Journal of Colloid and Interface Science 358 (2011) 109–115

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Fluorescent hollow/rattle-type mesoporous Au@SiO_2 nanocapsules for drug delivery and fluorescence imaging of cancer cells

Ting-Ting Wang [a], Fang Chai [a,b], Chun-Gang Wang [a,*], Lu Li [a], Hai-Yan Liu [a], Ling-Yu Zhang [a], Zhong-Min Su [a,*], Yi Liao [c]

[a] Institute of Functional Material Chemistry, Faculty of Chemistry, Northeast Normal University, Changchun 130024, People's Republic of China
[b] College of Chemistry & Chemical Engineering, Harbin Normal University, Harbin 150025, People's Republic of China
[c] Department of Chemistry, Capital Normal University, Beijing 100048, People's Republic of China

ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 8 February 2011
Available online 15 February 2011

Keywords:
Mesoporous silica nanocapsule
Gold nanoparticles
Controlled etching
Drug release
Fluorescent imaging

ABSTRACT

Multifunctional uniform and versatile hollow and rattle-type nanocapsules composed of spindle-shaped Au nanoparticles as cores and fluorescent mesoporous silica shells with tunable optical and fluorescent properties have been developed by controlled etching Au nanorods (AuNRs) coated with mesoporous SiO_2 (AuNR@mSiO_2) via a small amount of aqua regia (volume ratio HCl/HNO_3 = 3/1) as an etching agent in a facile way. The etching process can be tracked by UV–Vis absorption and fluorescence spectroscopy and the size of cavities in the hollow/rattle-type particles can be tuned by controlling the reaction time. The dye molecules incorporated in mSiO_2 walls enabled the nanocapsules to be utilized as a fluorescent imaging agent in cancer cell imaging. Furthermore, such hollow/rattle-structured nanocapsules have the merit of enhanced drug loading capacity acting as carriers for the loading and delivery of an anticancer drug, doxorubicin hydrochloride (DOX), with higher storage for cancer therapy. Herein, the combined functionalities of simultaneous cell imaging and drug delivery of the synthesized nanocapsules have been demonstrated, which provide a very promising candidate for application in optical imaging and drug delivery for cancer cells.

1. Introduction

Nanotechnology has been intensively explored in the constant battle against cancer in the past few decades. Cancer nanotherapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug-delivery systems [1]. Most of the clinically used imaging and therapeutic modalities are small molecules, such as gadolinium complexes and anticancer chemical drugs, which cause many unwanted side effects with the limitations of short blood circulation time, nonspecific biodistribution, and lack of water solubility [2]. Advances in nanobiotechnology and nanotechnology have led to the generation of novel multifunctional nanoparticles that enable the tumor-specific delivery of imaging probes and therapeutic agents in cancer imaging, early cancer cell diagnosis, and therapy [3]. Multifunctional nanostructured materials combining tumor-targeted imaging and therapy in an all-in-one system can be employed to overcome these limitations in the battle against cancer. The advantages of using a multifunctional nanomaterial system conjugating multiple components, such as fluorescent molecules, tumor-targeting moieties, anticancer drugs, could be manifold. They can simultaneously perform in anticancer drug delivery, optical imaging, and controllable cancerous cell death [4–18]. Among these various multifunctional nanomaterials, mesoporous materials based on silica matrices have been extensively highlighted for many biomedical applications as delivery carriers for anticancer drugs, DNA, and proteins, owing to their large surface area, tunable size, high accessible pore volume, and well-defined surface properties for modification. Mesoporous silica as a versatile solid support to construct hybrid material promises an unparalleled opportunity for enhancement of colloidal properties and functions by rational designs and profiting from its synthetic versatility [4,19–27]. Hyeon and co-workers have synthesized monodisperse mesoporous silica nanoparticles consisting of a single Fe_3O_4 nanocrystal core and mesoporous silica shell and demonstrated the multifunctional bioapplications of the core–shell mesoporous silica nanoparticles for simultaneous magnetic resonance and fluorescence imaging, and for drug delivery [18]. Recently, compared with composites with a metallic nanocrystal core such as magnetic core and mesoporous silica shell, hollow/rattle-type mesoporous structure has attracted special attention because of its excellent drug loading capacity, high permeability, and sustained-release property [28–31]. Chen et al.

