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Enhanced fluorescence imaging guided photodynamic therapy of sinoporphyrin sodium loaded graphene oxide

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ABSTRACT

Extensive research indicates that graphene oxide (GO) can effectively deliver photosensitives (PSs) by π−π stacking for photodynamic therapy (PDT). However, due to the tight complexes of GO and PSs, the fluorescence of PSs are often drastically quenched *via* an energy/charge transfer process, which limits GO-PS systems for photodiagnostics especially in fluorescence imaging. To solve this problem, we herein strategically designed and prepared a novel photo-theranostic agent based on sinoporphyrin sodium (DVDMS) loaded PEGylated GO (GO-PEG-DVDMS) with improved fluorescence property for enhanced optical imaging guided PDT. The fluorescence of loaded DVDMS is drastically enhanced *via* intramolecular charge transfer. Meanwhile, the GO-PEG vehicles can significantly increase the tumor accumulation efficiency of DVDMS and lead to an improved PDT efficacy as compared to DVDMS alone. The cancer theranostic capability of the as-prepared GO-PEG-DVDMS was carefully investigated both *in vitro* and *in vivo*. Most intriguingly, 100% *in vivo* tumor elimination was achieved by intravenous injection of GO-PEG-DVDMS (2 mg/kg of DVDMS, 50 J) without tumor recurrence, loss of body weight or other noticeable toxicity. This novel GO-PEG-DVDMS theranostics is well suited for enhanced fluorescence imaging guided PDT.

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1. Introduction

Photodynamic theranostics, due to the specific spatiotemporal selectivity and minimal invasiveness, is an emerging solution that promises simultaneous photosensitizer (PS) fluorescence imaging and photodynamic therapy (PDT) [1–6]. Based on the activation of light with appropriate wavelengths, PS can emit fluorescence *via* the relaxation of the excited-singlet-state PS back to the ground state, which can be employed for the photodiagnosis of disease, real-time visualization of *in vivo* PS delivery and distribution, and molecular imaging-guided PDT [1,2]. Additionally, PS can transfer the absorbed photon energy to surrounding oxygen molecules, resulting in the production of reactive oxygen species (ROS) including singlet oxygen or free radicals, which cause cancer cell death and tumor tissue destruction [7–9].

Most PSs used in PDT are limited by prolonged cutaneous photosensitivity, poor water-solubility and inadequate selectivity [10–12]. To improve the water solubility of PS molecules and increase their accumulation into cancer cells/tissues, various nanocarriers such as liposomes and polymer-, silica-, magnetic-, gold-, and carbon-based nanoparticles (NPs) have been actively

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developed for the delivery of PS [12–22]. Although these nanocarriers can improve tumor accumulation efficiency of PS *via* the enhanced permeability and retention (EPR) effect over free PS, the fluorescence of PS is often quenched in varying degrees by the nanocarriers [10,16,17,23–25]. Therefore, the development of novel PS delivery system without fluorescence quenching is desirable for optimizing PS fluorescence imaging and PDT.

Among the various nanocarriers, graphene oxide (GO), due to the large surface area, water solubility, abundant functional groups (epoxide, hydroxyl, and carboxylic groups) and easy surface modification, has been widely employed for biosensing, bioimaging, and drug/gene delivery [26–37]. GO shows great potential as an efficient quencher in GO-based nanosensors for many fluorescent moieties, including small molecule dyes, quantum dots and conjugated polymers *via* fluorescence resonance energy transfer or charge transfer [38–42]. Most recently, researchers have combined GO with PS for fluorescence imaging and PDT [16,23,24]. However, the drastic fluorescence quenching of PSs by GO in GO-based PS delivery system limits their potential as photodiagnostics. In our previous studies, we found that the fluorescence of chlorin e6 (Ce6) was drastically quenched by folic acid-conjugated GO [16]. Recently, we also observed 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-alpha (HPPH) fluorescence quenching by GO-PEG [24]. The similar results were also verified in the studies from other groups [23]. Therefore, there still remains a grand challenge to improve GO-based PS delivery system without fluorescence quenching.

In this study, we designed and validated a novel phototheranostic nanoplatform based on sinoporphyrin sodium (DVDMS) photosensitizer-loaded PEGylated GO (GO-PEG-DVDMS) for enhanced fluorescence imaging guided PDT. DVDMS (Scheme 1), a porphyrin dimer salt, has been used as a new type of PS with high yield of fluorescent emission at the wavelengths of 615–625 nm for diagnosis and treatment of cancer [43,44].

2. Experimental section

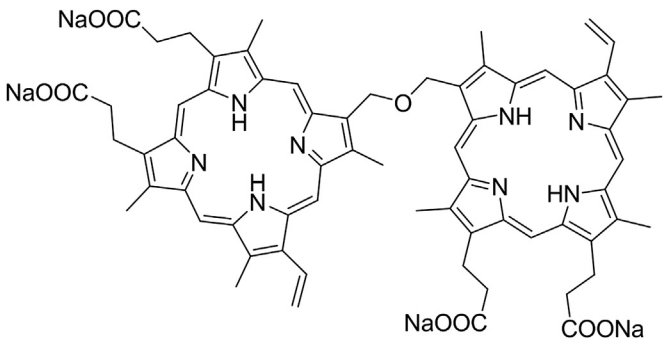
2.1. Materials

DVDMS photosensitizer was provided by Jiangxi Qinglong Group Co., Ltd. (Jiangxi, China). Amine-terminated eight-arm branched PEG (Mw: 15,000) was purchased from NOF America Corporation (White Plains, NY). *N*-(3-dimethylaminopropyl-N-ethylcarbodiimide) hydrochloride (EDC) was obtained from Fluka Inc. Singlet oxygen sensor green (SOSG), DAPI (SlowFade® Gold Antifade Reagent with DAPI, Molecular Probes), and live/dead stain kit were purchased from Invitrogen. All the above chemicals were used without any further purification. Water was purified with Milli-Q plus system (Millipore Co., Bedford, MA), and the resistivity was kept to be 18 MΩ cm.

