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Carrier-free photosensitizer nanocrystal for photodynamic therapy

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ABSTRACT

In recent years, many nanomaterials have been developed as drug carriers to overcome their low solubility. However, the poor drug loading (general $<5\%$) requires excessive use of carrier materials which may induce side effects and inhibit their clinical translation. Herein, hydrophobic meso-tetraphenylporphyrin (TPP) photosensitizer (PS) molecules are firstly assembled into pure nanocrystals by solvent exchange. Secondly, amphiphilic multidentate polymer ligand, PEG-grafted poly (maleic anhydride-alt-1-octadecene) (C_{18}PMH–PEG), is modified on the nanocrystal surface to enhance their stability in saline. The as-prepared drug delivery system (DDS) by the two-step strategy significantly improves the drug loading (over 87%). The DDS shows high stability in saline and is engulfed by cancer cells. Further study demonstrates that the DDS is an effective photodynamic therapeutic agent of cancer cells while the free PS molecules quickly precipitate without obvious destruction of cancer cells.

1. Introduction

The delivery of poorly water-soluble antitumor drugs has been intensely studied during the past decades because many available antitumor drugs are poorly water-soluble which results in many difficulties for clinical drug administration [1]. Water-dispersible nanopartilces, such as mesoporous silica nanoparticles [2], gold nanoparticles [3], carbon nanotubes [4], and nanographene [5], have been utilized as drug carriers for water-insoluble cargo by hosting several drug molecules in a single nanoparticle. Generally, these nanocarriers show poor drug loading ($<5\%$) which inevitably introduces excessive carrier-materials [6]. However, potential side effects, such as oxidative stress, have been found in these carrier-materials [7]. To avoid the side effects and reduce the excessive use of carrier-materials, several strategies have been developed to improve the drug loading recently, such as (1) acid–base ionic interaction between the cargo and the carrier [8]; (2) loading in porous metal-organic-framework nanocarriers [6,9]; (3) blending drugs and stabilizers together [10,11]. There is still plenty of room to improve the drug loading of drug delivery system (DDS).

Herein, meso-tetraphenylporphyrin (TPP), a hydrophobic photosensitizer (PS) molecule for photodynamic therapy (PDT) on tumor, is firstly assembled into pure nanocrystals by solvent exchange. To reduce the aggregation and precipitation of PS nanocrystals in saline, amphiphilic multidentate polymer ligand, PEG-grafted poly (maleic anhydride-alt-1-octadecene) (C_{18}PMH–PEG), is secondly modified on the nanocrystal surface by multi-noncovalent hydrophobic interaction. The as-prepared DDS by the two-step strategy shows significantly high drug loading of over 87% in total and can be engulfed by cancer cells. Further study with light irradiation demonstrates that the DDS is effective to kill cancer cells while the free PS molecules quickly precipitate without obvious destruction of cancer cells. Our DDS not only improves the drug loading of the nanocrystals but also disperses hydrophobic PS in water very well for effective PDT.

2. Experimental details

TPP was purchased from J&K Scientific Ltd. poly (maleic anhydride-alt-1-octadecene) and poly (ethylene glycol) (MW=5000) were purchased from Sigma-Aldrich (St. Louis, MO). Detailed synthetic procedure of C_{18}PMH–PEG is presented in the Supplementary material.

Dynamic Light Scattering (DLS) was used for characterizing nanocrystal sizes in PBS solution (Malvern, UK). TEM image was obtained on a FEI Tecnai G2 F20 S-Twin TEM (Hillsboro, OR). Emission spectrum of the nanocrystal was characterized with a fluorometer (Fluoromax4, Horiba Jobin Yvon, Edison, NJ). UV–vis-NIR spectrum was collected with a LAMBDA 750 UV/vis/NIR spectrophotometer (Perkin-Elmer).

TPP nanocrystals were prepared by quickly injecting 50 μL TPP solution (1 mM in THF) into vigorously stirred ultrapure water (18.2 MΩ cm) at 30 °C. After 10 min, the solution was filtered

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(0.22 μm pore size) to remove bulk aggregations. Then, C_{18}PMH–PEG was added and the excessive surfactant was removed by ultra-centrifugation.

KB cells (ATCC, Manassas, VA, USA) were cultured in a culture dish at 37 °C, 5% CO_2 in a DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin. After 24 h, coated TPP nanopartilces were added with a final concentration of 0.5 μM. After further incubation for 24 h, the cells were rinsed with PBS buffer for two times. The cells were fixed with 4% paraformaldehyde (water solution) for 30 min and then incubated with Hoechest 33258 (10 mg/mL) for 10 min. Finally, the cells were imaged with a confocal laser microscope (Leica, TCS-SP5).

