Journal of Drug Delivery Science and Technology 37 (2017) 38–45

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Fabrication of functionalized porous silica nanoparticles and their controlled release behavior

Xu Zhang [a], Yanbao Zhao [a, *], Songqiang Xie [b], Lei Sun [a]

[a] Engineering Research Center for Nanomaterials, Henan University, Kaifeng 475004, China
[b] Pharmaceutical College of Henan University, Henan University, Kaifeng 475004, China

ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form
18 November 2016
Accepted 20 November 2016
Available online 22 November 2016

Keywords:
Porous silica
Nanocarriers
Hemolytic activity
Redox-responsive
Drug delivery

ABSTRACT

Thiol and amine difunctionalized porous silica ($pSiO_2$-SH/NH_2) nanoparticles (NPs) were prepared by condensation. The obtained $pSiO_2$-SH/NH_2 NPs present uniform spheres with size of 55 nm. Folic acid (FA) as a tumor targeting agent was conjugated on the surface of $pSiO_2$ NPs by amide linkage ($pSiO_2$-SH/FA NPs), and captopril (Cap) as test drug was used to evaluate the releasing behavior. Results indicated that Cap is easily encapsulated into the pores of $pSiO_2$-SH/FA NPs, which can further react with the inner thiols to form disulfide bonds. The Cap release from $pSiO_2$-Cap/FA nanocarriers can be controlled by dithiothreitol (DTT) or reduced glutathione (GSH). The $pSiO_2$-SH/FA nanocarriers showed low cytotoxicity and redox-responsive drug release.

1. Introduction

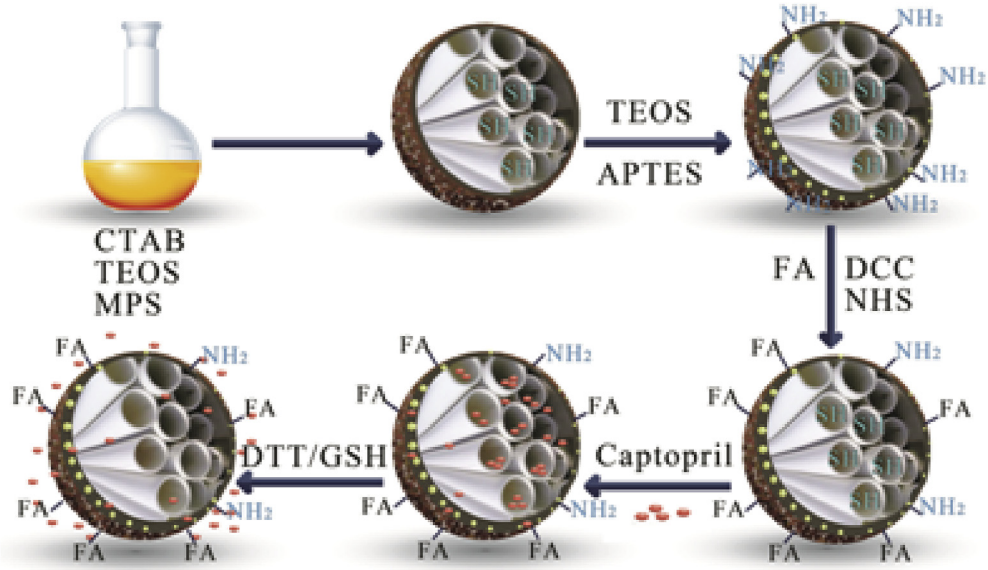
In the past years, nanoscale drug delivery systems (DDSs) have drawn tremendous attention for their unique properties in cancer therapies [1–3]. Drug encapsulated within nanocarriers is expected to improve therapeutic efficacy, prolong circulation and reduce side effect of drug. To date, a variety of nanoplatforms, such as polymeric micelles [4], hydrogels [5], liposomes [6] and silica nanoparticles (NPs) [7] have been employed as vehicles for drug delivery. Among these nanocarriers, mesoporous silica (MS) NPs have been extensively investigated as an excellent drug carrier due to their excellent biocompatibility, high stability, large surface area and the ease of surface modification [8]. Especially, MS NPs are capable of loading and protecting a wide range of therapeutic biomolecules within the pores [9,10].

Although the utilization of MS NPs has validated great potential in delivering drugs, this technique is facing tremendous challenge in many applications [11,12]. In particular, pure MS carrier in which drug molecules were simply physically adsorbed in the channels displays a premature and burst release, followed by a slow diffusion behavior, which is not in favor of achieving optimal local therapeutic efficacy. To improve the accumulation of the carriers at the tumor site, their surfaces are usually modified with targeting agents [13,14]. Folic acid (FA) possesses high selectivity and binding affinity to the folate receptor (FR) over-expressed in many cancer cells [15], thus FA modification facilitates the efficiency of cell uptake by cancer cells due to receptor-mediated endocytosis.

