The effect on the morphology of mesoporous silica nanoparticles modified with different functional groups

Youyun Wang1,2,3,4, Yu Wang1,2,3,4, Wanxia Wang1,2,3,4 and Mingxing Liu1,2,3,4,*

1Key Laboratory of Fermentation Engineering (Ministry of Education),
2Hubei Provincial Cooperative Innovation Center of Industrial Fermentation,
3National "111" Center for Cellular Regulation and Molecular Pharmaceutics,
4Hubei Key Laboratory of Industrial Microbiology, Hubei University of Technology, Wuhan, 430068 China
*Corresponding author: lmxing@hbut.edu.cn

Abstract. Three types of nanomaterials marked were successfully synthesized by grafting HA, HOOC-PEG-COOH and NH2-ZnO QDs on the surface of colloidal mesoporous silica nanoparticles modified with sulfydryl, amino and carboxyl groups, respectively, which could be marked as FRET-CMS, CMS-PEG-COOH and ZnO-CMS, respectively. Moreover, the structural and particle properties of these nanomaterials were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM) and nitrogen adsorption-desorption measurements. These nanoparticles presented with a relatively uniform spherical shape morphology with a diameter of about 100 nm. Furthermore, the mesoporous structure of the nanoparticles still remain but the pore diameter would decreased slightly after encapsulated with different functional groups, which would be no obvious effect on the capacity for the drug loading and releasing. So these results indicated that the properties of CMS nanoparticles with different functional groups were worth researching for its application in drug delivery system in the future.

1. Introduction
Nanoscaled colloidal mesoporous silica particles have excellent potential due to their high surface area and pore volume, tunable pore sizes, biocompatibility, easy of surface functionalization for diverse biomedical applications including bioimaging, diagnostics, biosensing, biocatalysis, bone repair and scaffold engineering, especially drug delivery [1][2]. In addition, colloidal mesoporous silica nanoparticles as cargo carriers have attracted substantial attention due to the inner or outer surface of the nanomaterials functionalised with organic molecules which are critical for successful drug delivery [3]. On the one hand, the functionalization of the internal pore system is needed to fine-tune the host-guest interactions and chemistry. On the other hand, the modification of the outer surface permits the attachment of large moieties without reducing the pore size and the available free pore volume, which can influence the colloidal stability of the nanomaterials and their interactions with the environment [4]. Moreover, surface functionalization using specifically designed stimuli-responsive switches or targetable ligands as gatekeepers endow mesoporous silica nanoparticles (MSN) with the capabilities of controlled or targeted drug delivery [5][6].

Herein, colloidal mesoporous silica nanoparticles were modified with sulfydryl, amino and carboxyl groups, respectively, and further successfully functionalized with HA, HOOC-PEG-COOH and NH2-ZnO QDs. These three types of nanomaterials were marked as FRET-CMS, CMS-PEG-COOH and ZnO-CMS, respectively. Moreover, the dynamic light scattering (DLS), transmission electron microscopy (TEM) and nitrogen adsorption-desorption measurements were used to characterize the
structural and particle properties of the nanomaterials, through which the effect on the morphology of nanomaterials with different functional groups could be analyzed. The properties of nanomaterials were worth researching for its application in drug delivery system.

2. Experimental Section

2.1. Materials
Tetraethyl orthosilicate (TEOS, 98%), 3-aminopropyltriethoxysilane (APTES, 98%), hexadecyl trimethyl ammonium Chloride (CTAC, 97%) rhodamine B were obtained from Aladdin Chemistry Co. (Shanghai). Mercaptopropyltriethoxysilane (MPTES, 95%), 8-Hydroxyquinoline (Hq), N-hydroxysuccinimide (NHS, 98%) and 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC•HCl, 99%) were supplied by J&K Chemical Co. (Beijing). Polyethylene glycol (PEG, Mn=2 kDa), Triethanolamine (TEA) pyridine (Py), Dichloromethane, Sodium hydroxide (NaOH), Zinc acetate dihydrate, zinc acetate, magnesium acetate, N,N-dimethylformamide (DMF), absolute ethanol, methanol, acetic acid, diethyl ether, hydrochloric acid (HCl, 37%), and maleic anhydride (MA) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium hyaluronate (HA) (Mw 10000 kDa) was obtained from Fusheng Industrial Shanghai Co., Ltd.

