Journal of Colloid and Interface Science 494 (2017) 363–372

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

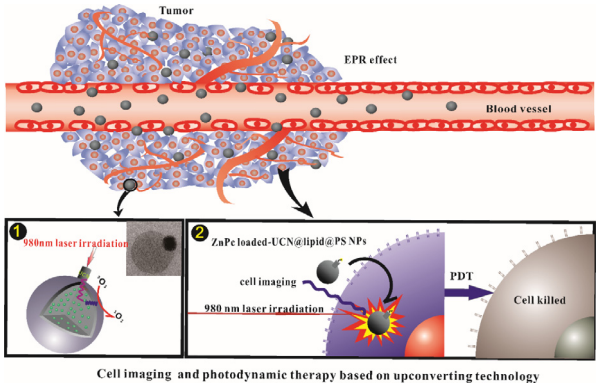
journal homepage: www.elsevier.com/locate/jcis

Construction of near infrared light triggered nanodumbbell for cancer photodynamic therapy

Beibei Hou, Bin Zheng, Weitao Yang, Chunhong Dong, Hanjie Wang *, Jin Chang

School of Life Sciences, Tianjin University, Tianjin 300072, PR China

GRAPHICAL ABSTRACT



Cell imaging and photodynamic therapy based on upconverting technology

ARTICLE INFO

Article history:
Received 30 August 2016
Revised 14 January 2017
Accepted 17 January 2017
Available online 19 January 2017

Keywords:
Photodynamic therapy
Upconverting nanocrystals
Remote controllability
Nanodumbbell
High loading amount

ABSTRACT

The application of photodynamic therapy (PDT) in deep tissue has been severely restricted by the poor photosensitizers loading and tissue-penetration of visible light for exciting the photosensitizers. How to prepare a nanocarrier with high drug loading amount and remote controllability still remains the challenge. In this article, a novel drug delivery system nanodumbbell was designed. The nanodumbbell was assembled from the hydrophobic upconverting nanoparticle (UCN) core and hydrophilic polymersome shell. The "nanodumbbell" offers possibilities to overcome the problem mentioned above. The UCN core works as a transducer to convert deeply penetrating near-infrared light to visible light to activate photosensitizers zinc (II) phthalocyanine (ZnPc) for photodynamic therapy. The polymersome lipid shell is used for loading ZnPc and protecting the whole system from nonspecific absorbance or corrosion during the transportation. The nanodumbbell is appealing because it can simultaneously achieve the high loading amount of ZnPc while avoiding UCNs aggregation. The reactive oxygen species (ROS) production test and PDT test *in vitro* suggested that the fluorescence emitted from the UCNs can be effectively transferred to the photosensitizers to produce cytotoxic ROS. When the UCN@lipid@polymersome nanodumbbell was decorated with targeting peptide (RGD), it presented better target specificity to cells. Our data suggest that this nanoparticle may serve as a useful nanoplatform for PDT treatment in deep-cancer therapy based on upconverting mechanism.

1. Introduction

Recently, photodynamic therapy (PDT) has been developed as an alternative to chemotherapy and radiotherapy for the clinical

* Corresponding author.
E-mail address: wanghj@tju.edu.cn (H. Wang).

http://dx.doi.org/10.1016/j.jcis.2017.01.053
0021-9797/

treatment of various diseases including cancer due to its minimal invasion and fewer side effects on healthy tissues [1–3]. It is generally accepted that PDT can cause damage to target cells by the interaction of photosensitizer with specific light and molecular oxygen [4–6]. The photosensitizer can be administrated and activated by certain light irradiation to produce reactive oxygen species (ROS) for sequential cancer eradication, leading to cellular death, vascular shutdown, and immune activation [7–9]. However, the clinical application of this technique is now limited in treatment of the superficial disease by the poor tissue penetration ability of the UV or visible light for the activation of photosensitizers, which hampered the application of PDT in the treatment of the internal tumors [10,11]. At the same time, most photosensitizers lose photochemical activity due to their poor solubility and easy aggregation in aqueous solutions, which also makes intravenous injection problematic for treatment of solid tumors [12,13].

It is now generally believed that near infrared light (NIR) has minimal light absorption by water (>900 nm) and hemoglobin (<650 nm) in living tissues, which can penetrate deeper than UV or visible light [14,15]. Applying NIR light to the activation of photosensitizers in the PDT treatment requires a transducer that can convert NIR light to visible light [16]. This conversion can be achieved by upconverting technology, which refers to a nonlinear optical process that converts two or more low-energy pump photons from the NIR spectral region to a higher-energy output photon with a shorter wavelength [17,18]. Thanks to the upconverting technology, the upconverting nanocrystals (UCNs) can convert NIR light into visible light, which matches the excitation range of most photosensitizers, thus solving the problem of shallow tissue-penetration of visible light needed for exciting the photosensitizer.

On the basis of UCNs, a series of nanocarriers for the PDT treatment were designed and reported over the last few years, such as photosensitizer loaded UCN@polymersome nanoparticles, photosensitizer loaded UCN@mesoporous silica and so on [19–23]. In the early stage of development, normally the hydrophobic photosensitizers was just physically adsorbed onto the surface or just loaded into opened holes of the nanocarriers [24,25]. All these methods made the whole delivery system unstable owing to photosensitizers leaking or unspecific absorbance, which impeded the application of PDT in complete tumor eradication. In addition, it was found that the ROS generated in the cytoplasm may vanish during the treatment due to their short lifetime ($\leqslant$3.5 μs) and limited diffuse distance ($\leqslant$0.02 μm) [26]. Consequently, an ideal nanoplatform would be a water-soluble nanosystem with a large loading of photosensitizers to generate ROS successively [27]. How to improve stability and the loading efficiency of the nanodevice is still one of the most urgent jobs in PDT treatment.

