disulfide bond (TPPC6SAM) was synthesized, and then it was utilized to construct a series of POEGMA-*b*-PTPPC6SAM block copolymers *via* reversible addition–fragmentation transfer (RAFT) polymerization with water-soluble and biocompatible monomer, oligo(ethylene glycol) methyl ether methacrylate (OEGMA). POEGMA-*b*-PTPPC6SAM block copolymers could self-assemble into spherical micelles and vesicles by varying the mass ratio of hydrophilic block to hydrophobic block. Confocal laser scanning microscopy (CLSM) and flow cytometry showed that POEGMA-*b*-PTPPC6SAM micelles could effectively accumulate in A549 cells, compared to that of free porphyrin. In addition, minimal dark toxicity and efficacious photo-toxicity in vitro were also evaluated by MTT assay. According to the result of MTT assay, POEGMA-*b*-PTPPC6SAM block copolymers had a higher photo-toxicity with a lower IC50 (below 3 μg/mL). Thus, reduction-responsive POEGMA-*b*-PTPPC6SAM block copolymers could well improve the release of porphyrin photosensitizers and enhance the efficiency of PDT.

1. Introduction

Over the past decades, photodynamic therapy (PDT) has been a promising modality for the treatment of malignant and nonmalignant cancers, because it has not only therapeutic performance in producing cellular death, immune activation, and vascular shutdown, but also minimally invasive for normal tissues [1–5]. In a typical process of PDT, specific photosensitizers (PSs) (e.g., porphyrin and phthalocyanine derivatives) induce the generation of cytotoxic reactive oxygen species (ROS) such as singlet oxygen and free radicals under light irradiation with appropriate wavelength, which could destroy cancer cells [6,7]. Thus, in contrast to conventional anticancer treatments such as chemotherapy and radiotherapy, PDT is more controllable, tumor-selective and minimally toxic for surrounding healthy tissues, since PSs could be light-activatable with different dose, time and location [8,9].

Currently, a number of conventional anticancer PSs such as Photofrin® and Metvixia® have been approved in clinical cancer treatments by the U.S. Food and Drug Administration (FDA) [10,11]. Porphyrin and its derivatives, as second-generation PSs, have aroused great attention in PDT due to their multiple advantages such as high singlet oxygen quantum yields and low toxicity to the body [12–14]. However, there are still some limitations of porphyrin PSs in their clinical application. Firstly, most of clinically used porphyrin PSs frequently suffered from inherent restriction in non-selectivity. Secondly, most of porphyrin PSs have a low water-solubility and tend to aggregate in aqueous solution, which results in a great decrease in the quantum yield of fluorescence and singlet oxygen generation [15–18]. To compensate for these limitations, a variety of nanostructured carriers have been developed for porphyrin PSs such as liposomes and polymeric nanoparticles (NPs). For example, Zheng et al. prepared phospholipid-porphyrin conjugates to form the porphysomes, which had excellent biocompatibility, high loading capacity, and further exhibited the great potential in the photodiagnosis and phototherapy [19–21]. Dai and *co*-workers developed a versatile theranostic porphyrin dyad nanoparticle by loading metal free porphyrin and Mn-porphyrin for magnetic resonance imaging guided PDT [22,23]. Lai et al. fabricated star-shaped porphyrin polylactide (PLLA) using porphyrin as an initiator. This porphyrin-PLLA PS could efficiently produce singlet oxygen under irradiation and be promise for cancer therapy [24]. Zhang and *co*-

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workers fabricated a dual-stage light irradiation strategy through a short PEG chain linked with protoporphyrin and mitochondria-targeted amphipathic proapoptosis peptide for cancer treatment [25]. Additionally, Na et al. designed a light-induced gene delivery system based on endo-lysosomal pH-responsive polymeric PS, which was further utilized for cancer gene therapy [26].