2.2. Synthesis of the Redox-Responsive Colloidal Mesoporous Silica Nanoparticles Drug Delivery Systems based on the fluorescence Resonance Energy Transfer (FRET-CMS)
The redox-responsive mesoporous silica nanoparticles drug delivery systems based on the fluorescence resonance energy transfer (FRET-CMS) have been successfully constructed for drug targeting and real-time monitoring and synthesized by the following steps: Znq was first introduced into the pore wall of mesoporous silica modified with mercapto groups (CMS-SH) by in-situ synthesis (Znq-CMS). Then Znq-CMS modified with the disulfide bond (Znq-CMS-SS-NH2) was prepared with the carboxyl modified on the surface of Znq-CMS (Znq-CMS-COOH) and cystamine dihydrochloride by the amidation reaction. Finally, the Rhodamine B (RhB) modified with HA was further grafted to the surface of Znq-CMS-SS-NH2 via amidation.

2.3. Synthesis of Carboxyl-Terminated Polyethylene Glycol Functionalized Colloidal Mesoporous Silica Nanoparticles (CMS-PEG-COOH)
A type of mesoporous silica nanoparticles functionalized with carboxy-terminated polyethylene glycol (CMS-PEG-COOH) was constructed by grafting HOOC-PEG-COOH onto the surface of CMS-NH2 via amidation. The CMS-NH2 was synthesized according to the published method with minor changes [7][8]. Water, ethanol and 25 wt % CTAC solution were premixed, followed by adding TEA until the pH value of the solution reached 10.5. TEOS was slowly added dropwise to the stock solution within 2-3 min under stirring at 60°C, and then APTES (0.152 ml, 0.65 mmol) was added dropwise into the solution 15 min after adding TEOS. The reaction mixture was cooled to room temperature after reacting for 2 h. Finally, the resulting products were centrifuged and washed with ethanol for two times. Extract the template from the products and then the CMS-NH2 was washed with ethanol and distilled water.

2.4. Synthesis of Redox/pH dual Stimuli-Responsive ZnO QDS-Gated Colloidal Mesoporous Silica Nanoparticles (ZnO-CMS)
A novel redox and pH dual stimuli responsive delivery system based on colloid mesoporous silica nanoparticles has been successfully constructed for on-demand drug release and synthesized by the following steps: The CMS-SH was synthesized by a co-condensation method with TEOS and MPTES by the former methods. The CMS-SS-NH2 was prepared with CMS-SH and S-(2-Aminoethylthio)-2-thiopyridine Hydrochloride (Py-SS-NH2). The CMS-SS-COOH was obtained by grafting maleic anhydride on the surface of CMS-SS-NH2 via amidation. Meanwhile, NH2-ZnO QDs was prepared with zinc oxide quantum dots (ZnO QDS) and 3-aminopropyltriethoxysilane (APTES) by ligand
The morphology of CMS-SH, FRET-CMS, CMS-NH$_2$, CMS-PEG-COOH and ZnO-CMS were analyzed by TEM. As shown in Figure 1, the spherical morphology and the wormhole arrangement mesoporous of CMS-SH, CMS-NH$_2$ and CMS-SS-COOH were obviously observed with the diameter of about 90 nm in Figure 1A, B, C. After grafting HA, HOOC-PEG-COOH and ZnO-CMS onto the surface of the corresponding nanoparticles, the mean diameter of FRET-CMS and CMS-PEG-COOH were grew to 100 nm and ZnO-CMS were slightly grown. Though there were no obvious differences in shape and diameter presented between CMS-SH and FRET-CMS, between CMS-NH$_2$ and CMS-PEG-COOH and between CMS-SS-COOH and ZnO-CMS, it was hard to observe the mesoporous structure of FRET-CMS, CMS-PEG-COOH and ZnO-CMS in Figure 1D, E, F. In this study, the size measured by DLS was slightly larger than that obtained from TEM on account of the hydration layer in aqueous condition [9].

Table 1. DLS measurements of CMS-NH$_2$ and CMS-HA.

| Materials     | Size(nm)    | PDI          | Zeta(mV)     |
|---------------|-------------|--------------|--------------|
| CMS-SH        | 113.2 ± 3.3 | 0.088 ± 0.015| -27.8 ± 2.3  |
| FRET-CMS      | 434.1 ± 4.6 | 0.128 ± 0.046| -28.7 ± 2.4  |
| CMS-NH$_2$    | 151.3 ± 2.3 | 0.101 ± 0.021| 37.5 ± 2.6   |
| CMS-PEG-COOH  | 238.1 ± 3.1 | 0.172 ± 0.032| -5.3 ± 1.5   |
| CMS-SS-COOH   | 149.4 ± 2.6 | 0.068 ± 0.025| -31.2 ± 2.1  |
| ZnO@CMS       | 158.5 ± 2.3 | 0.113 ± 0.024| 23.4 ± 2.3   |
Figure 1. TEM images of CMS-SH (A), CMS-NH$_2$ (B), CMS-SS-COOH (C), FRET-CMS (D), CMS-PEG-COOH (E) and ZnO-CMS (F).