In this paper, we designed and prepared thiol and amine difunctionalized porous silica ($pSiO_2$-SH/NH_2) NPs by two-step condensation, possessing high specific surface area and large pores for drug loading (Scheme 1). The inside thiols can anchor drug containing thiol by disulfide bond and the surface amine can conjugate FA through amide linkage, which would render the $pSiO_2$ NPs targeting feature. Captopril (Cap), as a model drug, was introduced into the pores of SiO_2-SH/FA NPs. The disulfide bond is stable in blood circulation, and it can only be cleaved by reduced glutathione (GSH) or other thiol compounds with certain concentrations. It is well documented that GSH is present in the intracellular matrix of cancer cells at levels two or three orders of magnitude higher than that found in extracellular environments [16]. The release behavior of Cap from $pSiO_2$-Cap/FA could be regulated by dithiothreitol (DTT) or GSH. In addition, the biocompatibility of the nanocarriers was evaluated by hemolysis and 3-(4,5-dimethylthiazol)- 2,5-diphenyltetrazoliumbromide (MTT) assay.

* Corresponding author.
E-mail address: yanbaozhao@126.com (Y. Zhao).

http://dx.doi.org/10.1016/j.jddst.2016.11.005
1773-2247/



Scheme 1. Synthesis protocol for $pSiO_2$-Cap/FA nanoparticles and picture showing release of drug.

2. Experimental section

2.1. Materials

Tetraethyl orthosilicate (TEOS, 98%), cetyltrimethylammonium bromide (CTAB), triethanolamine (TEA), *N*,*N*-Dimethylformamide (DMF), sodium acetate (NaAc), dimethyl sulfoxide (DMSO), hydrochloric acid (HCl) and ethanol were purchased from Tianjin Kermel Chemical Reagent (Tianjin, China). *N*-hydroxysuccinimide (NHS), *N*,*N*′-Dicyclohexylcarbodiimide (DCC), 3-aminopropyltriethoxysilane (APTES), folic acid (FA) and 3-mercaptopropyltrimethoxysilane (MPS) were obtained from Aladdin Chemistry Co., Ltd (Beijing, China). Captopril (Cap) and anhydrous ethanol (C_2H_5OH) received from Anhuiante Food Co., Ltd (Anhui China). Reduced glutathione (GSH), dithiothreitol (DTT) and 3-(4,5-dimethylthia-zol)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 culture medium and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY, USA). Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich (Missouri, America). All reagents were used without further purification.

2.2. Characterization

The functionalized porous silica NPs were characterized by transmission electron microscopy (TEM, JEM-2100 JEOL) and thermogravimetric analysis (TG, EXSTAR 6000). The optical properties were carried out using UV–visible (PE-Lambda 35, America) and Fourier-transform infrared spectroscopy (FTIR) (Bruker, Germany). The specific surface area (SSA) and pore volume were measured by the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods (Quanta chrome, Autsorb-1MP). Raman spectra were recorded by laser micro-Raman spectrometer (Renishaw, England). Zeta potential measurements were determined with the Zetasizer Nano Z (Malvern, UK). The absorbance of hemoglobin and living cell was measured using microplate reader (Bio Tek Instruments, USA). Fluorescent images were taken by Leica DMIL-LED (Leica Microsystems CMS GmbH, Germany).

2.3. Synthesis of $pSiO_2$-SH/NH_2 NPs and FA conjugation

In a typical synthesis, CTAB (0.142 g) was dissolved in 110 mL of water/ethanol solution (10/1), and then 4.4 mL of TEA was added to tune the pH value. After stirring for 10 min, the solution was heated to 60 °C, followed by dropwise addition of the mixed solution of TEOS (5.6 mL) and MPS (0.56 mL). After reaction for 5 h, thiol functionalized porous silica ($pSiO_2$-SH) NPs were obtained. Subsequently, a mixture of TEOS (1.4 mL) and APTES (0.28) mL was added to above solution. The mixture was stirred for another 5 h, the resultant product was centrifuged and washed to obtain thiol and amine difunctionalized porous silica ($pSiO_2$-SH/NH_2) precursor. Finally, the obtained precursor was dissolved in the mixed solution of HCl/ethanol (1/10) to remove CTAB and obtained the $pSiO_2$-SH/NH_2.

For FA conjugation, first, 180 mg of DCC and 150 mg of NHS were dissolved in 36 mL of DMSO/DMF (1:3) solution with stirring for 1 h. Next, 300 mg of FA was added into above solution to activate the carboxyl groups of FA for 24 h. Subsequently, the $pSiO_2$-SH/NH_2 NPs (370 mg) were added to the activated FA solution and allowed to react for 12 h at room temperature. After the reaction, the mixture was centrifuged and washed to obtain $pSiO_2$-SH/FA.

2.4. Cell culture, cell viability and hemolysis assay

Human SMMC-7721 hepatoma cells and MCF-7 breast cancer cells were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). Both cell lines were cultured in RPMI-1640 medium supplemented with fetal bovine serum (10%) and penicillin/streptomycin (100 mg/mL) in a humidified 5% CO_2 atmosphere at 37 °C.

The in vitro cell viability was measured using an MTT assay. First SMMC-7721 and MCF-7 cells were seeded respectively onto 96-well plates (5×10^3 cells per well) and incubated for 24 h. Then $pSiO_2$-SH/NH_2, $pSiO_2$-SH/FA and $pSiO_2$-Cap/FA NPs were introduced to the wells with a predetermined concentration in the culture medium. After incubation for 24 h, MTT (50 μL, 1 mg mL^{-1}) solution was added, and the cells were incubated for another 4 h. Upon removing MTT solution, the purple formazan crystals were dissolved with 100 μL DMSO and the absorbance was recorded at