The stability of TPP nanocrystals under white light irradiation (100 mW/cm^2) was measured by UV–vis-NIR spectra. For PDT efficacy, the KB cells were incubated with TPP nanocrystals for 24 h and later irradiated with 100 mW/cm^2 white light for 40 min. Then, the cell viability was measured by standard MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

3. Results and discussion

To obtain TPP nanocrystal, a stock solution of TPP (in tetrahydrofuran, THF) was injected into ultrapure water (18.2 MΩ cm) under vigorous magnetic stirring at 30 °C. Containing no hydrophilic group, the PS is hydrophobic and dissolves well in THF but insoluble in water. Upon injection into water, the PS molecules self-assembled into nanoscale particles (Fig. 1a). Unlike the traditional nanovehicle-based pharmaceutical formulation, the PS nanocrystals contain no carrier materials and are composed of pure TPP molecules which give rise to an ultra-high drug loading. Amphiphilic multidentate polymer ligand, C_{18}PMH–PEG, is further modified on the nanocrystal surface as stabilizer (Fig. 1b) to enhance the stability of the carrier-free PS nanocrystals in biological environment. The stabilizer was added after the PS nanocrystal formation which could avoid the stabilizer doping inside the nanocrystal. Such a two-step strategy effectively improves the drug loading of the final pharmaceutical formulation (over 87% in total) compared with that of drug and stabilizer coprocessing during forming nanocrystal which is in fact an intimate mixture of drug and excipient and lowers the drug loading as a result [12]. The UV–vis spectrum of the PS nanocrystal covers the entire visible region which facilitates the excitation by the white light source (Fig. 1c). The PS nanocrystal emits at a wavelength of 656 nm which would be helpful to monitor its endocytosis by cells with fluorescence microscopy (Fig. 1c).

To understand the surface modification effects of C_{18}PMH–PEG on TPP nanocrystals, the hydrodynamic diameter changes of surface modified TPP nanocrystals in saline were monitored over a period of 48 h. The hydrodynamic diameter of surface modified TPP nanocrystals is ~165 nm after 48 h incubation in saline which is slightly increased compared with that of original ~147 nm (Fig. 2a). The result demonstrates that the C_{18}PMH–PEG adsorbed on the surface of TPP nanocrystals could effectively reduce the aggregation of TPP nanocrystals in saline. Visibly, the uncoated TPP nanocrystals precipitate after 48 h incubation in saline while the surface coated TPP nanocrystals show no precipitation and maintain a clear suspension (Fig. 2b). These results indicate that the C_{18}PMH–PEG adsorbed on the surface of TPP nanocrystals functions as an effective stabilizer to stably disperse TPP nanocrystals in saline.

The surface coated TPP nanocrystals were incubated with KB cells for 24 h and imaged with fluorescence microscopy. It is clearly seen that many red and bright spots (the fluorescence of TPP nanocrystals) are shown around the nucleus (Blue, stained with Hoechest 33258) which demonstrate the effective endocytosis of TPP nanocrystals by KB cells (Fig. 3). Notable, the

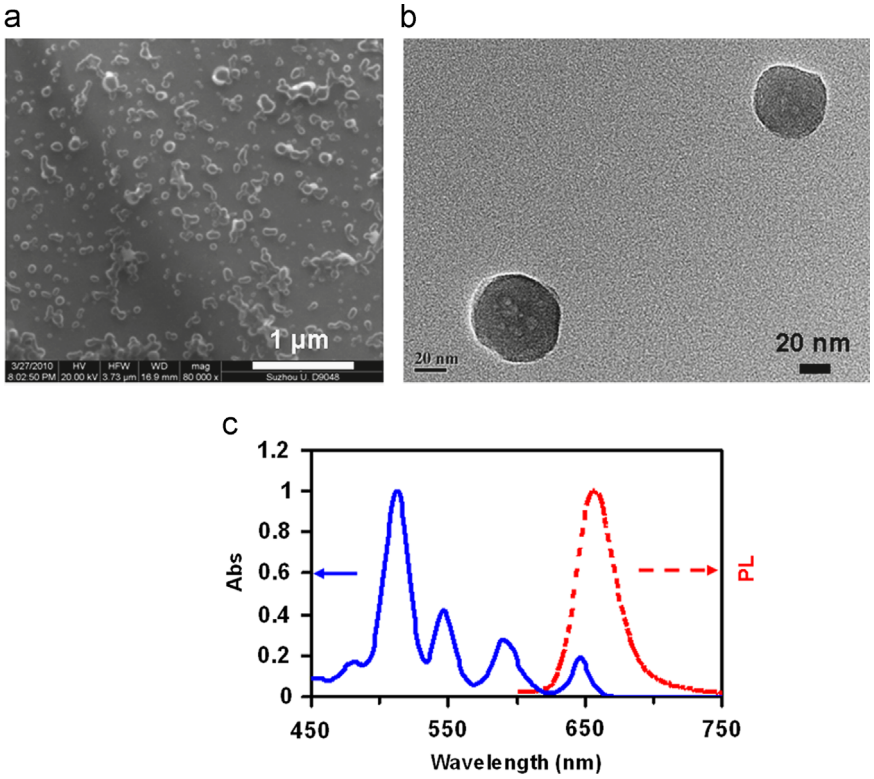

Fig. 1. (a) SEM image of TPP nanocrystals. (b) TEM image of surface coated TPP nanocrystals with C_{18}PMH–PEG. (c) Normalized UV–vis spectrum (blue) and fluorescence emission spectrum (red, *Ex*=480 nm) of surface modified TPP nanocrystal in water. (For interpretation of the references to color in this figure legend,the reader is referred to the web version of this article.)