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One-step synthesis of amine-functionalized hollow mesoporous silica nanoparticles as efficient antibacterial and anticancer materials

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Abstract

In this study, amine-functionalized hollow mesoporous silica nanoparticles with an average diameter of ~100 nm and shell thickness of ~20 nm were prepared by an one-step process. This new nanoparticulate system exhibited excellent killing efficiency against mycobacterial (*M. smegmatis* strain mc² 651) and cancer cells (A549).

Mesoporous silica nanomaterials with controlled morphology and structure have attracted increasing attention both for fundamental research on nano-interface and for their widespread potential applications.^{1–6} In recent years, much effort has been devoted to the fabrication of mesoporous silica nanoparticles with hollow interiors due to their superior advantages in confined-space catalysis, sustained drug release, renewable energy, and biosensing device.^{7–11}

In this regard, several strategies have been developed for constructing hollow mesoporous silica nanoparticles (HMSNs). Among them, the templating methods, including the use of hard templates (such as polystyrene latex particles¹² and hydroxyapatite nanoparticles¹³) and soft templates (such as emulsion droplets¹⁴ and vesicles¹⁵), are among the most popular. In these cases, the hollow interiors are formed after removing the template either by calcination at high temperature or by selective dissolution in an appropriate solvent. The template-removal process inevitably affects the shell structure, and additionally, the synthetic procedures is time-consuming. Self-templating is another strategy for preparing hollow silica particles. For example, Yin *et al.* synthesized hollow silica colloids through a spontaneous dissolution-regrowth process from solid silica spheres in alkaline solution with poly(vinylpyrrolidone) as the surface protector.^{16,17} Qi and co-workers fabricated gigantic hollow microspheres in mixed water-ethanol solvent using tetraethyl orthosilicate (TEOS) as emulsion droplets.¹⁸ Wang *et al.* obtained hollow silica particles from solid silica spheres under hydrothermal conditions in acid.¹⁹ However, these strategies either lead to disordered mesoporous channels in the shell, or need further surface modification to introduce reactive functional groups to the materials. Developing simple synthetic protocols that

simultaneously give ordered pore channels in the shell and reactive functionalized groups on the surface remains a challenge. Here, we report a simple procedure for the synthesis of amine-functionalized HMSNs from TEOS, 3-aminopropyltriethoxysilane (APTES), and cetyltrimethylammonium bromide (CTAB) in solution phase in a single step. In addition, the new HMSNs were used as efficient drug delivery system for mycobacteria and cells.

HMSNs were prepared by co-condensation of APTES and TEOS in the presence of CTAB as structure directing and pore-forming agent in the shell. A small amount of ammonia was added as the catalyst (see experimental details in ESI[†]). As shown in Fig. 1, nanosized HMSNs were successfully obtained with an average diameter of ca. 100 nm and shell thickness of ca. 20 nm. The dynamic light scattering (DLS) result shows a good dispersion of HMSNs in water with an average hydrodynamic size of 131.7 ± 8.1 nm (Fig. S1[†]). Zeta potential result shows that HMSNs has a high positive potential (+38.09 mV, Fig. S2[†]), which results from the positively charged amino groups on HMSNs. The peaks at 1473, 2865, and 2971 cm^{-1} in the FTIR spectra of HMSNs indicate the presence of organoalkoxysilane in the particles (Fig. S3[†], S4[†], and S5[†]).^{20,21} Thermogravimetric analysis (TGA) results (Fig. S6[†]) showed that HMSNs had a higher weight loss (25.6%) than conventional silica nanoparticles (15.1%) under same treatment conditions due to a relatively high percentage of organic moiety on HMSNs. The surface density of the active amino groups on HMSNs was measured to be about 3.4 amine/ nm^2 , using the 4-nitrobenzaldehyde assay following a literature protocol.²² Nitrogen adsorption-desorption measurement shows that HMSNs exhibited a typical type IV isotherm, as expected for HMSNs with ordered cylindrical pores in the shell (Fig. S7A[†]).²³ The specific surface area and pore volume of HMSNs were measured to be 410.73 m^2/g and 0.34 cm^3/g , respectively. Noted that there was a wide pore size distribution with a sharp peak at 3.1 nm in the HMSNs sample (Fig. S7B[†]), which could be attributed to its large interior and ordered pore channels have an average pore size of 3.1 nm in the shell layer.

