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Communication

Semiconducting polymer dots with photosensitizer loading and peptide modification for enhanced cell penetration and photodynamic effect

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ABSTRACT

This letter describes semiconducting polymer dots (Pdots) doped with a photosensitizer and modified with a cell penetrating peptide for photodynamic therapy (PDT). The resulting Pdots exhibited efficient singlet oxygen ($^1O_2$) generation mediated by intraparticle energy transfer. Experimental results indicated that the peptide-coated Pdots could promote the cellular uptake and increase the penetration efficiency *in vitro*, and effectively suppressed tumor growth and enhanced the photodynamic effect *in vivo*. Our results demonstrate that Pdots with photosensitizer loading and peptide modification hold great promise for cancer therapy.

Photodynamic therapy (PDT) has been accepted as an effective cancer treatment modality [1,2]. It involves the photosensitizer administration and local irradiation with appropriate excitation wavelength to activate the photosensitizer. The activated photosensitizer transfers the energy to molecular oxygen to generate singlet oxygen ($^1O_2$) through type II reaction mechanism [3], which can lead to tumor cells ablation and blood vessels destruction [4,5]. As PDT exploits the localized oxidative damage to implement the cancer treatment, making it much milder than conventional treatment modalities including surgery, chemotherapy and radiotherapy [6]. In the past few years, PDT has been demonstrated for treatment of different cancers such as skin cancer [7], bladder cancer [8], lung cancer [9], head and neck cancer [10]. Along with the development and practice of PDT, a variety of nanoparticle based photosensitizers have been developed to enhance the photodynamic effect, including polymeric nanoparticles [11], upconversion nanoparticles [12–14], gold nanoparticles [15], mesoporous silica nanoparticles [16,17] and quantum dots [18,19].

Recently, semiconducting polymer dots (Pdots) have attracted considerable attention in biomedicine field [20–22]. The rapid development of Pdots is owing to their outstanding properties, such as high brightness, excellent photostability, low cytotoxicity and good water solubility, *etc.* [23]. Meanwhile, Pdots with photosensitizer loading have been widely used for PDT to kill cancer cells [24]. For PDT applications, Pdots can be used as carrier to load hydrophobic photosensitizer, and served as energy donor for amplified $^1O_2$ generation. However, *in vitro* cellular uptake of Pdots is conducted by nonspecific endocytosis [25], which leads to low penetration speed and efficiency of Pdots in cancer cells, and required relatively long incubation time (more than 8 h) [24]. In addition, carboxyl Pdots with negative charge were difficult to be internalized by cancer cells (cell membrane was negatively charged) due to electrostatic repulsion, resulting in unsatisfactory therapeutic efficiency [26]. These issues need to be addressed for practical application of Pdots.

Here we utilized a semiconducting polymer as matrix to prepare photosensitizer-doped Pdots (carboxyl Pdots), and modified with cell penetrating peptides (CPPs). CPPs have a strong penetration ability to speed the carrier transfer into cells, thus they are used to promote the cellular uptake and increase the penetration efficiency [27,28]. *In vitro* experiments indicated that the peptide coated-Pdots promoted the cellular uptake and increased the penetration efficiency in fairly short time, and enhanced the photodynamic effect at low incubation concentration, in contrast with carboxyl Pdots. *In vivo* experiments indicated

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that the peptide coated-Pdots could suppress tumor growth and enhance the photodynamic effect.

We designed and prepared multifunctional photosensitizer-doped Pdots with CPPs modification. Zinc phthalocyanine (ZnPc) as a second-generation photosensitizer is widely used in PDT. However, photosensitizer ZnPc generally suffers severe aggregation in physiological environment due to poor water-solubility, which limits its practical application in cancer therapy [29]. Thus we employed the polymer do-PFDTBT as carrier to load hydrophobic ZnPc to overcome the problem of aggregation. Furthermore, we employed the polymer do-PFDTBT as energy donor for ZnPc to generate efficient $^1O_2$ generation [24]. The synthetic route of do-PFDTBT was described in our previous study [30], and the chemical structures of required materials were shown in Scheme 1. The ZnPc-doped Pdots (carboxyl Pdots) in aqueous solution were prepared by the nanoprecipitation method [20]. The resulting carboxyl Pdots were further used for preparation of peptide coated-Pdots.

Fluorescence spectroscopy indicates the efficient energy transfer from the polymer to photosensitizer. Fig. 1a showed the reasonable spectral overlap between the absorption spectra of ZnPc and the emission spectra of do-PFDTBT, which indicated the possibility for energy transfer to occur inside the Pdots. To maximize the energy transfer efficiency, we investigated the spectroscopic properties of ZnPc-doped Pdots (carboxyl Pdots). Fig. S1a (Supporting information) showed the absorption spectra of Pdots doping with different fractions of ZnPc. As the doping fraction was increased (0–5 wt%), the absorption intensity of ZnPc at 680 nm gradually increased, which indicated that ZnPc was doped into Pdots successfully. Fig. S1b (Supporting information) showed the emission spectra of Pdots doping with different fractions of ZnPc. At the doping fraction of 5 wt%, the energy transfer efficiency of ZnPc-doped Pdots reached the maximum, which was the prerequisite for efficient $^1O_2$ generation. Fig. 1b showed the absorption and emission spectra of ZnPc-doped Pdots at the doping fraction of 5 wt%. As expected, the ZnPc-doped Pdots presented broad emission spectra with a dominant emission peak of 685 nm, which indicated the efficient energy transfer between do-PFDTBT and ZnPc.

