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Thiadiazole molecules and poly(ethylene glycol)-block-polylactide self-assembled nanoparticles as effective photothermal agents

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ABSTRACT

A new photothermal nano-agent was obtained by the coprecipitation of 2,5-Bis(2,5-*bis*(2-thienyl)-*N*-dodecyl pyrrole) thieno[3,4-b][1,2,5] thiadiazole (TPT–TT) and a biodegradable amphiphilic block copolymer, methoxypoly(ethylene glycol)$_{2K}$-block-poly(D,L-lactide)$_{2K}$ (mPEG$_{2K}$-PDLLA$_{2K}$). TPT–TT, a donor–acceptor–donor (D–A–D) type small molecule, with *bis*(2-thienyl)-*N*-alkylpyrrole (TPT) as the donor and thieno[3,4-b]thiadiazole (TT) as the acceptor was a strong near infrared (NIR) absorber, which could convert the absorbed light energy into heat. The formation of TPT–TT nanoparticles (TPT–NPs), which possessed high stability in water, was confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). TPT–NPs showed high photothermal conversion efficiency (32%) and excellent photostability and heating reproducibility. The photostability of TPT–TT NPs was much better than that of indocyanine green (ICG), a federal drug administration (FDA) approved NIR dye. Besides, TPT–TT NPs exhibited significant photothermal therapeutic effect toward human cervical carcinoma (HeLa) and human liver hepatocellular carcinoma (HepG2) cells, while no appreciable dark cytotoxicity was observed. These results highlight the potential of TPT–TT NPs as an effective photothermal agent for cancer therapy.

1. Introduction

Photothermal therapy (PTT) has become an alternative to traditional cancer therapies because of the minimal invasiveness, low toxicity to normal tissues, and high specificity to tumor tissues [1–8]. For applications in clinic, photothermal agents with strong optical absorbance in the near-infrared (NIR) window (700–900 nm) are necessary to maximize light penetration depth and minimize the influence of biological chromophores in this region [1,9]. Besides, strong and stable NIR absorbance, high photothermal conversion efficiency, robust photostability and good biocompatibility are also indispensable for ideal PTT agents. PTT based on nanoparticles provides a rapid and efficient approach to selectively treat tumor tissues [8,10,11]. In recent years, a variety of NIR-absorbing inorganic nanomaterials, such as carbon materials [6,12–17], gold nanostructures [4,13,18–23], copper sulfide nanoparticles [24,25], palladium nanosheets [26,27], tungsten oxide nanowires [28], rare earth ions doped nanocrystals [29], porous silicon [30] and quantum dots [31] have been widely explored as photothermal agents [1,32]. Although most of these inorganic materials have shown high efficacies for treatment of cancers, the fact is that most of them are non-biodegradable and will be retained in the body for a long time, preventing their practical applications in clinical cancer treatment [1,32–35].

More recently, polymeric NIR-absorbing materials as PTT agents have attracted increasing research interests. Conjugated polymers (CPs), such as polypyrrole [36–41], polyaniline [42,43], poly(3,4-ethylenedioxythiophene):poly(4- styrenesulfonate) (PEDOT:PSS) [44] and dopamine-melanin [45] have been studied due to their large absorption coefficients and good photostabilities and shown encouraging photothermal therapeutic effects [46–48]. Even so, the biodegradation behaviors of CPs remain unclear [1,32]. In addition, porphyrin-lipids, or porphysomes, have also been reported as a new type of PTT agent for tumor therapy [49,50]. However, they have

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not shown very high photothermal conversion efficiency since part of the absorbed energy decays through radiative pathway [2]. Alternatively, small molecular organic dyes have been used not only as fluorescent probes in optical imaging, but also as PTT agents. For instance, indocyanine green (ICG), a federal drug administration (FDA) approved NIR dye, has shown high photothermal conversion efficiency [51–55]. Nevertheless, the further application of these small molecular PTT agents is limited by their poor photostability and short circulation time [1,56]. In view of the above, it is urgent to develop more photostable and biocompatible agents with high photothermal conversion efficiency in the NIR region to meet the severe requirements of future PTT.

2,5-Bis(2,5-*bis*(2-thienyl)-*N*-dodecyl pyrrole) thieno[3,4-b] [1,2,5] thiadiazole (TPT–TT), a donor–acceptor–donor (D–A–D) type small molecule, with *bis*(2-thienyl)-*N*-alkylpyrrole (TPT) as the donor and thieno[3,4-b]thiadiazole (TT) as the acceptor was reported earlier [57]. TPT–TT was a strong NIR absorber with high photostability, and it was non-fluorescent. In this work, a biodegradable amphiphilic block copolymer, methoxypoly(ethylene glycol)$_{2K}$-block-poly(D,L-lactide)$_{2K}$ (mPEG$_{2K}$–PDLLA$_{2K}$) was used as a stabilizer to solubilize TPT–TT and form TPT–TT nanoparticles (TPT–TT NPs) in aqueous solution. TPT–TT NPs exhibited high efficiency in converting light to heat upon 808 nm laser irradiation, and dramatically improved photothermal stability compared with ICG. TPT–TT NPs could act as an effective PTT agent to kill cancer cells under NIR light, while no appreciable dark toxicity was observed.

