Sensors and Actuators B 234 (2016) 21–26

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Silver nanoparticle-gated fluorescence porous silica nanospheres for glutathione-responsive drug delivery

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 9 April 2016
Accepted 24 April 2016
Available online 26 April 2016

Keywords:
Porous silica
Glutathione-responsive
Drug delivery

ABSTRACT

To reduce the premature release of encapsulated drug, glutathione-responsive fluorescent porous silica ($pSiO_2$) nanocarriers were developed by encapsulating Ag nanoclusters (Ag NCs) and capped with Ag nanoparticles (Ag NPs). Ag NCs/porous silica (Ag NCs/$pSiO_2$) nanospheres (NSs) have an average diameter of 45 nm and large specific surface with Brunauer-Emmett-Teller (BET) area of 453 $m^2\ g^{-1}$. After loading *N*-(2-Mercaptopropionyl) glycine (MPG), the absorbed Ag^+ ions were in situ reduced to Ag NPs and capped on the outer surfaces of Ag NCs/$pSiO_2$ NSs as gatekeepers to regulate the release of drugs. In the absence of glutathione (GSH), the release rate is below 8% within 12 h; while in the presence of 2 mM GSH, the amount released reaches 70% within 8 h. The Ag NPs-gated Ag NCs/$pSiO_2$ NSs displayed excellent GSH-responsive release. The incorporated Ag NCs presented novel drug-dependent fluorescence, which could be used to trace the drug release. In addition, the Ag NPs-gated Ag NCs/$pSiO_2$ NSs displayed excellent antibacterial activity against both Gram-positive and Gram-negative bacteria.

1. Introduction

There has been an increasing interest in developing drug delivery systems (DDS) in the past few years, and the encapsulation of drugs within nanocarriers is expected to depress the toxic side effects and simultaneously enhance therapeutic efficacy [1,2]. To date, various nanocarriers, including liposomes, polymers, hydrogels and inorganic nanoparticles (NPs), have been developed to achieve a controllable delivery of drugs [3–5]. Among these nanocarriers, porous silica nanospheres ($pSiO_2$ NSs) have been extensively investigated as an excellent drug carrier due to their high stability, good biocompatibility, large surface area, tunable pore sizes and the ease of surface modification [6,7]. In fact, the passive-diffusion release of loaded drug molecules from silica nanocarriers is not specific to cells, tissues or organs. To improve drug release efficiency, stimuli-responsive "smart" DDS has been developed [8,9]. A variety of gatekeepers have been fabricated and allow the release of loaded drug molecules into a specific environment in response to external or internal stimuli such as pH, redox, competitive binding, light and enzymes [10,11].

The other issue is to trace the release of drugs from the nanocarriers. However, the process of drug delivery cannot be monitored in vivo using the pure silica materials because of the lack of detectable signals. Fluorescent dyes are commonly applied to label carriers to obtain detectable fluorescent signals. As we all know, with the increase of temperature, the deactivation probability of fluorescent dyes becomes increase, results in the decrease of fluorescence intensity [12]. Metal nanoclusters (NCs) with discrete energy levels, bridging the "missing link" between atomic and nanoparticle behavior, show molecule-like electronic transitions within the conduction band and exhibit strong fluorescence [13,14]. Especially, Au and Ag NCs are promising optical probes for bioimaging and biosensing applications [15–17]. Therefore, Ag NCs incorporated $pSiO_2$ NSs would be used to monitor the responsive release of drugs.

An ideal DDS requires "zero" premature release and stimuli-responsive release of loaded drugs at the target sites. For instance, CdS nanoparticle-capped DSS would obtain low premature of loaded drugs [18]. Despite many efforts have been focused on this field, "zero" or minimal premature release of loaded drugs in DSS is still difficult to achieve [7,19]. It is well documented that glutathione (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. Generally, thiol-containing molecules have strong affinity with noble metal NPs, which can form the ultrastable metal-S bond by ligand-exchange process between the ligands on the surface of noble metal NPs and the thiol groups of thiol-containing molecules [20]. For example,

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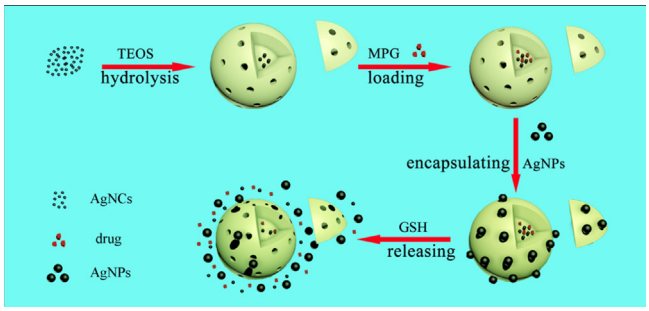
http://dx.doi.org/10.1016/j.snb.2016.04.136
0925-4005/



Scheme 1. Synthesis process of Ag NPs-gated Ag NCs/$pSiO_2$ NSs.

Ag NPs capped mesoporous silica NSs via DNA linkage exhibit GSH-respective controlled release [21]. Therefore, Ag NPs gated DSS could be governed by thiol-mediated dismantle of Ag NPs.

In this paper, an effective strategy is proposed to construct the thiol-responsive Ag NPs gated fluorescent Ag NCs/$pSiO_2$ based DDS (Scheme 1). *N*-(2-Mercaptopropionyl) glycine (MPG) as model drug was loaded into the Ag NCs/$pSiO_2$ cavities. The outer Ag NPs were in situ formed and capped on outer surface through amine linkages as the gatekeeper for high-loading and minimal premature release of drugs. The inner Ag NCs functions as a fluorescent label to monitor the drug release. The thiol-triggered release mechanism, which is different from chemical cleavage of S-S linkage connecting the cap and $pSiO_2$ NSs, is governed by thiol-mediated dismantle of Ag NPs through ligand exchange, that is, a special Ag-S interaction [22].