2.2. Synthesis of GO-PEG-DVDMS

GO was synthesized using a modified Hummers method as previously reported [36,45,46]. In brief, NaOH (0.05 M) was added into the GO solution and the mixture was heated for 4 h at 55 °C. The solution was adjusted to pH = 1 by adding HCl (37% v/v). Then the solution was obtained by centrifugation after being neutralized and purified with water. Then 25 mg of amine-terminated eight-arm branched PEG was added into the base-treated GO solution (1 mg/mL, 5 mL) in a 25 mL glass vial. The mixture was sonicated for 15 min. *N*-(3-dimethylaminopropyl-*N*-ethylcarbodiimide) hydrochloride (EDC) was added to give a final concentration of 10 mg/mL. After stirring overnight at room temperature, the solution was centrifuged to remove any aggregates and the supernatant was collected. The PEGylated GO was further purified with 100 kDa filter (Millipore Inc.) and washed with distilled water. To determine the GO-PEG concentration, the absorbance at 230 nm was recorded with a weight extinction coefficient of 47.6 mg mL^{-1} cm^{-1}.

DVDMS was dissolved in water at 10 mg/mL as the stock solution for further use. DVDMS (0.4 mg/mL), and PBS (0.02 M) were slowly added into GO-PEG (0.1 mg/mL) solution and incubated overnight to produce GO-PEG-DVDMS. Unbound free DVDMS was removed by filtration through a 100 kDa cutoff spin filter and washed with water for 6–8 times.



Scheme 1. Chemical structure of DVDMS.

2.3. Characterization of GO-PEG-DVDMS

The size and thickness of GO-PEG-DVDMS were characterized using atomic force microscopic (AFM) images. The ultraviolet-visible (UV–vis) spectra was measured to determine the concentration of DVDMS loaded onto GO-PEG by the characteristic absorption peak at 516 nm with a molar extinction coefficient of 2.9×10^4 $M^{-1}cm^{-1}$ after subtracting the absorbance contributed by GO-PEG at the same wavelength. Fluorescence spectra of GO-PEG-DVDMS and free DVDMS were measured with an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) under 385 nm excitation.

2.4. Detection of singlet oxygen

Singlet oxygen sensor green (SOSG) was used to detect the singlet oxygen generation (SOG) of GO-PEG-DVDMS or DVDMS [47]. The GO-PEG-DVDMS or DVDMS solutions were mixed with SOSG in water containing 2% methanol with the final concentration of 1.0 μM. For each sample, the DVDMS concentration was fixed at 0.5 μg/mL. Then the solutions were irradiated with a 630 nm laser (Research Electro-Optics Inc., Colorado, US.). SOSG fluorescence emission was measured with an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) under 485 nm excitation. The sample's SOG was evaluated by the SOSG fluorescence enhancement compared with the background or control samples.

2.5. Cell uptake and internalization

U87MG human glioma cells, obtained from the American Type Culture Collection (ATCC, Manassas, VA), were cultured in Dulbecco's Modified Eagle Medium containing 10% FBS and 1% penicillin/streptomycin at 37 °C under 5% CO_2. For cell uptake assay, the cells were seeded in Lab Tek II 8-well chamber slides (Nalge Nunc International, Rochester, NY) with a density of 1×10^4 cells/mL and were allowed to grow for 24 h. Then, medium was replaced and cells were incubated in the dark with GO-PEG-DVDMS or DVDMS at DVDMS concentration of 1 μg/mL for 24 h. Then cells were washed with phosphate buffered saline (PBS) for three times. After being mounted with a mounting solution containing DAPI (SlowFade® Gold Antifade Reagent with DAPI, Molecular Probes, Invitrogen) for nuclear staining, the cells were observed by an IX81 epifluorescence microscope (Olympus, Japan).

2.6. Flow cytometry

The U87MG cells were incubated with GO-PEG-DVDMS or DVDMS at DVDMS concentration of 1 μg/mL for 24 h. The cells were then washed and resuspended in PBS. The cells were measured by Accuri C6 flow cytometer using C Flow Plus software (BD, Ann Arbor, MI). The data were analyzed by FlowJo version 7.6.5 (FlowJo, Ashland, OR).

2.7. Cytotoxicity assay

The U87MG cells were seeded into 96-well plates (5×10^3 cells per well) for 24 h, and then darkly incubated with different concentrations of free DVDMS, GO-PEG and GO-PEG-DVDMS at 37 °C. After 24 h incubation, cells were washed twice and 10 μL of MTT solution (5 mg/mL MTT in PBS, pH 7.4) was added to each well and the plate was incubated for another 4 h. After removing the medium, the wells were washed by PBS, and then the intracellular formazan crystals were dissolved into 100 μL DMSO. The absorbance was recorded at 490 nm by a plate reader, and the percentage of cell viability was determined by comparing cells treated with untreated control.

2.8. In vitro photodynamic therapy

The U87MG cells were grown in 96-well plates at a density of 5×10^3 cells/well in MEM complete medium. The cells were incubated with GO-PEG-DVDMS or free DVDMS at different concentrations for 24 h. The wells were then washed with PBS for three times. 100 μL of fresh medium was added into each well, which were immediately irradiated by the 630 nm laser (5 J/well). After irradiation, the cells were kept for another 24 h. Cell viability was estimated by the standard MTT assay.