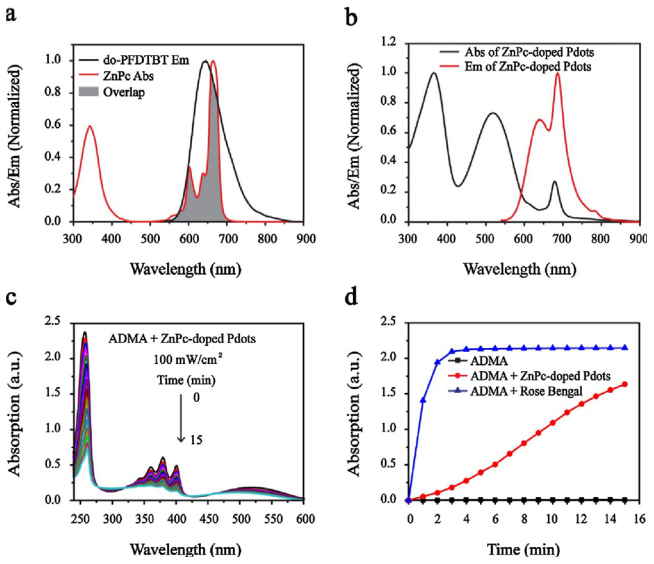
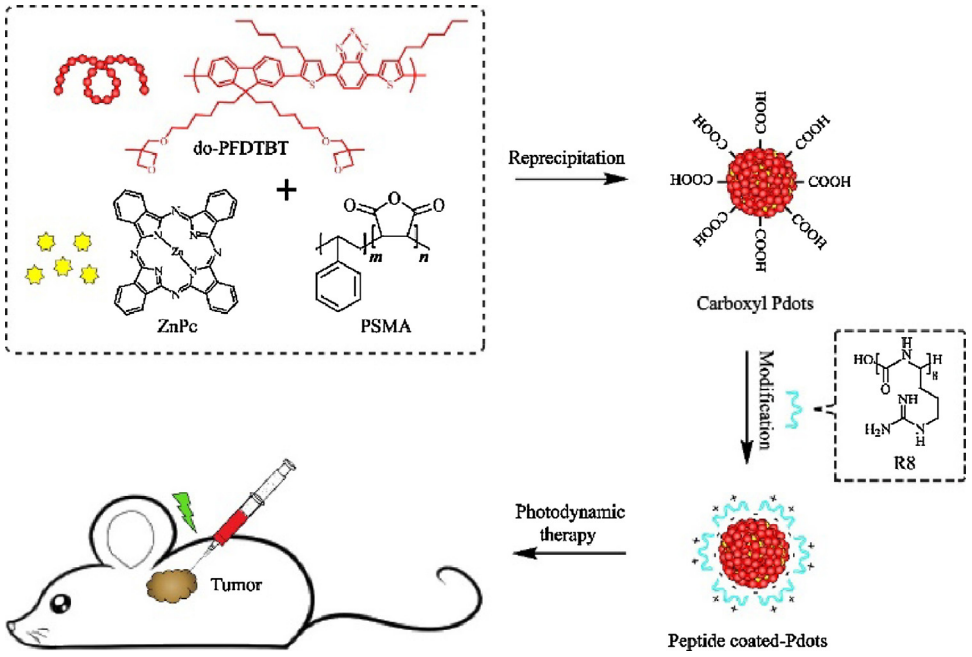


Fig. 1. Preparation and characterization of ZnPc-doped Pdots. (a) Spectral overlap between the absorption spectra of ZnPc and the emission spectra of do-PFDTBT. (b) Absorption and emission spectra of ZnPc-doped Pdots at 5% doping fraction. (c) Absorption spectra changes of ADMA mixed with ZnPc-doped Pdots. (d) Absorption spectra quenching of ADMA mixed with RB, ZnPc-doped Pdots, and ADMA alone.

$^1O_2$ generation yield of pure conjugated polymer is generally low [31,32]. Incorporation of photosensitizer into the Pdots makes energy transfer occur inside the Pdots, which can significantly enhance the $^1O_2$ generation yield. 9,10-Anthracenediylbis(methylene)dimalonic acid (ADMA) is widely used to detect the $^1O_2$ generation, and the photodegradation of ADMA is revealed by its absorption spectral bleaching. Therefore, the $^1O_2$ generation can be indirectly measured by monitoring the absorbance changes of ADMA [33]. In the present of ZnPc-doped Pdots, the absorption spectra of ADMA at 259 nm occurred obvious bleaching due to a large amount of $^1O_2$ generation (Fig. 1c). Meanwhile, we utilized Rose Bengal (Φ ($^1O_2$) = 0.76) as a standard substance to precisely calculate the $^1O_2$ quantum yield of ZnPc-doped Pdots [34]. The $^1O_2$



Scheme 1. Preparation and photodynamic effect of the peptide coated-Pdots.

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