Colloids and Surfaces B: Biointerfaces 161 (2018) 442–448

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full Length Article

pH-Sensitive mesoporous silica nanoparticles for chemo-photodynamic combination therapy

Tingsheng Yan[a], Jinju Cheng[b], Zongjun Liu[a], Feng Cheng[a], Xinjing Wei[a], Jinmei He[a,*]

[a] MIIT Key Laboratory of Critical Materials Technology for New Energy Conversion and Storage, State Key Laboratory of Urban Water Resource and Environment, School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, Heilongjiang 150010, China
[b] Food Science College, Northeast Agricultural University, Harbin, Heilongjiang 150030, China

ARTICLE INFO

Article history:
Received 13 July 2017
Received in revised form 21 October 2017
Accepted 1 November 2017

Keywords:
pH-Sensitive
Chemo-photodynamic therapy
Mesoporous silica nanoparticle
Rose bengal
Doxorubicin

ABSTRACT

In order to optimize the chemotherapeutic efficacy of doxorubicin (DOX) and improve the photodynamic therapeutic effectiveness of rose bengal (RB), a mesoporous silica nanoparticle system was designed as the carrier of RB and DOX for chemo-photodynamic combination therapy. A pH-sensitive strategy has been exploited to enhance the delivery efficiency. Our results suggested that the production of singlet oxygen was independent of the release of RB while strongly influenced by the external DOX layer. This method showed several benefits, including accelerating cellular uptake of the payloads and enabling chemo-photodynamic combination therapy for synergistic cancer treatment. Our study provides a new way for co-delivery of chemotherapy agents and photosensitizers.

1. Introduction

Photodynamic therapy (PDT) has attracted broad attention as a non-invasive therapeutic technique. In the PDT treatment, photosensitizers can transfer the photon energy to produce reactive oxygen species (ROS) under specific wavelengths of light [1,2]. Rose bengal (RB) is one kind of photosensitizer which possessed a high singlet oxygen quantum yield under 532 nm light irradiation [3]. However, the drawbacks of poor intracellular uptake ability limited its application in solid tumor treatment. Nanoparticle vehicles have been used to deliver RB into cancer cells [4]. Among the various drugs used in chemotherapy, doxorubicin (DOX) is a well-known chemotherapy agent, which can interact with DNA by intercalation and inhibition of macromolecular biosynthesis. DOX shows an obvious inhibiting effect on many tumors, including breast cancer, brain cancer and prostate cancer. However, DOX may cause drug resistance of the cancer cells and life-threatening cytotoxicity after repeated sessions of chemotherapy [5,6]. New strategies are needed for better treatment of cancers. Targeted drug delivery systems (DDS) and combined treatments were often used to minimize these problems [7–10].

Combining chemotherapy and PDT treatments in one system has been demonstrated to promote the therapeutic effect [11,12]. The excellent DDS might be the key factor for chemo-photodynamic combination therapy. Among various DDSs, mesoporous silica nanoparticles (MSNs) with different sizes, shapes, and functionalities were demonstrated to be one of the top priority candidates for drug delivery [13–16]. Appropriate size can help to optimize the penetration and local accumulation in tumor tissue through the widely known enhanced permeation and retention (EPR) effect. Also, MSNs are easily functionalized to enable stimuli-responsive release of payloads, such as reducing environment, pH and temperature responsive [17–19]. The controlled release capacity of MSNs can be enhanced by the modification with stimuli-responsive gatekeepers, such as polymers, dendrimers and polysaccharides [20,21].