2. Materials and methods

2.1. Materials

All chemicals and reagents were used as received from commercial sources without any purication. Solvents for chemical synthesis were purified by distillation. Ultrapure water was prepared from a Milli-Q system (Millipore, USA). Chemical reactions were carried out under an argon atmosphere. The synthesis of 2,5-Bis(2,5-*bis*(2-thienyl)-*N*-dodecyl pyrrole) thieno[3,4-b] [1,2,5] thiadiazole (TPT–TT) has been reported [57]. Methoxypoly(ethylene glycol)$_{2K}$-block-poly(D,L-lactide)$_{2K}$ (mPEG$_{2K}$–PDLLA$_{2K}$) was prepared according to the method reported by our group previously [58,59].

2.2. Characterizations

The Ultraviolet–visible-NIR (UV–vis–NIR) absorption spectra were obtained by using a Shimadzu UV-2450 PC UV–vis spectrophotometer. Size and size distribution of the nanoparticles were determined by Malvern Zeta-sizer Nano for dynamic light scattering (DLS). The measurement was carried out at 25 °C and the scattering angle was fixed at 90°. The morphologies of the nanoparticles were measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. To prepare the specimen for TEM, a drop of micelle solution was deposited onto a copper grid with a carbon coating. The specimen was air-dried and measured at room temperature.

2.3. Synthesis of TPT–TT and preparation of TPT–TT NPs

TPT–TT was synthesized by the previous method [57]. The TPT–TT NPs were prepared through a nanoprecipitation method. Briefly, TPT–TT and mPEG$_{2K}$–PDLLA$_{2K}$ at a weight ratio of 1:4 were dissolved in 5 mL tetrahydrofuran (THF). After 5 min of stirring, the solution was added dropwise to 10 mL of deionized water and stirred to evaporate THF. The solution was filtered with a syringe filter (pore size: 0.45 μm) and powders of TPT–TT NPs were obtained by freeze-drying. MPEG$_{2K}$–PDLLA$_{2K}$ micelles only were also prepared with the same procedure just without TPT–TT.

2.4. Loading content of TPT–TT in the nanoparticles

For determination of drug loading content (DLC), the powders of TPT–TT NPs were dissolved in THF and analyzed with a UV–vis spectrophotometer at 713.5 nm by using a standard curve method. The DLC was calculated by the following equation:

$$\mathrm{DLC(wt\%)} = \frac{\text{the weight of TPT} - \text{TT in the nanoparticles}}{\text{the weight of nanoparticles}}$$

2.5. Photothermal evaluation and measurement of photothermal conversion efficiency (η)

The stock dispersion of TPT–TT NPs was diluted to various concentrations, and their photothermal effects were evaluated under irradiation of an 808 nm laser at a power of 2.0 W/cm^2, or at a fixed concentration of 50 μg/mL with irradiation at various laser intensities. Every sample was irradiated for 10 min. A thermocouple probe with an accuracy of 0.1 °C was used to measure the temperature of the solutions immediately before and after laser application.

For measuring the photothermal conversion performance of the TPT–TT NPs, 0.3 mL aqueous dispersion of TPT–TT NPs at 50 μg/mL was irradiated with an 808 nm laser at a power of 2.0 W/cm^2 for 10 min. The thermocouple probe was inserted into the solution parallel to the path of the laser. The temperature was recorded every 10 s by a digital thermometer with a thermocouple probe. Then, the photothermal conversion efficiency of TPT–TT NPs was determined according to previous method [45,60]. The photothermal response of TPT–TT NPs in water and the linear time data versus $-\ln\theta$ obtained from the cooling period were shown in Fig. S1 (Supporting information, SI). θ is defined as the ratio of ΔT to ΔT_{max}, where ΔT is the temperature change, and ΔT_{max} is the maximum temperature change.

2.6. Photostability and heating reproducibility of TPT–TT NPs

The photostability of TPT–TT NPs and ICG was investigated by monitoring their absorbance changes upon continuous 808 nm laser irradiation at a power of 2.0 W/cm^2 for different time periods. The UV–vis–NIR spectra were tested every minute.

Heating/cooling curve of the TPT–TT nanoparticles at a concentration of 50 μg/mL was completed over 8 cycles. Each cycle includes 10 min of laser irradiation to generate heat with a cooling time of 25 min before the start of the next cycle. Temperatures were recorded immediately after 10 min of laser irradiation and following the 25 min of cooling period.

2.7. Cell culture and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays

All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), and the culture medium was replaced once every day.

For MTT assays, cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells per well and incubated in DMEM for 24 h. The medium was then replaced by mPEG$_{2K}$–PDLLA$_{2K}$ micelles or TPT–TT NPs at various concentrations. The incubation was continued for 24 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added