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Rational engineering of semiconductor QDs enabling remarkable $^1O_2$ production for tumor-targeted photodynamic therapy

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ABSTRACT

Semiconductor quantum dots (QDs) have served as superior optically active nanomaterials for molecular imaging and photodynamic therapy (PDT), but the low singlet oxygen ($^1O_2$) quantum yield and lack of tumor selectivity have limited their applications for tumor PDT *in vivo*. Here, we report the rational engineering of QDs into tumor-targeting hybrid nanoparticles through micelle-encapsulating a pre-assembled unique QD-Zn-porphyrin complex, a highly fluorescent organic photosensitizer rhodamine 6G (R6G), and a near-infrared fluorophore NIR775 with folic acid labeled phospholipid polymers. These nanoparticles have large porphyrin payloads and strong light absorption capability, thus contributing to an extremely high $^1O_2$ quantum yield (~0.91) via an efficient dual energy transfer process. *In vivo* studies show that they can preferably accumulate in tumors through folate receptor-mediated active delivery, permitting non-invasive fluorescence imaging and effective PDT of tumors in living mice. This study highlights the utility of hybrid semiconductor QDs for both tumor imaging and PDT *in vivo*.

1. Introduction

Photodynamic therapy (PDT) is an effective therapy modality with non-invasive and high spatiotemporal precision for various malignant tumors [1–4]. It involves the application of photosensitizers (PSs) that can be activated by light to generate cytotoxic reactive oxygen species (ROS, e.g., single oxygen $^1O_2$), ultimately destroying tumors through multifactorial mechanisms [5–8]. To achieve effective PDT, it is essential to develop PSs which are nontoxic in the absence of light, but are able to selectively localize at tumor tissues and produce $^1O_2$ in high quantum yield upon light irradiation. A myriad of PSs capable of killing tumor cells in the presence of light have been actively developed [9–11]. Among them, porphyrins are the most widely used PSs for the tumor treatment in clinical because of the high ability to produce ROS [12–14]. However, the PDT with these porphyrins in clinical is still not very efficient as they largely suffer from low absorption coefficients, poor water solubility and limited tumor selectivity [15–17]. As such, recent efforts have been made to integrate porphyrins into various nanoparticles, aiming to take the advantages of nanotechnology to overcome these limitations [8,18–21].

Semiconductor quantum dots (QDs) are one of the most versatile optically active nanomaterials that have been widely used for molecular imaging and biosensing due to their prominent characteristics, including large absorption coefficients, high emission quantum yields, high photostability, broad absorption bands, and easy surface functionalization [22–25]. In essence, the superior properties of QDs have also motivated people to consider their applications for PDT [26–29]. One of the intriguing examples has been demonstrated by Weiss et al. who employed a covalent conjugation strategy to develop water-soluble peptide-coated QD-PS conjugates, affording a $^1O_2$ quantum yield of 0.31 for PDT [30]. Encouraged by this, follow up works have successfully explored other kinds of QD-PS conjugates, aiming to improve energy transfer efficiency to enlarge $^1O_2$ production for killing cancer cells upon light excitation [31–33]. Despite these efforts, currently reported QD-PS conjugates are still subject to the tedious conjugation process, insufficient $^1O_2$ generation capacity and limited tumor selectivity. Moreover, there is still lack of evidence that QD-PS conjugates could trigger effective PDT in living systems, requiring the development of new strategy to engineer QDs capable of

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enhancing $^1O_2$ generation and ablating tumors *in vivo*.

Herein, we report the design of a unique QD-Zn-porphyrin nanocomplex, and further engineer it into a tumor targeting hybrid nanoparticles through micelle encapsulating it with a highly fluorescent organic PS rhodamine 6G (R6G) and a near-infrared fluorophore NIR775 by amphiphilic phospholipid polymers. Encapsulation of porphyrins within micelles is a well-explored method capable of improving the water solubility and pharmacokinetic properties *in vivo*, while the occurrence of porphyrin aggregation within the micelles to cause self-quenching effects could generally lower the ROS generation capacity, thus compromising the PDT outcome [34–39]. We demonstrate that, by using the unique QD-Zn-porphyrin complex, the fluorescence of porphyrins within the hybrid nanoparticles is not quenching but enhancing through a dual energy transfer process, thus contributing to strong fluorescence and remarkable $^1O_2$ quantum yield for fluorescence imaging and efficient PDT of tumors in living mice.

2. Experimental sections

2.1. Preparation of TMPyP-Zn-QD nanocomplexes

Freshly prepared *N*-acetyl-L-cysteine (NAC)-CdTe/ZnS QDs (29.4 μM, 34.0 μL) and meso-tetrakis(1-methyl 4-pyridinio) porphyrin (TMPyP) (20 mM, 3.4 μL) were mixed in D.I. water (462.6 μL). The mixture was sonicated at room temperature (r.t.) for 5 min, and then stirred at r.t. for another 30 min to promote the extensive complexion. Then, the resulting solution was transferred into a 10 KD Millipore, centrifuged (4000 rpm), and washed with D.I. water three times. Finally, TMPyP-Zn-QDs were obtained, and the concentration of the stock solution of TMPyP-Zn-QDs was determined by TMPyP.