Therefore, in order to incorporate the benefits of chemotherapy and PDT treatments, we focused on the developing of a mesoporous silica nanoparticle system as the carrier of RB and DOX for chemo-photodynamic combination therapy (Fig. 1). In this work, the amino-functionalized MSNs were firstly modified with a pH sensitive linker 4-Hydrazinobenzoic acid (HBA). We found that RB could be effectively loaded in the nanoparticles with a high loading capacity. DOX was then attached to the linker by hydrazone bond, acting as both chemotherapy reagent and protection cover to prevent the leakage of RB. This method showed several benefits, including accelerating cellular uptake of the payloads and enabling chemo-photodynamic combination therapy for synergistic cancer treatment. Our study provide a new way for co-delivery of chemotherapy agents and photosensitizers.

* Corresponding author.
E-mail address: hejinmei@hit.edu.cn (J. He).

https://doi.org/10.1016/j.colsurfb.2017.11.006
0927-7765/

2. Methods and materials

2.1. Materials

DOX (purity >99%) and RB (purity >95%) were purchased from Aladdin Reagent Database Inc. 4-(2-N-Boc-hydrazino) benzoic acid (HBA-Boc) was purchased from Chem-Impex Inc. (USA). (Shanghai, China). Tetraorthoethylsilicate (TEOS), cetyltrimethylammonium bromide (CTAB), 3-aminopropyl-trimehoxy silane (APTMS), Hoechst 3334, luorescein isothiocyanate (FITC), 1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS) and trifluoroacetic acid (TFA) were purchased from Sigma (USA). Singlet oxygen sensor green were purchased from Thermo Fisher Scientific (USA). Human breast cancer cell line MCF-7 was provided by Sanggon biotech Co. (China).

2.2. Synthesis of amino-functionalized MSN

MSNs were synthesized according to published procedures [22]. CTAB (500 mg, 1.4 mmol) was dissolved in H_2O (240 mL) and NaOH (1.75 mL, 2 M). The mixture was adjusted to 80 °C for 30 min. Then, 2.5 mL TEOS (1.2 mmol) was added dropwise into the solution under stirring vigorously for 2 h. The crude product was filtered, washed and dried. The surfactant template (CTAB) was removed by refluxing in a methanolic solution (HCl (37%): methanol = 1:100 (v/v)) for 8 h. The surfactant free MSNs were refluxed in 50.0 mL of anhydrous toluene with 1.00 mL of APTMS for 24 h to yield the amino-functionalized MSNs (MSNs-APTS).

2.3. Synthesis of MSNs-AH-Boc conjugates

MSNs-APTS (100 mg) was suspended with HBA-Boc (50 mg) in a pH 7.4 PBS solution (100 mL), then EDC·HCl (100 mg) and NHS (40 mg) were added. The mixture was allowed to react at room temperature for 24 h. Then, anhydrous ethanol was used to remove the unreacted reagents. The resulting solid products were dried in a vacuum.

2.4. Loading of RB and DOX

The loading of RB into MSNs-AH-Boc nanocomposite was accomplished by mixing certain amount of RB in MSNs-AH-Boc aqueous solution (0.2 mg/mL, pH 7.4). The mixture was stirred at 4 °C in the dark overnight to form MSNs-AH-Boc@RB. The free RB was removed by centrifuging and washing with deionized water.

For the loading of DOX, TFA was used to deprotect the MSNs-AH-Boc@RB. 200 mg of MSNs-AH-Boc@RB was dissolved in 60 mL DCM. 4 mL TFA was added, stirring occasionally in ice bath for 30 min, to remove the Boc group. MSNs-AH@RB purified by centrifuging and washing with methanol. MSNs-AH@RB (100 mg) and a certain amount of DOX were suspended in 25 mL DCM, stirring at room temperature in the dark for 24 h, to form MSNs-AH-DOX@RB.

