Sensors and Actuators B 257 (2018) 105–115

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Research paper

Fabrication of degradable lemon-like porous silica nanospheres for pH/redox-responsive drug release

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ARTICLE INFO

Article history:
Received 12 August 2017
Received in revised form 16 October 2017
Accepted 17 October 2017
Available online 28 October 2017

Keywords:
Porous silica
pH/redox-responsive
Drug delivery
Degradation

ABSTRACT

In this work, pH/redox-responsive silica-based nanocarriers were constructed for drug delivery. Lemon-like porous silica (L-pSiO_2) sample presents uniform sphere with size of 160–180 nm and processes thiol-functionalized center-radial inner pores and amine-functionalized shell. Cystine (Cys) molecules were conjugated on the surface of L-pSiO_2 to seal the drug-loaded pores. To minimize the premature release, ZnO quantum dots (QDs) were further used to seal the nanopores of pSiO_2 NSs, which could act as gates and fluorescence probes. Both in *vitro* cellular cytotoxicity and hemolysis assay demonstrated that the L-pSiO_2/Cys nanospheres (NSs) were highly biocompatible. The L-pSiO_2/Cys NSs have high specific surface area (201 $m^2\ g^{-1}$) and large pore volume (0.426 $cm^3\ g^{-1}$) and are suitable to utilize as drug carriers. The DOX-loaded L-pSiO_2/Cys NSs displayed more efficient cytotoxic to HepG2 cells than free DOX. Both L-pSiO_2/Cys and L-pSiO_2/Cys/ZnO carriers displayed low premature and pH/redox-responsive release. The DOX release from L-pSiO_2/Cys followed the first-order kinetics model with high correlation coefficient. Interestingly, the L-pSiO_2/Cys carrier displayed GSH-responsive degrading the inner core and pH-responsive dissolving silica shell at physiological environment.

1. Introduction

Chemotherapy is the most widely used frontline strategy for cancer therapy in clinic. However, the direct administration of conventional anticancer drugs results in limited efficacy and exerts serious adverse side effects on the patient. Nanoparticle-based drug delivery system provides emerging and promising opportunities to improve cancer therapy [1,2]. An idea delivery system encapsulating drugs should accumulate preferentially in tumors because of leaky tumor vasculature and as reservoirs provide sustained drug release. To date, various nanocarriers have been designed for the administration of anticancer drugs, due to their capabilities of enhancing drug solubility, improving pharmacokinetics and transporting them to target sites [3–7]. Among drug carriers explored, mesoporous silica carriers have attracted tremendous attention due to their high surface area, tunable pore size, excellent biocompatibility and readily functionalized surface [8,9]. The large pore volume could accommodate drug molecules within the pore channels with a high payload and the easily modified surface could tune the drug-support interaction and pharmacokinetics. Importantly, the pores could be gated with various valves such as nanoparticles [10], polymer [11,12], macromolecules [13], DNA, or proteins [14,15], which were designed to trigger the release of the entrapped drug in the presence of external or internal stimuli including light [16], pH [13,17], thermos [18], redox [15,17], and enzyme [19].

For biomedical application, drug carriers should be capable of "zero" premature release and stimuli-responsive release of loaded drugs at the target sites. This performance may be achieved using responsive carriers and great efforts have been made on the responsive silica carriers for on-demand intracellular anticancer drug release in the past decade [20]. In particular, multi-responsive carriers hold greater potential for controlling the drug release process and improving therapeutic performance [14–17,20]. Additionally, the elimination of the silica nanocarriers from the biologic system after their carrying out the therapeutic functions is important aspect to be considered [21,22]. Although silica was recognized as safe by the US Food and Drug Administration, the accumulation of silica nanoparticles within an organism would lead to increased toxicity at system level due to their poor degradability [23]. For instance, silica nanoparticles have been found to accumulate in the liver, bladder, kidneys, spleen, and lungs, which may cause severe problems and would do harm to living organs [24]. Therefore, it is of great significance and highly desirable to fabricate degradable silica drug carriers.

