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Shell-by-shell synthesis of multi-shelled mesoporous silica nanospheres for optical imaging and drug delivery

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ABSTRACT

Self-templated synthesis involving interior channel wall protection as well as outermost surface passivation was crucial to successful synthesis of multi-shelled mesoporous silica nanospheres. The shell-by-shell fabrication of double- and triple-walled mesoporous silica nanospheres downsized to ~100 nm. The multi-shelled mesoporous silica can be built as rattle-type or hollow structures with ~110 nm of double-shelled and ~140 nm of triple-shelled sizes. Notably, the shell-to-shell distance can be tuned by controlling the etching period from the self-templation processes without changing the multi-shelled size or interior core diameter. The multi-shelled mesoporous nanostructures provide a platform for the development of a multifunctional vector by the inclusion of functional species into shell-to-shell cavities and porous shells. The encapsulation of the fluorophore and drug in shell-to-shell space and mesoporous shells showed that multi-shelled silica spheres can be used in dual-modality for imaging and drug co-delivery vectors through the appropriate selection of pH-dependent molecules. The in vitro evaluation in triple-shelled silica indicated that an anti-cancer doxorubicin (DOX), loaded in the outer periphery space, was successfully carried and released in the cytoplasm, then entered nuclei while fluorescein FITC (primarily distributed in inner periphery space) was effectively encapsulated inside the spheres. The double- and triple-shelled nanospheres consistently provided imaging probes with visible tracking capability in vitro and in vivo.

1. Introduction

Multi-shelled spheres formed as rattle-type or hollow structures are unique hierarchical structures comprise of multiple concentric shells with different diameters [1–3]. With the void spacing between the shells ideally suited to load distinct functional species, the fantastic architectures stimulate technological importance because of their potential applications in controlled delivery, confined nanoreactors, and catalysis. Template synthesis has been an effective means of manufacturing such shell-in-shell architectures [4–10]. The choice of template composition and size, and the corresponding preparation strategy strongly influence the final designed multi-shelled materials. Because of its easy fabrication and derivatization, silica composition is often used as a sacrifice template, either treated as core support [5,6] or as an intermediate layer between the shells [7,8], to develop other composites in multi-shelled structures. For example, the ~250 nm of silica acting as a sacrifice core was employed to generate double-shelled SnO_2 [5] and coaxial SnO_2@C [6] hollow structures. With the same composition, the ellipsoidal double-shelled SnO_2 created by the introduction of silica as removal layer was developed by Archer et al. [7]. The development of shell-by-shell synthetic technology for multi-shelled silica with rattle-type and hollow nanospheres remains an attractive challenge. Shell-by-shell construction of multi-shelled nanostructures itself is representative of the advancement of nanofabrication technology. An additional challenge to the manufacture of multi-shelled structures is achieving control of the space between the shells. For a certain particle size, tuning shell-to-shell distance is critical in varying the physical and chemical features of multi-shelled nanospheres and may provide better understanding of how to control the local chemical micro/nano environments.

Self-templated synthesis involving surface-protected etching provides an effective approach for creating rattle-type or hollow structures from sol–gel derived colloids because of their porosity [11–13]. With appropriate protection of the outermost surface, a selected etchant is able to dissolve the unprotected interior while sustaining the protected outer layer. Although self-templated strategy presents an elegant route to create single-shelled rattle-type or hollow silica nanostructures, there has been difficult to form multi-shelled silica colloids. The carefully optimizing outermost layer by the

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increase of thickness was necessary in order to sustain extensive etching for the formation of multi-shelled nanostructures. As shown by Yin et al., the resulting shell thickness was at least 100 nm for double-shelled nanoparticles [13]. Therefore, the creation of the complex building block like multi-shelled nanostructures with intact sizes near to 100 nm remains a challenge. In this study, the shell thickness could be down to 10–20 nm to yield double- and triple-walled silica nanospheres. Additionally, the presented strategy was developed to directly build up the *mesoporous* shell with the tunable pore sizes by the selection of different alkytrimethylammonium bromide (C_nTAB) in chain lengths, which is different from Yin and co-workers methods by the introduction of base etching forming *mesoporous* silica layer. We found that interior channel wall protection with APTES modification as well as outermost surface with PVP passivation was crucial to successful synthesis of multi-shelled mesoporous silica. Briefly, the inner mesoporous silica shells were protected by APTES prior to fabricate outer mesoporous silica layers. Subsequently, the as-prepared outer silica layers were passivated with PVP and treated with base etchant to from shell-in-shell nanostructures. The multi-shelled mesoporous silica can be built as rattle-type or hollow structures with ~110 nm of double-shelled and ~140 nm of triple-shelled sizes. Notably, the shell-to-shell distance can be easily tuned by controlling the etching period from the self-templation processes without changing the multi-shelled size or interior core diameter. For the multi-shelled structures, previous studies primarily focused on the development of synthetic technologies. However, a unique aspect of these multi-shelled materials is their attractive interior architectures. It would be anticipated to encapsulate different functional molecules into respective shell-to-shell space, showing that multi-shelled silica spheres can be used in dual-modality for imaging and drug delivery vectors.

