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Multifunctional polycationic photosensitizer conjugates with rich hydroxyl groups for versatile water-soluble photodynamic therapy nanoplatforms

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ABSTRACT

Photodynamic therapy (PDT) has already shown immense potential in antitumor fields due to its low systemic toxicity and negligible drug resistance. However, the clinical application of current photosensitizers is still restricted by the low singlet oxygen yield or insolubility. Herein, series of star-like hydroxyl-rich polycations (Pc-PGEA/Pc) with flanking phthalocyanine (Pc) were proposed for effective water-soluble photosensitizers. The designed Pc-PGEA/Pc polymers consist of one Pc core and four ethanolamine and Pc-difunctionalized poly(glycidyl methacrylate) arms. The strong π-π stacking and hydrophobicity of introduced Pc units drive the amphipathic Pc-PGEA/Pc polymers to self-assemble into well-defined cationic nanoparticles. Such Pc-PGEA/Pc nanoparticles present impressive photodynamic therapy effects under moderate irradiation and remarkable photoacoustic imaging (PAI) ability. These kinds of nanoparticles also exhibit good performance as gene vectors. The PAI ability given by the proper wavelength absorbance of Pc units provides one promising method for PAI-guided combined antitumor therapy. The present work would contribute valuable information for the development of new strategies of visible antitumor therapy.

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1. Introduction

Due to its controllable and efficient damage to tumor tissues, photodynamic therapy (PDT) has been considered as a promising approach to tumor therapy [1–5]. The highly reactive singlet oxygen produced by photosensitizers under controlled irradiation could interrupt important biochemical reactions and destroy vital organelles. Such lethal processes directly lead to the death of tumor cells meanwhile minimizes the damage of adjacent healthy tissues [6,7]. As the crucial part of PDT, various photosensitizers were synthesized, such as hematoporphyrin [8,9], 5-aminolevulinic acid [10], and phthalocyanine [11,12]. As a member of zinc(II) phthalocyanine derivatives, zinc(II) tetraaminophthalocyanine (TAPc-Zn) could be considered as an outstanding candidate for sensitizer, due to their extraordinary singlet oxygen productivity, low toxicity and strong absorbance at red light region which provides a better tissue penetrability [13]. However, the insolubility of TAPc-Zn is still a serious obstacle for its widespread application.

On the other hand, gene therapy (GT) also proved its reliability in treating series of serious diseases such as cancers [14–18]. Flexible design strategies of polycations ensure the safety and efficiency of GT [19–23]. The representative members of polycations, such as polyethylenimine (PEI) [24,25], polyamidoamine (PAMAM) [26], and poly(L-lysine) [27], have already been verified as effective vectors of nuclei acid in many occasions. Atom transfer radical polymerization (ATRP) is considered as a reliable method to

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produce functional polymers with controllable molecular weight and narrow distribution [28–30]. As a typical monomer, glycidyl methacrylate (GMA) presents excellent living polymerization characteristic during the ATRP processes. The epoxy unit of GMA suggests the possible modification of the resultant polymer, poly(glycidyl methacrylate) (PGMA). A practical and meaningful application of epoxy groups is the ring-opening reaction by amino species to produce cationic units with hydroxyl groups for gene delivery. A series of aliphatic amine-functionalized PGMAs, such as ethanolamine (EA)-functionalized PMGA (denoted by BUCT-PGEA) [31,32], and piperazine (PP)-, N-(aminoethyl)piperazine (AEPP)-, or N-(3-aminopropyl)-2-pyrrolidinone (APP)-functionalized PGMA [33], were reported. Such kinds of cationic PGMA derivatives exhibit good gene transfection performances.

In this work, series of star-like PGMA-based cationic vectors (Pc-PGEA/Pc) with flanking Pc units were proposed via ATRP and ring opening reaction for developing photodynamic active water-soluble photosensitizers with gene delivery abilities (Fig. 1(A)). Four amino groups of TAPc-Zn could be converted into ATRP initiation sites (TBrPc-Zn) by amidation. The resultant Pc-PGEA/Pc polycations consisted of one Pc core and four EA/Pc-difunctionalized PGMA arms. Such polycations could readily self-assemble into well-defined cationic nanoparticles powered by π-π stacking and hydrophobicity of Pc units. Such kind of multifunctional Pc-PGEA/Pc nanoparticles were systematically investigated via a series of *in vitro* and *in vivo* assays. The present work would provide valuable information for the exploration and multifunctionalization of high effective photosensitizers.