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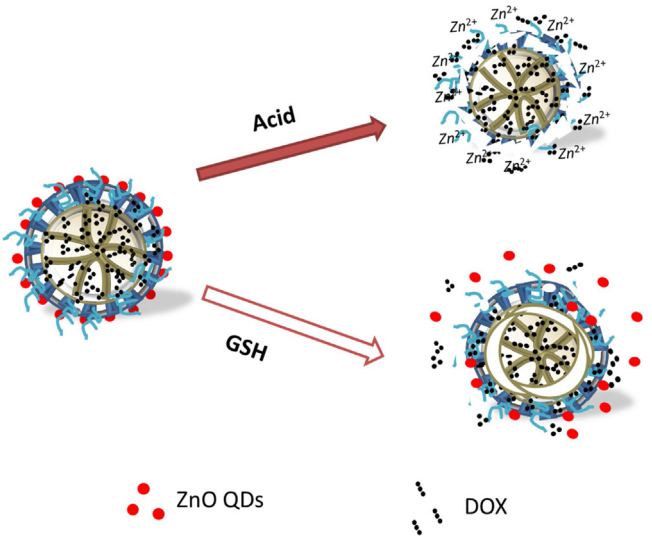
https://doi.org/10.1016/j.snb.2017.10.104
0925-4005/



Scheme 1. The release and degradation of L-pSiO_2/Cys/ZnO NSs.

Here, a novel degradable and pH/redox dual-responsive carrier based on lemon-like porous silica (L-pSiO_2) nanospheres (NSs) has been prepared for on-demand drug release. The L-pSiO_2 NSs possess thiol-functionalized inner pores and amine-functionalized outer layer. Cystine (Cys) was grafting onto the surface of L-pSiO_2 NSs through amide linkage to construct the responsive shell. Doxorubicin (DOX) as a model drug was used to assess the loading and release behavior of this carrier. After incorporating DOX into the pore channels, the inside thiols would form disulfide bonds under the environment of oxidation, which would be conducive to reduce the diffusion of the loading DOX and stabilize the DOX. It is well known that the pH value in tumor tissues is lower than that of normal tissue, and the intracellular concentration of glutathione (GSH) is about 2−3 orders more than that in extracellular plasma. The Cys layer not only acts as'gate-keeper'to alter the release of transported drug in response to the intracellular pH change and reducing microenvironment, but also improves its biocompatibility. In addition, acid-decomposable ZnO quantum dots (QDs) were used to block the drug-loaded pores of the L-pSiO_2 carriers to further reduce the premature release. The DOX-loaded L-pSiO_2 carrier would display pH/redox dual-stimuli release behavior due to the cleaving the disulfide bond cleaved by GSH, the dissolution of ZnO QDs and the conformational change of Cys in acid environment (Scheme 1). Meanwhile, we also studied the pH/GSH-responsive degradation of silica carriers. In addition, the biocompatibility of the L-pSiO_2 carrier was also evaluated by hemolysis and 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium- bromide (MTT) assay.

2. Materials and methods

2.1. Reagents

Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), triethanolamine (TEA), N,N-dimethylformamide (DMF) and anhydrous ethanol were purchased from Tianjin Kermel Chemical Reagent (Tianjin, China). Disodium hydrogen phosphate (Na_2HPO_4), monopotassium phosphate (KH_2PO_4), potassium chloride and sodium citrate were purchased from Deen Reagent Co. (Tianjin, China). 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimidehydrochloride (EDC), Reduced glutathione (GSH), N-hydroxysuccinimide (NHS), L-cystamine dihydrochloride (Cys), 3-mercaptopropyltrimethoxysilane (MPS) and 3-aminopropyltriethoxysilane (APTES) were purchased from Aladdin. RPMI 1640 culture medium, 3-(4,5-dimethylthia-zol)−2,5-diphenyltetrazoliumbromide (MTT), trypsin, fetal calf serum (FCS) and dimethyl sulfoxide (DMSO) were purchased from Gibco (Grand Island, NY, USA). Doxorubicin hydrochloride (DOX) was purchased from Solarbio (Beijing, China). All chemicals were of analytical reagent grade and used as received without any further purification. Distilled water was used throughout the experiment.

