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Dual imaging-guided photothermal/photodynamic therapy using micelles

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ABSTRACT

We report a type of photosensitizer (PS)-loaded micelles integrating cyanine dye as potential theranostic micelles for precise anatomical tumor localization *via* dual photoacoustic (PA)/near-infrared fluorescent (NIRF) imaging modalities, and simultaneously superior cancer therapy *via* sequential synergistic photothermal therapy (PTT)/photodynamic therapy (PDT). The micelles exhibit enhanced photostability, cell internalization and tumor accumulation. The dual NIRF/PA imaging modalities of the micelles cause the high imaging contrast and spatial resolution of tumors, which provide precise anatomical localization of the tumor and its inner vasculature for guiding PTT/PDT treatments. Moreover, the micelles can generate severe photothermal damage on cancer cells and destabilization of the lysosomes upon PTT photoirradiation, which subsequently facilitate synergistic photodynamic injury *via* PS under PDT treatment. The sequential treatments of PTT/PDT trigger the enhanced cytoplasmic delivery of PS, which contributes to the synergistic anticancer efficacy of PS. Our strategy provides a dual-modal cancer imaging with high imaging contrast and spatial resolution, and subsequent therapeutic synergy of PTT/PDT for potential multimodal theranostic application.

1. Introduction

Photodynamic therapy (PDT) is extensively considered as an intriguing treatment modality in the field of cancer therapy [1,2]. However, there are still several issues that restrict its clinical potential. Firstly, PDT generally requires to a pinpoint light irradiation at tumor, and thus tumor localization and monitoring are essential to achieve efficient PDT with low side effect. Secondly, singlet oxygen has a short half-life of <40 ns and diffusion range of 10–20 nm in biological system [3], which requires efficient subcellular translocation of photosensitizer (PS) to improve the accessibility of singlet oxygen to the cytoplasm and target organelles in cancer cells [1,3,4]. Additionally, photo-bleaching of PS under photoirradiation and limited penetration depth of light in the excitation range of 600–700 nm may also hinder their anticancer efficacy owing to their insufficient photocytotoxicity [2]. Therefore, to overcome these limitations of PDT, it is highly necessary to develop a multimodal nanoplatform for achieving both precise tumor localization and enhanced PDT efficacy of PS.

Recently, various imaging or anticancer agents have extensively been integrated with PS using nanoparticles for achieving cancer imaging and enhanced PDT efficacy *via* combinational treatments [5–12]. However, the generation of imaging modality and

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enhanced efficacy demands the incorporations of additional imaging agent and anticancer drugs within nanoparticles, which thus result in a ternary or more complex composition. Moreover, so far there are only a few strategies to synergize efficacy on anticancer agents *via* intracellular synergistic mechanisms in addition to some inorganic nanomaterials such as gold nanoparticles and superparamagnetic iron oxide nanoparticles in our previous studies [5,13]. Consequently, it is highly desired to develop a simple strategy that can provide both cancer imaging and synergistic effect on PDT.

Near-infrared organic cyanine dyes such as indocyanine green and Cypate have been considered as clinically potential theranostic agents, which possess promising multiple properties including near-infrared fluorescence (NIRF) emission above 800 nm, photoacoustic (PA) effect, photothermal therapy (PTT) after excitation by near-infrared light [14–21]. Cyanine dyes exhibit NIRF and PA imaging modalities with good imaging sensitivity and spatial resolution, respectively [14,20,21], and are also able to generate obvious photothermal damage *via* hyperthermia [6,22–24]. To date, cyanine dyes have been integrated into theranostic nanoparticles for both cancer imaging and therapy [5,14,25–31]. In our previous study, polymeric micelles encapsulating cyanine dye were found to generate severe photothermal damage on tumor and simultaneously induce the disruption of lysosomal membranes upon photoirradiation [32], which are presumably potential to facilitate the cytoplasmic delivery of anticancer agents.

In this study, we report a PS (Ce6)-loaded micelle system encapsulating cyanine dye (Cypate) (Cy/Ce6-Micelles) (Scheme 1), which was evaluated for the NIRF and PA imaging modalities with precise anatomical tumor localization and high contrast. The synergistic anticancer efficacy of Cy/Ce6-Micelles was further elucidated under sequential PTT/PDT treatments.

2. Materials and methods

2.1. Materials

β-Benzyl-L-aspartate N-carboxyanhydride (BLA-NCA) was obtained from Enlai Biological Technology Company (Chengdu, China). mPEG-NH_2 (12000 MW) was purchased from Jenkem Technology Company (Beijing, China). mPEG-*b*-PAsp was synthesized as mentioned method in the reference (The degree of polymerization of PAsp segment was calculated to be 64 according to ^{1}H NMR spectrum, Figs. S1 and S2) [32,33]. AO was purchased from Amresco. Decylamine were obtained from Sigma–Aldrich. Cypate was synthesized as reported in Refs. [34,35].

2.2. Synthesis

mPEG-*b*-PAsp was reacted with decylamine in anhydrous N-methyl pyrrolidone (NMP) at 45 °C overnight. Then, the solutions were dialyzed in the mixture of ethanol and water for removing excess amines and then were dialyzed in water. Finally, mPEG-*b*-PAsp(DA) was obtained by lyophilizing the dialyzed solutions, and their degree of polymerization was calculated to be 54 according to ^{1}H NMR spectrum (Fig. S2) [32].