* Corresponding authors.
E-mail addresses: wangcg925@nenu.edu.cn (C.-G. Wang), zmsu@nenu.edu.cn (Z.-M. Su).

0021-9797/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2011.02.023

put forward a novel "structural difference-based selective etching" strategy to successfully fabricate hollow/rattle-type mesoporous silica spheres with either homogeneous solid silica or various heterogeneous inorganic nanocrystals (Au, Fe_2O_3, and Fe_3O_4) as core and mesoporous silica as shell (Au@mSiO_2, Fe_2O_3@mSiO_2, and Fe_3O_4@mSiO_2). The nanomaterials showed high drug loading and ultrafast immobilization of biomolecules [31]. Zhu et al. fabricated rattle-type Fe_3O_4@SiO_2 hollow mesoporous spheres with large cavities and excellent monodispersity by using carbon spheres as templates. The results indicated that this kind of material is very promising for targeted drug delivery [30].

To date, most of the rattle-type nanoparticles used in drug delivery and cancer diagnosis and therapy are mainly concentrated on rattle-type Fe_3O_4@SiO_2 hollow mesoporous spheres. Au nanospheres and AuNRs possess many unique physical properties and have been employed in many bioapplications, such as medical imaging, biological sensors, drug delivery, and disease therapy. However, the reports on rattle-type Au@mSiO_2 nanocapsules with a mesoporous silica shell used in drug delivery and cancer therapy are few. Very recently, Au@mSiO_2 rattle structure developed by Chen et al. has been deposited silica shells twice (one is dense shell and the other is mesoporous shell) followed by treatment of Na_2CO_3 or ammonia solutions and calcination to obtain cavities. This procedure seems too complex and time-consuming [31].

Herein, we report the controllable synthesis of fluorescent hollow/rattle-type Au@mSiO_2 nanocapsules with tunable optical and fluorescent properties from AuNR@mSiO_2 by aqua regia etching with sonication. The sizes of hollow interiors of Au@mSiO_2 nanocapsules can be easily tuned by etching time and monitored by UV–Vis absorption spectroscopy due to the unique localized surface plasmon resonance (LSPR) of AuNR. Furthermore, DOX, an anticancer drug, was loaded into the hollow/rattle-type Au@mSiO_2 nanocapsules to evaluate the capacity as drug delivery vehicles and cytotoxic effects on human hepatocellular liver carcinoma cell line (HepG-2). Besides, small dye molecules incorporated in the silica matrices of Au@mSiO_2 nanocapsules imparted a simultaneous optical imaging modality of HepG-2. In addition, we presume that the hollow/rattle-type Au@mSiO_2 nanocapsules also have the potential to be used in surface-enhanced Raman scattering (SERS) diagnosis in the presence of a Au core, if this kind of nanocapsules encapsulates Raman active molecules which can diffuse into the hollow interiors from a mesoporous silica shell, and acted as a contrast agent in optical cancer cell imaging by dark-field microscopy due to the strong light scattering of Au nanoparticles.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$, ⩾99.99%), 3-aminopropyltrimethoxysilane (APTMS, 95%), tetraethyl orthosilicate (TEOS, ⩾98%), cetyltrimethylammonium bromide (CTAB, ⩾99%), sodium borohydride ($NaBH_4$, ⩾98%), silver nitrate, ascorbic acid (AA, ⩾99.7%), Rhodamine B isothiocyanate (RITC), and doxorubicin hydrochloride were purchased from Sigma (USA). All other reagents were used as received. All the glassware was cleaned with aqua regia (volume ratio HCl/HNO_3 = 3/1) and thoroughly rinsed with Millipore water (18.0 MΩ cm^{-1}) prior to the experiments.