The adsorption-desorption isotherms and pore size distributions of nanomaterials were shown in Figure 2. The $S_{BET}$, $V_t$, and $W_{BJH}$ of the corresponding materials were summarized in Table 2. All CMS materials exhibited characteristic type IV isotherms in Figure 2A, corresponding to the mesoscaled pore structure shown in Figure 2B. The $S_{BET}$ and $V_t$ of FRET-CMS, compared with CMS-SH, were obvious decreased from 1225 cm$^2$/g to 599 cm$^2$/g and 1.26 cm$^3$/g to 0.83 cm$^3$/g, respectively. The $S_{BET}$ and $V_t$ of CMS-PEG-COOH, compared with CMS-NH$_2$, were decreased from 655 cm$^2$/g to 475 cm$^2$/g and 0.98 cm$^3$/g to 0.62 cm$^3$/g, respectively. As the same, the $S_{BET}$ and $V_t$ of ZnO-CMS, compared with CMS-SS-COOH, were decreased from 864 cm$^2$/g to 363 cm$^2$/g and 1.26 cm$^3$/g to 0.83 cm$^3$/g, respectively. The pore diameter of FRET-CMS and ZnO-CMS were all decreased from 2.1 nm to less than 2 nm, respectively. While, the $W_{BJH}$ of CMS-PEG-COOH was 5.25 nm that did not significantly decreased from 5.95 nm. These results indicated that the meso porous structure of the nanoparticles still remain after encapsulated with different functional groups such as HA, HOOC-PEG-COOH and NH$_2$-ZnO QDs, but the pore diameter were decreased slightly, respectively.

Figure 2. The nitrogen adsorption-desorption isotherms (A) and pore size distributions (B) of CMS-SH, FRET-CMS, CMS-NH$_2$, CMS-PEG-COOH, CMS-SS-COOH and ZnO-CMS.
Table 2. The nitrogen adsorption-desorption analysis of CMS-SH, FRET-CMS, CMS-NH₂, CMS-PEG-COOH, CMS-SS-COOH and ZnO-CMS.

| Materials         | \( S_{\text{BET}} \, [\text{m}^2/\text{g}]^a \) | \( V_t \, [\text{cm}^3/\text{g}]^b \) | \( W_{\text{BJH}} \, [\text{nm}]^c \) |
|-------------------|---------------------------------|---------------------------------|---------------------------------|
| CMS-SH            | 1225                            | 1.26                            | 2.1                             |
| FRET-CMS          | 599                             | 0.83                            | <2                              |
| CMS-NH₂           | 655                             | 0.98                            | 5.95                            |
| CMS-PEG-COOH      | 475                             | 0.62                            | 5.25                            |
| CMS-SS-COOH       | 864                             | 1.21                            | 2.1                             |
| ZnO@CMS           | 363                             | 0.78                            | <2.1                            |

\( a \) \( S_{\text{BET}} \) is the B-E-T surface area calculated at a relative pressure of \( P/P_0 \) from 0.064 to 0.199.

\( b \) \( V_t \) is the total pore volume measured at a relative pressure of 0.974.

\( c \) \( W_{\text{BJH}} \) is the pore size distribution calculated by the B-J-H method on the desorption branches of the nitrogen isotherms.

4. Conclusions
We have successfully prepared three kinds of nonmaterial’s by grafting HA, HOOC-PEG-COOH and NH₂-ZnO QDs on the surface of colloidalmesoporous silica nanoparticles modified with sulfydryl, amino and carboxyl groups, respectively. The nanoparticles presented with a relatively uniform spherical shape morphology with a mean diameter of about 100 nm. Furthermore, the mesoporous structure of the nanoparticles still remain but the pore diameter would decreased slightly after encapsulated with different functional groups. In addition, the terminated carboxy group of FRET-CMS and CMS-PEG-COOH could couple with many targeting moleculars containing amino functional groups and the ZnO QDS could act as the capping agent of nanoparticles. Therefore, the properties of CMS nanoparticles with different functional groups was worth researching for their potential application as drug delivery carriers.

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