2.2. Preparation and modification of L- pSiO_2 NSs

The L-pSiO_2 sample was synthesized by two-step method. First, 0.142 g of CTAB was dissolved in 65 mL of water-ethanol solution (5/1) to form emulsion under magnetic stirring. Then, 4.4 mL of TEA was added to tune the pH value to 10. Subsequently, 3 mL of the mixed solution of TEOS and MPS (10/1) was added to the above solution and heated to 60 °C. After reaction for 5 h, thiol functionalized porous silica NSs were obtained. Final, the mixture of TEOS (2.8 mL) and APTES (0.28 mL) was added to the above porous silica solution and reacted for another 5 h to get L-pSiO_2 sample with thiol functionalized core and amine functionalized shell. The CTAB surfactant was removed by hot ethanol-HCl solution.

The L-Cys molecules were conjugated onto the surface of L-pSiO_2 NSs according to protocols described in our previous work with modification [25]. In brief, 180 mg of EDC and 138 mg of NHS were dissolved in 36 mL of DMSO/DMF (1:3) solution and stirring for 30 min at 25 °C. Then, 110 mg of L-Cys was added to above solution to active the carboxyl group and the solution was stirred at 25 °C for 24 h. Subsequently, 55 mg of L-pSiO_2 was added to the activated L-Cys solution. After the reaction for 24 h at 25 °C, the mixture was centrifuged, washed with ethanol and water several times and dried in a vacuum oven at 25 °C for 24 h to obtain L-pSiO_2/Cys.

2.3. Drug loading, ZnO capping and in vitro release

DOX was chosen as a model drug to assess the release behavior of L-pSiO_2/Cys. Typically, 90 mg of L-pSiO_2/Cys and 30 mg of DOX were dispersed in 12 mL of deionized water. Then, the mixture was shaken at 25 °C for 24 h in darkness to reach the equilibrium state. During this process, 2 mL of mixed solution was taken out at 4, 6, 8, 12, 18, 24 h respectively to search for the best equilibrium state time. The DOX-loaded L-pSiO_2/Cys NSs were then collected via centrifugation at 8000 rpm for 10 min, and washed several times with deionized water. The supernatant and washing solution were collected and the residual DOX amount was determined by UV–vis spectroscopy.

For capping ZnO QDs, 6 mL of ZnO QDs PBS solution (10 mg/mL) was added to 10 mL of DOX-loaded L-pSiO_2/Cys solution (10 mg/mL). Here, ZnO QDs were synthesized using reported method [24]. The resulting suspension was shaken for another 8 h at 25 °C to obtain DOX-loaded L-pSiO_2/Cys/ZnO sample followed by filtration and washing thoroughly with PBS buffer solution.

In *vitro* drug release experiment, 5 mg of DOX-loaded L-pSiO_2/Cys powder was dispersed into 5.0 mL of buffer solution and sealed a dialysis bag (cut-off molecular weight 8000 Da). The sealed dialysis bag was submerged into 50 mL of phosphate buffer saline (PBS) (pH 7.4) and shaken at 37 °C. At predetermined time intervals, 5 mL of released solution was taken out and an equal volume of fresh medium was added to keep the volume constant. To investigate the stimuli-responsive controlled release profiles of the drug, the above-mentioned operations were performed, and then the pH of the buffer solution was changed from 7.4 to 5.0, and additionally to 3.0, or GSH solutions with different concentrations were added to the buffer solution (pH 7.4). The released amount of DOX was measured by a UV–vis spectrophotometer at 481 nm. The release experiments were repeated three times.