2.2. General procedure for the preparation of HyNPs

1,2-Distearoyl-*sn*-glycero-3-phosphoethanol-amine-*N*-[methoxy (polyethylene glycol)-2000] (DSPE-PEG-2000, 1.9 mg) and 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[folate(poly ethylene glycol)-2000] (DSPE-PEG-2000-FA, 0.1 mg) were dissolved in 1.5 mL CH_2Cl_2/CH_3OH (9/1). The solution was then rapidly added into a 1.5 mL aqueous solution containing 45.3 μM TMPyP-Zn-QDs and 20 μg R6G, and the resulting mixture was sonicated at r.t. for 5 min, followed by stirring under dark overnight. After removal of the organic solvents under vacuum, the aqueous solution containing HyNPs was then transferred into a 10 KD Millipore, centrifuged (4000 rpm), and washed with D.I. water three times. HyNPs stock solution was obtained and kept under dark at 4 °C. The concentration of HyNPs were determined by TMPyP.

2.3. General procedure for the measurement of $^1O_2$ production capacity in vitro

The $^1O_2$ Production Capacity of HyNPs in aqueous solution was evaluated using three independent methods. First, singlet oxygen sensor green (SOSG) was used as the $^1O_2$ indicator. A solution of HyNPs at different concentration and SOSG (20.0 μM) in D.I. H_2O was exposed to a white light (LED lamp, 400 nm long pass filter, 20 mW/cm^2) and irradiated for different times. The fluorescence spectra of SOSG were then acquired with excitation at 488 nm. The $^1O_2$ generation capacity was evaluated by comparing the fluorescence intensity of SOSG at 525 nm. Second, electron spin resonance (ESR) experiment was conducted using 2,2,6,6-tetramethylpiperidine (TEMP) as the $^1O_2$ scavenger. A solution of TEMP (60 mM) and HyNP3 (8.0 μM) in D.I. H_2O was exposed to a white light (LED lamp, 400 nm long pass filter) at a power of 20 mW/cm^2 for 115 s. After irradiation, the ESR spectra were then acquired by an electron paramagnetic resonance spectrometer (EMX-10/12, Bruker, Germany). Third, the $^1O_2$ emission at ~1270 nm was examined as a direct evidence to identify the $^1O_2$ generation. Briefly, a solution of 8.0 μM TMPyP, 8.0 μM TMPyP-Zn QDs, 8.0 μM HyNP1, or 8.0 μM HyNP3 in D_2O was irradiated with a 530-nm excitation laser, and the luminescence of $^1O_2$ in each solution was immediately acquired with a FLS 920 time-resolved spectroscope (Edinberge, UK) equipped with a NIR detector. The $^1O_2$ generation capacity was directly compared by the different fluorescence intensity at ~1270 nm.

2.4. Cell culture

Human carcinoma KB cell lines were cultured in RPMI-1640 medium supplemented with fetal bovine serum (10%), streptomycin (100.0 mg L^{-1}), and penicillin (100 IU·mL^{-1}). Human colon carcinoma HCT116 cell lines were cultured in McCoy's 5A (modified) medium supplemented with fetal bovine serum (10%), streptomycin (100.0 mg L^{-1}), and penicillin (100 IU·mL^{-1}). Human embryonic kidney HEK293 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%), streptomycin (100.0 mg L^{-1}), and penicillin (100 IU·mL^{-1}). All these cells were cultured in a humidified incubator containing 5% CO_2 and 95% air at 37 °C. The medium was replenished every other day and the cells were subcultured after reaching confluence.

2.5. Fluorescence imaging of tumor cell using HyNPs

KB cells (~200 k) were seeded into 35.0 mm confocal dishes (Glass Bottom Dish) and incubated at 37.0 °C for 24 h. The medium was then replaced with fresh FA free RPMI-1640 medium containing different concentration of HyNP3. After incubation at 37.0 °C for different time, the cells were washed with cold phosphate buffer saline (PBS, pH 7.4) three times, and the epifluorescence images of cells were acquired under an IX73 optical microscope (Olympus, Japan). The R6G fluorescence in HyNP3 was acquired with excitation at 530 nm, and emission collected from 570 to 600 nm. The fluorescence of TMPyP-Zn-QDs in HyNP3 was acquired with excitation at 430 nm, and emission collected from 600 to 650 nm. For the colocalization assay, KB cells were incubated with HyNP3 (1.5 μM) for 0.5 h, washed with PBS (pH 7.4), and further incubated with 1.0 μM Lyso-Tracker@Red DND-99 and 1.0 μM Hoechst 33342 at 37.0 °C for 20 min. After washed with PBS three times, fresh RPMI-1640 medium was added, and the fluorescence images were acquired. The fluorescence of HyNP3 was acquired with excitation at 530 nm, and emission from 570 to 600 nm. Hoechst 33342 was excited at 340–390 nm, and emission was collected from 420 to 460 nm. Lyso-Tracker@Red DND-99 was excited at 540–580 nm, and the emission was collected from 600 to 650 nm.

2.6. Fluorescence imaging of intracellular $^1O_2$ levels

The intracellular $^1O_2$ levels upon light irradiation were examined using a cell permeable $^1O_2$ indicator 2′,7′-dichlorfluorescein-diacetate (DCFH-DA). KB or HCT116 cancer cells in 35.0 mm Glass bottom dishes were incubated with different concentration of HyNP3 under dark for 30 min. The medium was then removed, washed with fresh medium once, and 1.0 mL fresh medium containing DCFH-DA (20.0 μM) was then added into the cells. After incubation at 37 °C for 20 min, the cells were irradiated with white light (LED lamp, 400 nm long pass filter, 20 mW/cm^2) for different time. After irradiation, the cells were washed with 1 × PBS (pH 7.4)