Additionally, Ag NPs could enhance the effect of radiation treatment in killing cancer cells [23,24]. Moreover, Ag NCs (NPs) are recognized as a promising antimicrobial and disinfectant agent and can be used to treat infectious diseases [25]. Their antibacterial efficiency was also evaluated against gram-positive *Staphylococcus aureus* (*S. aureus*) and gram-negative *Escherichia coli* (*E. coli*), respectively.

2. Materials and methods

2.1. Reagents

Reduced glutathione (GSH), 3-aminopropyltrimethoxysilane (APTMS) and *N*-(2-Mercapto- propionyl) glycine (MPG) were purchased from Aladdin (China). Cetyltrimethylammonium bromide (CTAB), isopropyl alcohol, sodium hydroxide (NaOH) and hydrazine hydrate (N_2H_4, 80 wt%) were purchased from Tianjin Kermel Chemical Reagent Co. (Tianjin, China). Tetraethyl orthosilicate (TEOS) and silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co. (China). Dehydrated alcohol was from Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). All chemicals were of analytical reagent grade and were used as received without any further purification. Distilled water was used throughout the experiment.

Nutrient broth (NB, BR) and nutrient agar (NA, BR) were received from Beijing Aoboxing Biotech Company (Beijing, China). Gram-positive *S. aureus* (ATCC 35696) and Gram-negative *E. coli* (ATCC 23282) were selected as bacterial strains (China center of industrial culture collection, China).

2.2. Synthesis of GSH-Ag NCs and Ag NCs/$pSiO_2$ NSs

10 mL of GSH solution (50 mM) and 10 mL of $AgNO_3$ solution (50 mM) were mixed to form GSH-Ag(I) complex, and then 1 M NaOH solution was added until the pH value was tuned to 7. Then, 2 mL of N_2H_4/H_2O solution (80%) as the reducing agent was added to the above solution and the pH value of the solution rose to 10. Afterward, the solution was incubated at room temperature without stirring for 11 h. At last, the yellow GSH-Ag NCs were precipitated by addition of isopropyl alcohol, and collected through centrifugation.

The prepared GSH-Ag NCs was dispersed into 5 mL of CTAB solution (0.55 mM). Then 45 mL of NaOH solution (15 mM) was added dropwise to it, followed by addition of mixed solution of TEOS (0.5 mL) and APTMS (50 uL). After reaction for 3 h at 30 °C, the porous silica encapsulated Ag NCs were collected through centrifugation, washed with ethanol and dried in vacuum at room temperature.

2.3. Drug loading, Ag NPs capping, and drug release

Ag NCs/$pSiO_2$ NSs and MPG ethanol solution were mixed together and stirred at room temperature for 12 h to reach the equilibrium state. The MPG-loaded Ag NCs/$pSiO_2$ NSs sample was collected by centrifugation to remove the excess unloaded MPG.

Then, 100 mg of the prepared MPG-loaded Ag NCs/$pSiO_2$ NSs were dispersed in a mixed solution of ethanol (100 mL) and ammonia (5 mL). Then 20 mL of $AgNO_3$ ethanol solution (0.1 M) was rapidly injected and the reaction was continued under ultrasonication for 40 min. The obtained Ag NPs-gated Ag NCs/$pSiO_2$ NSs sample was washed with water and ethanol. The precipitate was collected using centrifugation, and then dried in an oven at room temperature.

To study the premature release of MPG, 0.1 mg of MPG-loaded, Ag NPs-gated Ag NCs/$pSiO_2$ NSs sample was dispersed in 5 mL of water in a dialysis bag and the sealed dialysis bag was submerged in 50 mL of water. To investigate the GSH-responsive release profiles of the drug, the release medium changed to 50 mL GSH solution (2 mM), and the above mentioned operations were performed. The released amount of MPG was calculated through detecting the absorbance of the released medium at a certain time.

2.4. Antibacterial tests

The antibacterial activity of the Ag NPs-gated Ag NCs/$pSiO_2$ NSs was tested against *S. aureus*, and *E. coli* by determining the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The LB medium was used as a carrier to dilute the Ag NPs-gated Ag NCs/$pSiO_2$ NSs solutions to different concentrations. The MIC was defined as the lowest concentration of sample that inhibits the visible growth of bacteria. Briefly, the same amount of bacteria (20 μL, 10^8 CFU/mL) were grown overnight in serial dilutions of sample and the minimum concentration at which no bacterial growth was the MIC. Similarly, the MBC was determined by spreading 100 μL of the mixture that incubated overnight but with no observable bacteria growth from MIC analysis on the agar plate, subsequently incubating at 37 °C for 24 h. The number of survival colonies was counted to get the MBC value.

2.5. Characterization

The morphology was characterized by transmission electron microscopy (TEM, JEM-2010, Japan) at 200 kV. Absorption spectra were collected on a Lambda 950 spectrometer (PE, America) at room temperature. Fluorescence (PL) spectra were investigated using a Fluorolog-3 fluorescence spectrophometer (JY-HORIBA, France) at the excitation wavelength of 260 nm. Fourier transfer infrared (FTIR) spectra were collected on a VERTEX 70 spectrometer (Bruker, Germany). The surface area and pore size distribution were determined from N_2 adsorption/desorption isotherms measured on a full-automatic specific surface and porosity analyzer (Quadrasorb SI, America) at 77 K.