To explore the formation mechanism of HMSNs by this one-step process, we evaluated the specific roles of APTES, CTAB, and the addition order of the reagents (Fig. S8[†], S9[†], and S10[†]). In the absence of APTES, mesoporous silica nanoparticles of ~40 nm without the hollow structure were obtained (Fig. S8[†]), which indicates that APTES plays a significant role in maintaining the morphology of HMSNs. Without CTAB, the obtained HMSNs aggregated and undefined shell structure was formed (Fig. S9[†]), which suggests that CTAB regulates the shell-forming process. When switching the addition order of reagents, the hollow structure was not observed (Fig. S10[†]), which implies that TEOS and APTES are the determining factors in the formation of the hollow interior. Based on these observations, a possible mechanism is proposed to account for the nucleation and growth of HMSNs (Fig. 2). Due to the solubility difference of APTES and TEOS in water (APTES is soluble whereas TEOS is insoluble in water),²⁴ when they are added to the CTAB solution, the protonated APTES acts as a stabilizer surrounding the TEOS droplets (Fig. 2A, Fig. S8[†]). CTAB micelles could also be captured at the water-oil interface and act as structure-directing agent (Fig. 2B).^{18,25} The addition of ammonia initiated and accelerated the

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hydrolysis of TEOS, and the formed silica fragments that are negatively charged prefer to deposit on the positively charged CTAB micelles. APTES also co-condenses with TEOS to form the shell layer in addition to acting as a morphological stabilizer (Fig. 2C). Since no additional TEOS can be supplied to the oil droplet due to the APTES protecting layer, the condensed materials will form a shell, leading a hollow structure (Fig. 2D). As the reaction progresses, the shell thickness increases until TEOS in the droplet is completely consumed (Fig. 2E).

These HMSNs have abundant amino groups for further functionalization, large hollow interior that can be used as a reservoir, and well-defined mesoporous shell which could act as accessible channels for the reagents to diffuse in and out of the interior. Herein, we explore the application of HMSNs as drug delivery vehicle in killing bacteria and cancer cells. Isoniazid (INH), an antituberculosis drug,²⁶ was loaded into HMSNs. The loading capacity could achieve 315.8 mg per gram of HMSNs (Fig. S11[†]). Fig. 3A shows the release profile of INH from INH-loaded HMSNs (HMSNs-INH) in pH 6.6 PBS (simulating the environment of 7H9 broth medium²⁷). It can be seen that INH displayed a sustained release profile from HMSNs, and the drug release reached ~60% after 72 hours. Mycobacteria-killing kinetics were studied by treating *M. smegmatis* strain mc² 651 with different concentrations of free INH and INH-loaded HMSNs (INH drug concentration from 10 to 1280 µg/mL) over 24 h (Fig. 3B) and 72 h (Fig. 3C), and a dose-response relationship was observed. Free INH-treated mycobacteria were completely inhibited at the concentration of 1280 µg/mL for both 24 h and 72 h incubation, while HMSNs-INH-treated mycobacteria were completely inhibited at the concentration of 640 µg/mL and 320 µg/mL for 24 h and 72 h incubation, respectively. In addition, the half inhibitory concentration (IC₅₀) of mycobacteria treated by free INH drug was 3.3- and 4.1-fold of HMSNs-INH-treated bacteria at 24 h and 72 h respectively. This enhanced antibacterial activity might be probably due to increased intrabacterial accumulation of sustained INH drugs from HMSNs-INH and partly by the strong interactions between HMSNs and bacteria.²⁶ HMSNs alone had bacteria-killing effect to some extent, especially at high particle concentrations of > 1 mg/mL (Fig. 3D). This is anticipated since quaternary ammonium species are known to have antimicrobial activities.^{28,29}

We next investigated the interactions between HMSNs and mycobacteria. As shown in Fig. 4, HMSNs could efficiently bind to the bacteria surface likely due to the nonspecific adsorption of the positively charged amine-nanoparticles onto the negatively charged bacteria cell surface.³⁰ Further examination of the images revealed that the interactions of HMSNs also led to bacteria injury (Fig. 4A), incomplete or missing bacteria cell wall (Fig. 4B), and even bacteria rupture and death (Fig. 4C), which is consistent with the results in Fig. 3D. This is in clear contrast to the negatively charged conventional mesoporous silica nanoparticles (Fig. S12[†]) which had minimal interactions with mycobacteria (Fig. S13[†]). The strong interaction between HMSNs and bacteria was also confirmed using FITC-labeled HMSNs fluorescent nanoparticles, where the mycobacteria became fluorescent after treating with HMSNs-FITC (Fig. 4D, Fig. S14[†] and S15[†]). These preliminary results demonstrated that HMSNs has the potential to be used as an effective nanovehicle for treating drug resistant bacteria.