2. Experimental section

2.1. Materials

All reagents were of analytical purity and used without further purification: tetraethyl orthosilicate (TEOS, 98%, Acros), polyvinylpyrrolidone (PVP, M_w. 4,0000, Sigma–Aldrich), styrene (stabilized 99%, Acros), 4-styrenesulfonic acid sodium salt hydrate divinylbenzene (Sigma–Aldrich), Potassium peroxodisulfate ($K_2S_2O_8$, 98%, Showa), sodium bicarbonate ($NaHCO_3$, J. T. Baker), ethanol (EtOH, 99.9%, J.T. Baker), aminopropyl–ethoxysilane (APTES, 99%, Acros), sodium hydroxide (NaOH, Fullin), ammoniun hydroxide solution (NH_3, 33%, Sigma–Aldrich), tetradecyltrimethylammonium bromide (C_{14}TAB, 99+, Acros), hexadecyltrimethlammonium bromide (C_{16}TAB, 99+, Acros), octadecyltrimethylammonium bromide (C_{18}TAB, 98%, Aldrich), doxorubicin hydrochloride (DOX·HCl, 98%), fluorescein 5(6)-isothiocyanate (FITC, approx 90%, Sigma), and indocyanine green (ICG, MARK).

2.2. Synthesis of multi-shelled silica nanoparticles

2.2.1. Synthesis of polystyrene nanoparticles as a template

Synthesis of polystyrene nanoparticles as a template: Poly(styrene-co-styrene sulfonate) (denoted as PS) nanoparticles were prepared by following the previous methods with slight modification [14,15]. 0.025 g of $NaHCO_3$ and 0.1 g of 4-styrenesulfonic acid sodium salt hydrate were pre-dissolved in 50 mL deionized water and heated at 75 °C under vigorous stirring. 5 mL of styrene solution was subsequently added. After reacting for 1 h, the initiator $K_2S_2O_8$ (0.025 g) was added to start polymerization under magnetic stirring for 18 h.

2.2.2. Preparation of single-shelled silica nanospheres

Preparation of single-shelled silica nanospheres: To prepare single-shelled silica nanoparticles, we dispersed 0.2 mL of as-prepared PS solution (~12.4 mg in dry powder) into 10 mL of ethanol solution, including 10 μL of APTES. After vigorously stirring (2 h), 100 μL of TEOS and 250 μL of NH_3 were then introduced into the above solution accompanying by stirring and reacted at room temperature. The solution became milky when it reacted overnight. The white color product of PS@silica nanoparticles was collected using centrifugation (9500 rpm) and rinsed several times with ethanol. The as-obtained product was PS@silica nanoparticles which were dispersed into 8 mL pure water before further use. We obtained single-shelled hollow silica nanoparticles after removing PS core with calcination at 580 °C for 2 h.

2.2.3. Preparation of double-shelled silica nanospheres

Preparation of double-shelled silica nanospheres: The double-shelled silica nanoparticles were synthesized by coating a mesoporous silica layer on the resultant PS@silica nanoparticles via a CTAB surfactant-assisted reaction followed by a self-templating etching process. In a typical synthesis, 3 mL of the as-obtained PS@silica nanoparticles solution was mixed with 5.75 mL of pure water, including with CTAB (8.7 mM) and NaOH (2.17 mM). Then 100 μL TEOS was added as a droplet under a vigorous stirring and then the mixture solution was reacted at 55 °C for 3 h. Consequently, PS@SiO_2 covered with mesoporous silica layer nanoparticles was generated. The supernatant was removed by centrifugation and the white precipitate was collected. After washing the white precipitate with water three times, the final product was re-dispersed in the 10 mL of PVP solution (1 mM) to modify the mesoporous silica with PVP polymer coverage. The mixture solution was heated at 100 °C again for 3 h. After cooling to room temperature, 8 mL of the PVP-modified silica nanoparticles solution was taken and reacted with 1 mL of 0.25 M NaOH for 1 h, resulting in a final 27.8 mM NaOH. TEM images demonstrated that the resulting nanoparticle exhibited a double-shelled configuration. The resulting double-shelled silica nanospheres were subsequently dispersed in ethanol (10 mL), after which 10 μL of APTES was added under stirring for a 2 h reaction. After washing, the final product was stored in 8 mL deionized water before loading the drug or next silica layer coating. The shell-in-shell hollow silica nanoparticles could be formed by a calcination process at 580 °C for 2 h.