2.3. Preparation of micelles

2 mg Cypate, 2 mg Ce6 and 6 mg mPEG-*b*-PAsp(DA) were mixed at the ratio of 1:1:3 in the mixture (0.5 mL) of DMSO, and then the solution was dispersed into 4 mL distilled water. Subsequently, Cy/Ce6-Micelles were prepared by dispersing the mixture into de-ionized water and purified using dialysis (Cut-off 3.5 K MW). The mixture of free Cypate and Ce6 (free Cypate/Ce6) was prepared by dissolving Cypate and Ce6 in 5% DMSO.

2.4. Characterization

The hydrodynamic diameter of micelles was measured using DLS (Malvern, UK) at 25 °C. The morphology of the micelles was observed using TEM (Tecnai-G20). The absorbance and fluorescent spectrums of Cypate or Ce6 were measured using UV–vis Spectrophotometer (UV2600, Shimadzu) and Fluorescence Spectrophotometer (LS 55, Perkin Elmer), respectively. The photoacoustic intensity of Cy/Ce6-Micelles was measured at the wavelengths of 680, 780 and 880 nm using PA system (Endra Nexus 128, Ann Arbor, MI).

2.5. Monitoring of singlet oxygen

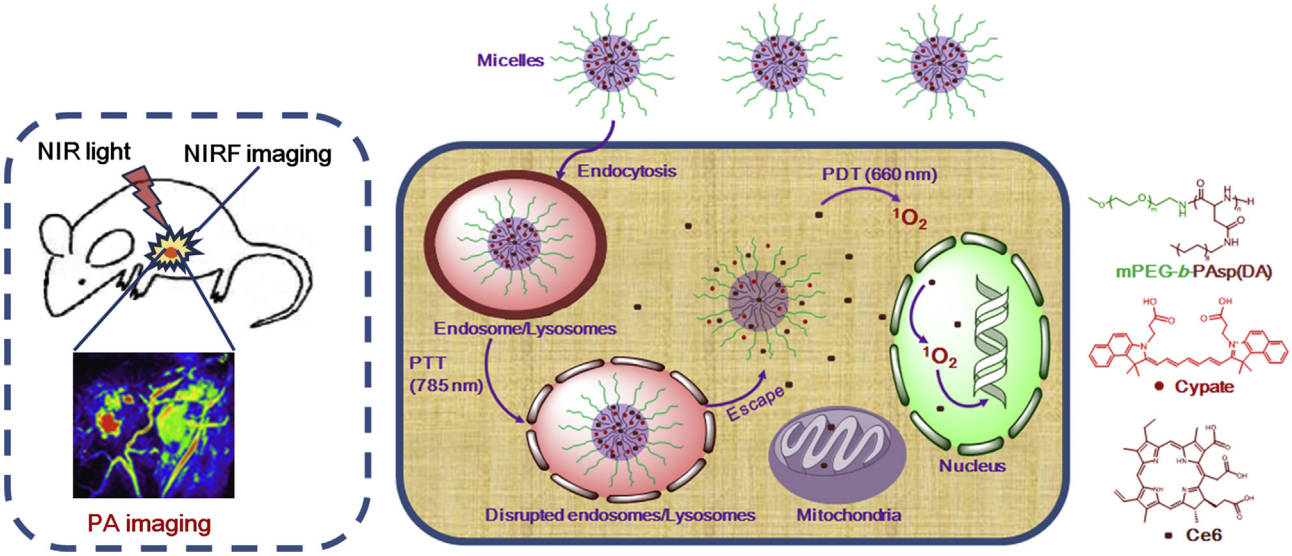
Free Cypate/Ce6 and Cy/Ce6-Micelles containing various concentrations of Cypate and Ce6 were dispersed in PBS solutions containing 32.0 μg/mL DPBF (1.0 mL), and were further irradiated for 3 min at 1.5 W/cm^2 (785 nm) or 10 min at 1.0 W/cm^2 (660 nm) for singlet oxygen measurement. The fluorescent spectrum was scanned from 430 to 550 nm using an excitation wavelength of 403 nm. Then, the fluorescence intensity at 485 nm was recorded. The solution of DPBF without Cypate or Ce6 in darkness was used as the control.

2.6. Photostability

Cy/Ce6-Micelles and free Cypate/Ce6 (10 μg/mL Cypate or Ce6, each 0.5 mL) were irradiated at 1.5 W/cm^2 (785 nm) or at 1.0 W/cm^2 (660 nm) for 0, 0.5, 1, 2, 3, and 10 min, respectively. The absorbances of Cypate or Ce6 were measured using UV–vis Spectrophotometer (UV2600, Shimadzu).

2.7. Photothermal effect

The solutions of Cy/Ce6-Micelles and free Cypate/Ce6 (each 0.5 mL) at the concentrations of 1, 2, 5, 10, 25, 50, and 100 μg/mL Cypate were stored in the glass vials with the volume of 0.5 mL, respectively, and then the solutions were irradiated at the excitation wavelength of 785 nm *via* a optical fiber (1.5 W/cm^2, FS-Optics, China). Simultaneously, the temperature of the solutions was measured during



Scheme 1. Schematic illustration of PS-loaded micelles integrating cyanine dye for dual-modal cancer imaging and synergistic therapy of PTT and PDT *via* an enhanced cytoplasmic delivery of PS.