2.2. Characterization

TEM was performed on a JEOLFETEM-2100 transmission electron microscope under 200 kV accelerating voltage. UV–Vis absorption spectroscopy was obtained on a U-3010 spectrophotometer (Hitachi, Japan). Fluorescence spectra were performed with an Eclipse fluorescence spectrophotometer (Varian, USA). Confocal laser scanning microscopy (CLSM) was operated on an Olympus Fluoview FV1000. Power X-ray diffraction (XRD) patterns were obtained on a D8 Focuss diffractometer (Bruker) with Cu Kα radiation (λ = 0.15405 nm).

2.3. Synthesis of AuNRs

CTAB-stablized AuNRs were synthesized using the seed-mediated growth method improved by El-Sayed and co-workers [33]. Briefly, the seed solution was prepared by reducing $HAuCl_4$ (0.5 mM, 5 mL) in CTAB (0.2 M, 5 mL) with ice-cold $NaBH_4$ (10 mM, 0.6 mL). After 5 h, 120 μL of this seed solution was injected into a growth solution of $AgNO_3$ (10 mM, 1140 μL), CTAB (0.2 M, 50 mL), $HAuCl_4$ (1 mM, 50 mL), and AA (0.1 M, 550 μL). Then this solution was left overnight. The solution was then centrifuged at a time, at 10,000 rpm for 20 min to remove excess CTAB surfactant. The as-prepared AuNRs were collected and dispersed in 50 mL of pure water by sonication for further use.

2.4. Synthesis of RITC-labeled AuNR@mSiO_2

RITC was incorporated in the silica coating on the AuNR surface by first making a RITC/APTMS/ethanol solution. Ten milligrams of RITC was covalently linked to 44 μL of APTMS in 0.75 mL ethanol under dark conditions for 2 days. The amino groups of APTMS are able to easily react with the isothiocyanate moieties of RITC molecules to yield thiourea groups. The prepared RITC-APTMS stock solution was kept at 4 °C [19]. CTAB-stabilized AuNRs coated with mesoporous silica were synthesized according to previously reports [32,34]. Briefly, 10 mL of the as-prepared AuNRs and 10 mL pure water were mixed followed by adding 250 μL of 0.1 M NaOH on stirring. Then 5 μL of the RITC/APTMS/ethanol solution was introduced into the mixture. After that, 45 μL of 20% TEOS in ethanol was injected four times at a 30-min interval. The reaction mixture was reacted for 24 h under gentle stirring. The obtained RITC-labeled AuNR@mSiO_2 were then centrifuged and washed with ethanol at least five times to remove the unreacted species as well as CTAB molecules. Then the nanoparticles were dispersed in 8 mL of ethanol.

2.5. Synthesis of RITC-labeled hollow/rattle-type Au@mSiO_2

Aqua regia was used as an etching agent to form a hollow/rattle-type structure. In a typical process, 30 μL of aqua regia was added into 3 mL of RITC-labeled AuNR@mSiO_2 solution under sonication. The equivoluminal products at different reaction times (10, 20, 60, and 150 min) were shut down to centrifuge and dispersed in pure water and measured by UV–Vis absorption spectroscopy and TEM microscopy. The particles etched by aqua regia for 10, 20, 60, and 150 min were designated as RITC-labeled rattle-Au@mSiO_2-10, 20, and 60 and RITC-labeled hollow mSiO_2, respectively.

2.6. Loading DOX into RITC-labeled hollow/rattle-type Au@mSiO_2

UV–Vis spectroscopy was used to determine the amount of drug molecules DOX loaded into the nanocapsules and to calculate the loading efficiency. The absorption increase was directly proportional to the concentration of DOX solution. The drug-loaded nanocapsules were prepared by mixing DOX aqueous solution (2 mg/mL, 8 μL) with the capsules of RITC-labeled rattle-Au@mSiO_2-60 aqueous solution (6.3 nM, 0.8 mL) for 12 h and then centrifuged at 12,000 rpm for 20 min. To evaluate the DOX-loading