The drug loading content (DL) and drug encapsulation efficiency (EE) of DOX and RB were calculated by the following equation:

$$\text{DL}\ (\%) = \frac{\text{weight of loaded drug}}{\text{weight of drug loaded nanoparticles}} \times 100\%$$

$$\text{EE}\ (\%) = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100\%$$

2.5. Characterization

The size and morphology of the MSNs, MSNs-AH@RB and MSNs-AH-DOX@RB were characterized by TEM on a JEM-2100F (JEOL, Japan) and dynamic light scattering (DLS; Malvern, UK). The structure of the samples was measured by a UV–vis spectrophotometer (Model UV-1603), Fourier transform infrared spectrophotometric (FTIR; Bruker Tensor 27 spectrometer) and the multipoint BET (Brunauer-Emmett-Teller) method from the N_2 adsorption data.

2.6. In vitro DOX and RB release

The in vitro release of DOX and RB was evaluated by a dialysis method. 1 mg of MSNs-AH-DOX@RB was dissolved in 2 mL PBS and subsequently transferred into a dialysis bag (molecular cut-off 3500 Da). The mixture was incubated in 80 mL PBS under different pH values (pH = 5.5 and 7.4) with continuous shaking (100 rpm) at 37 °C. At scheduled time points (0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 h), 1 mL of the release medium was taken for testing, and then replenished with equal volume of fresh PBS. The accumulative release of DOX and RB was detected by UV–vis spectra at 480 nm for DOX and at 560 nm for RB. The cumulative release amount (*Er*) of DOX or RB was calculated by the following equation:

$$E_r = \frac{V_e \sum_1^{n-1} C_i + V_0 C_n}{m_D}$$

Where as, V_0 is the whole volume (V_0 = 82 mL), V_e is the volume of the replaced medium (V_e = 1 mL), C_n is the concentration of DOX or RB at scheduled time intervals and m_D is the amount of DOX or RB in MSNs-AH-DOX@RB.

2.7. Determination of singlet oxygen

A highly sensitive singlet oxygen sensor green (SOSG) was employed to detect the singlet oxygen levels. Free RB, MSNs-AH@RB and MSNs-AH-DOX@RB were dissolved in 3 mL of water mixed with 2.5 mM SOSG, and then irradiated by a 532-nm laser (0.5 W/cm^2) for 10 min. The concentration of singlet oxygen was detected by measuring the fluorescence intensity of SOSG at 494 nm.

2.8. In vitro cell experiments

Human breast cancer cell line MCF-7 was cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin/streptomycin at 37 °C. For confocal fluorescence imaging (CLSM), MCF-7 cells were cultured in 96-well plates and allowed to adhere for 24 h. Then, the medium was replaced with a fresh medium containing free RB, free DOX, FITC-labeled MSNs, FITC-labeled MSNs-AH@RB and FITC-labeled MSNs-AH-DOX@RB. After 4 h, the cells were washed three times with PBS and then fixed with 2.5% glutaraldehyde at 4 °C for 30 min. The cell nuclei were stained with Hoechst 33342 at room temperature for 15 min. The intracellular distribution was observed by confocal laser scanning microscope (CLSM, LSM 510Metanlo, Zeiss).

For flow cytometry (FCM) measurements, MCF-7 cells (1.0×10^5 cells per well in 6-well plates) were cultured in medium for 24 h, and co-incubated with free RB, free DOX, FITC-labeled MSNs, FITC-labeled MSNs-AH@RB and FITC-labeled MSNs-AH-DOX@RB for 4 h. After washed with PBS, the cells were trypsinized and resuspended with 0.5 mL of PBS for flow cytometry measurements.

MCF-7 cells (5×10^3 cells per well) were seeded into 96-well cell plate. After 24 h incubation, a serial concentrations of free RB (2.5–25,000 ng/mL), free DOX (2.5–25,000 ng/mL), MSNs (2.5–25,000 ng/mL), MSNs-AH@RB (equivalent RB 2.5–25,000 ng/mL), MSNs-AH-DOX (equivalent DOX 2.5–25,000 ng/mL) and MSNs-AH-DOX@RB (equivalent DOX 2.5–25,000 ng/mL). After incubation in the dark for 12 h, one group was kept in the dark to test the dark toxicity, and the other