It is well-known that polymer micelles formed *via* amphiphilic block copolymers have attracted great attention in the drug delivery system [27–34]. These micelles could provide distinct advantages including longer circulation time, improved systemic stability, and minimum toxicity [35–38]. Among these amphiphilic block copolymers, poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) as the hydrophilic block, has been widely used in construction of drug carriers, since it has uncharged, cheap, non-toxic, non-immunogenic, excellent water-soluble and biocompatible advantages [39]. Furthermore, POEGMA conjugated with drugs can efficiently prolong their circulation time of therapeutics in vivo blood, resulting in diminished reticuloendothelial system uptake of nanostructured materials due to desirable sizes in cancer therapy. For instance, Liu et al. fabricated amphiphilic block copolymers based on hydrophiphilic POEGMA block, which had been applied for the drug delivery of doxorubicin (Dox) or paclitaxel (PTX) with magnetic resonance imaging agents [40–42]. Yan et al. synthesized stimuli-responsive amphiphilic POEGMA multiblock copolymers, which could be incorporated with Dox and near-infrared fluorescence (NIR) probe for theranosis and therapy of liver cancer [43]. To our best knowledge, the PS based on POEGMA-containing block copolymers has not been reported in PDT yet.

More recently, reduction-responsive nano-carriers have received considerable interests because of the significant difference reduction potential in the reducing environments of intracellular and extracellular milieu [44–48]. This strategy relies on the fact that some tumor cells have a larger amount of glutathione (GSH) (approximately 10 mM) in the cytosol [49,50]. After the cell-internalization of reduction-responsive drug carriers, disulfide bonds could be fast cleaved by GSH, thus triggered release of the drugs into the intracellular environment. Zhong et al. have reported several reduction-responsive copolymers based on a disulfide linkage for efficient intracellular release of DOX [51–53]. Huh et al. developed a GSH-responsive PS system based on biarmed poly(ethylene glycol)-(pheophorbide a)$_2$ conjugate, which exhibited selective release and efficient activation of the PSs in tumor cells [54]. We also constructed an amphiphilic reductive-responsive supramolecular photosensitizer bearing a disulfide bond, which could self-assemble into spherical micelles in aqueous solution, and the photosensitizer could be well released from spherical micelles by GSH [55].

In this contribution, a novel reduction-responsive porphyrin monomer with a disulfide linkage, TPPC6SAM, was firstly synthesized. Then it was further utilized to prepare the amphiphilic POEGMA-*b*-PTPPC6SAM block copolymers *via* reversible addition–fragmentation chain transfer (RAFT) polymerization (Scheme 1). The assembly and reduction-responsive disassembly behavior of amphiphilic block copolymers in aqueous solution were studied by dynamic light scattering (DLS) and transmission electron microscopy (TEM), respectively. The biodistribution of POEGMA-*b*-PTPPC6SAM micelles was characterized by confocal microscopy and flow cytometry. Their cellular dark cytotoxicity and phototoxicity were separately evaluated in A549 cells by using a MTT assay.

2. Experimental section

2.1. Materials

Oligo(ethylene glycol) methyl ether methacrylate (OEGMA, M_n~475 g/mol, Aldrich) was purified by passing over a neutral aluminum oxide column and then stored at −20 °C prior to use. The mono-hydroxyl porphyrin derivative, TPPC6-OH was synthesized according to our previous work [56], and the results were given in Supporting Information (Fig. S1 and S2). Tetrahydrofuran (THF) was dried by refluxing with the fresh sodium–benzophenone complex under N_2 and distilled just before use. *S*-1-dodecyl-S′-(α, α′-dimethyl-α′′-acetic acid) trithiocarbonate (DDAT) was prepared according to the previous literature [57]. 4, 4′-Azobis(isobutyronitrile) (AIBN) (Fluka, 98%) were purified by recrystallization from ethanol. 4′, 6-Diamidino-2-phenylindole (DAPI), glutathione ethyl ester (GSH-OEt), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), *N*, *N*′-dicyclohexylcarbodiimide (DCC), 4-dimethyaminopyridine (DMAP), and all other reagents were purchased from Aladdin and used as received. All other chemicals used were of analytical reagent grade.