2. Materials and methods

2.1. Materials

Triethylamine (TEA, 99.5%, extra dry, with molecular sieves), ammonium molybdate (98%), zinc acetate dihydrate ($ZnAc_2 \cdot 2H_2O$, 99%) and 1,3-diphenylisobenzofuran (DPBF, 97%) were obtained from Energy-Chemical Co. (China). 4-Nitrophthalimide (>98%), urea (>99%), 2-bromoisobutyryl bromide (BIBB, >98%), 2,2′-bipyridyl (bpy, >99%), heparin sodium, glycidyl methacrylate (GMA, >95%) and acridine orange (AO, >90%) were obtained from Tokyo Chemical Industry Co. Ltd. (Japan). Anhydrous *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), copper(I) bromide (CuBr, 99%), ethanolamine (EA, 98%), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, 97%), annexin V-FITC apoptosis detection kit, fluorescein diacetate (FDA, European Pharmacopoeia Reference Standard) and propidium iodinate (PI, 98%) were obtained from Sigma-Aldrich Chemical Co. (USA). C6 and HEK293 cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). Plasmid pRL-CMV encoding Renilla luciferase, plasmid pEGFP-N1 encoding enhanced green fluorescent protein and tumor suppressor plasmid p53 were amplified in Escherichia coli and purified according to the supplier's protocol (Omega Biotek, Norcross, USA).

2.2. Preparation of TBrPc-Zn initiator

Zinc(II) tetraaminophthalocyanine (TAPc-Zn) was prepared according to the literature [34]. The detailed preparation process was illustrated in Supporting Information. TAPc-Zn (0.638 g, 1 mmol) and TEA (832 μL, 6 mmol) were added into a 50 mL flask containing 8 mL of anhydrous DMF. After TAPc-Zn was dissolved thoroughly, BIBB (742 μL, 6 mmol) in 2 mL of anhydrous DMF was added dropwise into the aforementioned mixture under ice bath condition for 30 min. Then, the reaction solution was stirred at 30 °C for another 24 h. After that, the reaction was quenched by pouring into 100 mL of $NaHCO_3$ saturation solution. The precipitate was centrifuged and washed with deionized water to neutralize. Finally, TBrPc-Zn initiator (1.09 g, 88% yield) was obtained by lyophilization.

2.3. Preparation of Pc-PGEA/Pc

Pc-PGMA polymers composed of one Pc core and four PGMA arms were firstly prepared under the typical condition of ATRP. TBrPc-Zn (123 mg, 0.1 mmol, 1 equiv), GMA (2.0 mL, 15 mmol, 150 equiv) and bpy (124 mg, 0.8 mmol, 8 equiv) were added into a 50 mL flask containing 5 mL of DMSO. The reaction solution was degassed by nitrogen for 8 min before adding CuBr (58 mg, 0.4 mmol, 4 equiv). In order to manipulate the molecular weights of resultant Pc-PGMAs, the polymerization was conducted with different time (15, 30 or 45 min) and terminated by exposure to air. Resultant polymers were precipitated with 150 mL of methanol to remove the excess monomer and catalyst complexes. The crude products were further purified by re-precipitation cycles with methanol and dried under reduced pressure.

The procedures of preparing EA-functionalized polycations (Pc-PGEA) followed our previous work [31]. Pc-PGMA (100 mg), TEA (0.2 mL) and excess EA (1.0 mL) were added into 8 mL of DMSO. Reaction mixture was degassed by nitrogen for 6 min, and then stirred at 80 °C for 1 h. The Pc-PGEA product was purified through dialysis (MWCO 3500) and lyophilization. For the preparation of EA/Pc-difunctionalized Pc-PGEA/Pc, Pc-PGMA (100 mg) and TEA (0.2 mL) were dissolved into 8 mL of DMSO, and then TAPc-Zn (28 mg) and EA (200 μL) were added into the prior mixture to achieve the designed ratio (1:0.25:4.75) of [epoxy groups of Pc-PGMA]:[amino group of TAPc-Zn]:[amino group of EA]. The reaction solution was degassed as aforementioned by nitrogen for 6 min and stirred at 40 °C for 24 h. Then, the temperature was raised to 80 °C for additional 1 h to complete the reaction. The Pc-PGEA/Pc product was purified by dialysis (MWCO 3500) and removing the insoluble substance (mainly remaining Pc), prior to lyophilization.

2.4. Characterization

The chemical structures of synthesized materials were characterized by UV–Vis, FT-IR and NMR spectra. X-ray photoelectron spectra (XPS) was also used to certify the structures of main products. UV–Vis spectra were recorded on a Shimadzu UV-2600 UV–Vis spectrometer. FT-IR spectra were recorded on a Thermo Nicolet iS10 FT-IR spectrometer. NMR spectra were recorded on a Bruker ARX 400 MHz spectrometer using DMSO-d_6 with tetramethylsilane (Me_4Si) as an internal standard. XPS spectra were recorded on a Kratos AXIS His X-ray photoelectron spectrometer (Al Kα source). Molecular weights of Pc-PGMAs were determined by gel permeation chromatography (GPC) equipped with a Waters Styragel columns and a Waters-2414 refractive index detector. DMSO was used as the eluent at 40 °C with the flow rate of 1.0 mL min^{-1}, where monodispersed polyethylene glycol (PEG) standards were used to generate the calibration curves.

2.5. Singlet oxygen assay

1,3-Diphenylisobenzofuran (DPBF) was used as a sensor to measure the generation of singlet oxygen according to the literature [35]. 3.0 mL of methanol solution containing Pc-based materials (6.7 μg/mL) and DPBF (200 μg/mL) was irradiated by a simulated sunlight source (optical filter 700 ± 10 nm) with a power density of 30 mW/cm^2. Meantime, the same condition without Pc

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