To further explore the potential of HMSNs, anticancer drug doxorubicin (Dox) was loaded into HMSNs, and its efficacy towards A549 cells was evaluated. After treating the Dox-loaded HMSNs-FITC, the particles were efficiently internalized into A549 cells (Fig. 5 and Fig. S16[†]). The loading capacity of Dox was 261.5 mg per gram of nanoparticles (Fig. S17[†]). The sustained release profiles of Dox from Dox-loaded HMSNs (HMSNs-Dox) were measured in pH 7.4 and pH 4.5 PBS to simulate the environment of extracellular incubating medium and intracellular endosomal-lysosomal acidification, respectively.³¹ The cumulative drug release were 21.4% and 89.1% after 72 hours in pH 7.4 and pH 4.5 PBS, respectively (Fig. 6A). Cell viability test using A549 cells shows no significant adverse effect even at high HMSNs particle concentration of 1 mg/mL (Fig. 6B). When A549 cells were incubated with a series of equivalent drug concentrations of free Dox and HMSNs-Dox for 24 h and 72 h, both free Dox and HMSNs-Dox shows obvious cell inhibition after 24 h (Fig. 6C) and 72 h incubation (Fig. 6D) with Dox concentration from 0.08 to 50 μ M. However, the IC₅₀ values of Dox are 3.9- and 5.0-fold of HMSNs-Dox at 24 h and 72 h respectively. The increased cytotoxicity of HMSNs-Dox can be attributed to the sustained release of Dox from HMSNs-Dox, and perhaps also to the enhanced Dox uptake by A549 cells when Dox encapsulated into amine-enriched HMSNs.³²

In summary, we have successfully developed an one-step strategy to synthesize amine-functionalized HMSNs with well-defined mesoporous shell. When loaded with an antibiotic or a cancer drug, these HMSNs exhibited excellent antibacterial (*M. smegmatis* strain mc² 651) and anticancer (A549) activities lowering the IC₅₀ values by several folds. The increased activities can be attributed to its unique structures including the amine surface for enhanced interactions with cells, typical hollow interior cavity as drug reservoir, and well-defined mesoporous shell for sustained drug release. This general synthetic strategy can be extended to fabricate hollow nanomaterials of different structure and shape, paving the way for the applications of these in many fields such as catalysis, adsorption, separation, and microreactors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. (A)–(C) TEM images of HMSNs at different magnifications. (D) SEM image of HMSNs. In the inset, the arrow points to a hollow interior of a HMSN.

Fig. 2.
The proposed mechanism for the formation of HMSNs.

Fig. 3.

(A) Release profile of INH from drug-loaded HMSNs in PBS solution (pH 6.6); (B) and (C) Antibacterial efficacy of free INH and INH-loaded HMSNs (concentration of INH from 10 to 1280 $\mu\text{g/mL}$) on *M. smegmatis* strain mc² 651 for 24 h (B) and 72 h (C), respectively; (D) Antibacterial efficacy of HMSNs at a concentration range from 0.25 to 8 mg/mL for 24 h and 72 h.

Fig. 4. (A–C) TEM images of HMSNs incubated with *M. smegmatis* strain mc² 651. The inset in (A) is a magnified image of nanoparticles around bacterium. Arrows in (A) show bacteria injury, and the arrows in (B) indicate missing bacteria cell wall. (D) Fluorescence image of *M. smegmatis* strain mc² 651 treated with HMSNs-FITC.

Fig. 5.
(A–C) Fluorescence and (D) bright-field images of A549 cells after treating with Dox-loaded FITC-labeled HMSNs: (A) FITC channel; (B) Doxorubicin channel; (C) merged FITC and Doxorubicin channels. Scale bars: 50 μm .

Fig. 6.

(A) Release profile of Dox from HMSNs-Dox in PBS buffer (pH 4.5 and pH 7.4); (B) A549 cell viability with concentrations of HMSNs from 0.0625 to 1 mg/mL for 24 h and 72 h. (C) and (D) are inhibition rates of Dox and HMSNs-Dox (concentration of Dox from 0.08 to 50 μ M) on A549 cells for 24 h and 72 h respectively.