2.2.4. Preparation of triple-shelled silica nanospheres

Preparation of triple-shelled silica nanospheres: The synthesis procedure to prepare triple-shelled silica nanospheres was similar to the preparation of the 2nd mesoporous silica shell, except for the addition of 200 μL of TEOS in the synthesis of 3rd mesoporous silica layer. Once again, the triple-shelled hollow silica nanoparticles were formed after calcination at 580 °C for 2 h.

2.3. Encapsulation of fluorescence and anti-cancer drugs in multi-shelled silica nanoparticles

Molecules encapsulated into inner cavities of multi-shelled silica vehicles were prepared as follows. The FITC drugs were loaded into rattle-type double-shelled silica nanostructures by physically mixing the materials (8 mL) with an aqueous solution of FITC (2.6 mg/mL, 80 μL) to achieve saturation absorption at room temperature for 24 h. Then, the FITC-loaded double-shelled nanostructures were purified by centrifugation/washing processes with water more than three times. The amount of FITC-loaded in double-shelled nanostructures was calculated from the difference between the initial amount of FITC and the residue in the supernatants collected from centrifugation and subsequent washing processes. The fluorescent signal of FITC (excitation at 450 nm and emission at 515 nm) was recorded using a fluorescence spectrophotometer. We used FITC-loaded silica nanostructures (31.7 μg mg^{-1}) as a starting material and repeated the self-templeting process (as described above) to synthesize triple-shelled silica nanostructures. In the course of 3rd shell preparation, the loss of FITC was carefully quantified to estimate the residual amount of FITC-loaded within multi-shelled silica nanoparticles. Subsequently, the FITC-loaded triple-shelled silica nanostructures (8 mL) were physically mixed with a DOX solution (4 mg/mL, 80 μL) to achieve saturation adsorption at room temperature for 24 h. To determine the amount of DOX in the triple-shelled silica nanospheres, the supernatants of the residual drug molecules were collected and subjected to signal quantification. The fluorescent signals of DOX (excitation at 480 nm and emission at 585 nm) were recorded using a fluorescence spectrophotometer. In the triple-shelled silica nanospheres, the loading amount of FITC and DOX were 14.6 μg mg^{-1} and 30.8 μg mg^{-1}, respectively.

2.4. In vitro release studies of multi-shelled silica nanostructures

We used FITC-loaded rattle-type double-shelled silica nanostructures (31.7 μg mg^{-1}) as the starting materials to synthesize triple-shelled nanospheres (as described above). In the course of 3rd shell preparation, the loss of FITC was carefully quantified to estimate the residual amount of FITC within triple-shelled nanospheres. Subsequently, the as-prepared FITC-loaded silica nanospheres were resuspended in 25 mL PBS at pH = 4.3 and 7.2. The released FITC was evaluated by fluorescence detection (excitation at 450 nm and emission at 515 nm). The drug release behavior of FITC-loaded rattle-type double-shelled silica nanostructures was carried out in 25 mL PBS at pH = 4.3 and 7.2 as well.

For DOX-loaded in rattle-type triple-shelled silica nanostructures, the double-shelled silica nanospheres without inclusion of FITC as the starting materials to synthesize triple-shelled silica nanospheres. Once the fabrication of 3rd shell was completed, the triple-shelled nanostructures (8 mL) were mixed with a DOX solution (4 mg/mL, 80 μL) at room temperature for 24 h. Subsequently, the as-prepared DOX-loaded silica nanospheres were resuspended in 25 mL PBS at pH = 4.3 and 7.2). The released DOX was estimated by the fluorescent signal of DOX (excitation at 480 nm and emission at 585 nm).