2.2. Synthesis of 6-(5′-(4′-phenoxyl)-10′, 15′, 20′-triphenylporphyrin) succinate (TPPC6SA)

TPPC6SA was synthesized by the esterification between TPPC6-OH and succine anhydride. TPPC6-OH (0.73 g, 1 mmol), succine anhydride (0.873 g, 8.73 mmol), and 4-dimethyaminopyridine (DMAP) were dissolved in THF (30 mL) at room temperature. Water (3 mL) was added to quench the reaction after the mixture stirring for 24 h. After a day, the solution was extracted with dichloromethane (40 mL) and washed thrice with saturated NaCl solution. The organic layer was dried by anhydrous $MgSO_4$ and the solvent was distilled in vacuo. The crude product was purified by column chromatography (silica gel, eluent: ethyl acetate/petroleum ether = 1:1). Yield: 0.76 g (91.6%). ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S3), δ ppm: 8.84 (m, 8H, *β*-H), 8.20 (m, 6H, 10, 15, 20-Ar-*o*-H), 8.11 (m, 2H, 5-Ar-*o*-H), 7.76 (m, 9H, 10, 15, 20-Ar-*m*- and *p*-H), 7.27 (m, 2H, 5-Ar-*m*-H), 4.19 (t, 4H, −O−C*H*$_2$−CH$_2$−), 2.69 (t, 4H, −CO−C*H*$_2$−⊜C*H*$_2$−CO−), 1.98 (m, 2H, −O−CH$_2$−C*H*$_2$−CH$_2$−), 1.77–1.52 (m, 6H, −CH$_2$−(C*H*$_2$)$_3$−CH$_2$−OH), −2.80 (s, 2H, −N*H*−).

2.3. Synthesis of 2-((2-Hydroxyethyl) disulfanyl) ethyl methacrylate (HSEMA)

HSEMA was prepared from 2, 2′-dithiodiethanol and methacryloyl chloride in the mono-esterification manner [58]. Firstly, 2, 2′-dithiodiethanol (0.75 mL, 100 mmol) and triethylamine (4.2 mL, 30 mmol) were dissolved anhydrous THF (160 mL) in a 500 mL flask, cooled to 0 °C in an ice-water bath, and then methacryloyl chloride (0.75 mL, 15 mmol) in anhydrous THF (40 mL) was added dropwise into the solution. The reaction mixture was allowed to react for 24 h at room temperature under stirring. The solution was filtrated and the solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography (silica gel, eluent: ethyl acetate/petroleum ether = 1:1). Yield: 2.74 g (82.3%). ^{1}H NMR (400 MHz, $CDCl_3$, Fig. S4) δ ppm: 6.16, 5.63 (s, 2H, C*H*$_2$=C(CH$_3$)−), 4.46 (t, 2H, −OC*H*$_2$CH$_2$SS−), 3.88 (t, 2H, −C*H*$_2$OH), 2.98, 2.89 (t, 4H, −C*H*$_2$S-SC*H*$_2$−), 1.92 (s, 3H, CH$_2$=C(C*H*$_3$)−).

2.4. Synthesis of the reduction-responsive porphyrin monomer, TPPC6SAM

TPPC6SAM was prepared according to the esterification between TPPC6SA and HSEMA in the presence of EDC/DMAP. A typical reaction procedure was given below: TPPC6SA (1 g, 1.2 mmol), HSEMA (0.53 g, 2.4 mmol) and DMAP (0.15 g, 1.2 mmol) were dissolved in anhydrous THF (20 mL) in a 100 mL Schlenk flask under nitrogen, and then stirred in a 0 °C bath. EDC (0.3 g, 1.56 mmol) was dissolved in anhydrous THF (10 mL) and added dropwise into the