Herein, in our lab UCN@lipid@polymersome (UCN@lipid@PS) nanodumbbell was designed and synthesized. The UCN@lipid@PS nanodumbbell is particularly attractive because it not only can improve the stability of UCNs and photosensitizers, but also greatly increase the photosensitizers loading amount. The hydrophobic UCNs nanocrystals were transferred into water by an amphiphilic lipid named octadecyl-quaternized poly-glutamicacid (OQPGA) synthesized in our lab as reported (Fig. S1, ESI†) [28], to form UCN@lipid. Polystyrene-block-poly(acrylic acid) (PS-PAA, PS: PAA 70,000:13,000) was selected to form the big polymeric polymersome container to carry photosensitizers, avoiding just adsorbing them onto the surface or loading into opened holes of the nanocarriers. The UCN@lipid and photosensitizers were both coated into polymersome shell to form UCN@lipid@PS nanodumbbell. Since the exterior of the polymersome possesses many carboxyl functional groups available for functionalization, the decorating with functional molecules such as RGD peptide not only can enhance the nanoparticle target specificity to cancer cells, but also improve its physiological stability. Thus, the nanoparticle has three parts: (1) The upconverting nanocrystal core works as energy transducer, which can transfer the energy from NIR to UV or visible light for activating the photosensitizer to produce ROS; (2) The lipid middle layer works for stabilizing the UCNs and preventing them aggregation; (3) The polymersome shell can load the photosensitizers and prevent leaking. The RGD peptide decoration on the polymersome outside shell can help the whole system to target the lesions.

We compared the drug loading efficiency and drug encapsulation efficiency between the amphiphilic lipid OQPGA and PS-PAA. Experiment results demonstrate that compared with the UCN@lipid nanoparticle, with the help of the macromolecule PS-PAA, the whole drug delivery system can load much higher amount of photosensitizers. The properties, such as structure, size distribution, morphology, ROS production test, cell uptake test and PDT treatment effect *in vitro* were also evaluated. Our data suggest that the UCN@lipid@PS nanodumbbell could serve as a useful nanoplatform for PDT treatment in deep-cancer therapy based on the upconverting mechanism.

2. Experimental section

2.1. Materials

Ytterbium (III) chloride hexahydrate ($YbCl_3{\cdot}6H_2O$, 99.99%), Yttrium (III) chloride hexahydrate ($YCl_3{\cdot}6H_2O$, 99.99%), Erbium (III) chloride hexahydrate ($ErCl_3{\cdot}6H_2O$,99.99%), octadecene (ODE, 90%), zinc (II) phthalocyanine (ZnPc, 97%), oleic acid (OA, 90%), ammonium fluoride (NH_4F, 99.99%), polystyrene-block-poly (acrylic acid) (PS-PAA, PS:PAA 70,000:13,000) and tetrahydrofuran (THF, 99.0%) were obtained from Sigma-Aldrich. RGD peptide (arginine–glycine–aspartic acid sequence) was purchased from Shanghai GL Biochem Ltd. Amphiphilic lipid named octadecyl-quaternized poly-glutamicacid (OQPGA) was synthesized following a previously protocol in our lab.

2.2. Synthesis of $NaYF_4$ (Y:Yb:Er = 78%:20%:2%) upconverting nanocrystals

$NaYF_4$:Yb:Er nanocrystals were synthesized using thermal decomposition method reported in our lab [29]. $YbCl_3{\cdot}6H_2O$ (20 μL, 1 M), $YCl_3{\cdot}6H_2O$ (780 μL, 1 M), and $ErCl_3{\cdot}6H_2O$ (20 μL, 0.1 M) in deionized water were mixed with 6 mL oleic acid and 15 mL 1-octadecene in a 100 mL flask under continuous stirring. After the mixture was heated to 120 °C to get rid of water under argon atmosphere, the solution was cooled down to room temperature and the mixture of 4 mmol NH_4F, 2.5 mmol NaOH and 10 mL methanol was added. Then, the solution was slowly heated to remove the methanol, degassed at 100 °C for 30 min, heated to 300 °C and kept under N_2 flux for 1 h. When cooled down to room temperature, the UCNs were precipitated by adding the same volume of ethanol and purified by centrifugation. Finally, the final product was re-dispersed in cyclohexane.

2.3. Synthesis of UCN@lipid nanoparticles

The UCN@lipid nanoparticles were prepared by the reverse phase evaporation method. Briefly, lipid molecule OQPGA (12 mg) was dissolved in 2 mL of water at room temperature. Then, 1 mL stock solution of UCNs (2 mg/mL in dichloromethane) was mixed with OQPGA solution to form emulsion under sonication. Later on, the solvents were evaporated on a rotary evaporator to form a gel-like, highly concentrated suspension. After centrifugation to remove the free OQPGA, the UCN@lipid nanoparticles suspensions were kept at 4 °C. ZnPc loaded UCN@lipid was set as a