Synthesis and Characterization of Hyaluronic Acid Modified Colloidal Mesoporous Silica Nanoparticles

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Abstract: The colloidal mesoporous silica nanoparticles functionalized with hyaluronic acid (CMS-HA) were successfully synthesized by grafting hyaluronic acid onto the external surface of the amino-functionalized mesoporous silica nanoparticles (CMS-NH2). Moreover, the particle properties of CMS-HA were characterized by fourier transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS) and transmission electron microscopy (TEM). The nanomaterials were negatively charged and had a relatively uniform spherical morphology with about 100 nm in diameter, which could make it more compatible with blood. So the results suggested that the CMS-HA might be a critical nanomaterial for applying in target drug delivery system.

1. Introduction
Spherical mesoporous silica particles with nanoscale as a promising drug delivery system have attracted much attention owing to their excellent properties including high loading capacity, high colloidal stability, facile surface modification ability and excellent biocompatibility [1]. However, although considerable progress has been made, it is still necessary and urgent to overcome the lack of target selectivity of drugs [2]. Here, the intention is to develop an effective material to achieve high targeting efficiency and ensure the high dispersity of CMS in physiological condition [3].

Hyaluronic acid (HA) attached to the surface of nanoparticles can promote specific target and improve drug delivery efficiency via the overexpression of the transmembrane glycoprotein CD44 on cancer cells [4]. HA has been widely reported as a targeting ligand in biomedical field due to its excellent biocompatibility, biodegradability and targeting specificity [5]. Furthermore, HA acting as gatekeepers can be grafted onto various nanoparticles for controlled drugs release [6] since it can respond to hyaluronidase (HAase) which is found to be upregulated in many tumor matrices and endolysosomes [7].

Herein, a novel drug delivery systems based on CMS modified with HA (CMS-HA) has been synthesized via grafting HA onto amino functionalized colloidal mesoporous silica nanoparticles (CMS-NH2) into one system by an amidation reaction (figure 1). Furthermore, doxorubicin (Dox) will be loaded into the CMS-HA and the drug loading efficiency and controlled release properties will be further researched. The CMS-HA which combined the advantages of CMS and HA as a drug carrier might make the delivery process more controllable [8].
2. Experimental Section

2.1. Materials
Tetraethyl orthosilicate (TEOS, 98%), 3-aminopropyltriethoxysilane (APTES, 98%), hexadecyl trimethyl ammonium Chloride (CTAC, 97%) and hyaluronic acid (HA, Mw=10-20 KDa) were obtained from Aladdin Chemistry Co. (Shanghai). N-hydroxysuccinimide (NHS, 98%) and 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimidehydrochloride (EDC•HCl, 99%) were supplied by J&K Chemical Co. (Beijing). Triethanolamine (TEA) was obtained from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2. Synthesis of Amino-functionalized Colloidal Mesoporous Silica Nanoparticles (CMS-NH₂)
The CMS-NH₂ was synthesized according to the published method with minor alterations [9]. Water (64 mL, 3.55 mol), ethanol (10.5 mL, 0.179 mol) and 25 wt % CTAC solution (10.4 mL, 7.86 mmol) were premixed, followed by adding TEA (12.4 mL, 0.093 mol). The mixture prepared as stock solution was stirred at room temperature until all TEA was dissolved, and the pH value of the solution was about 10.5. Typically, the stock solution (20mL) was heated to 60°C, to which TEOS (1.454 mL, 6.5mmol) was slowly added dropwise within 2-3 min under stirring, 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 (10000 rpm, 15 min), washed with ethanol for two times. Extraction of the template from the products was performed by the means of refluxing for two times in a solution containing 5 mL of concentrated hydrochloric acid in 45 mL of ethanol at 60°C for 2 h, and then the CMS-NH₂ was centrifuged (10000 rpm, 15 min), washed with ethanol and distilled water, and redispersed in distilled water (5 mg/mL) for following use.

![Figure 1](image_url). The synthesis route of mesoporous silica nanoparticles modified with hyaluronic acid (CMS-HA).

2.3. Synthesis of Hyaluronic Acid Functionalized Mesoporous Silica Nanoparticles (CMS-HA)
The CMS-HA was synthesized by the amidation reaction between CMS-NH₂ and HA. Briefly, 15 mg HA was hydrated in 3mL distilled water at room temperature and activated by EDC (9 mg) and NHS (6 mg) for 1 h. Then, 5mL of CMS-NH₂ suspension (5 mg/mL) was added to the mixed solution. The solution was stirred for another 24h at room temperature. Finally, the obtained samples were centrifuged, washed with water and ethanol, and redispersed in distilled water to preserve.
2.4. Characterizations
The zeta potential and particle size of the nanoparticles were determined with a Malvern Zetasizer Nano ZS90. The zeta potential and hydrodynamic diameter were measured at 25°C and pH 7. Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100F transmission electron microscope. Fourier transform infrared (FT-IR) spectrum was obtained on Nicolet Nexus 470 using the KBr pellet technique.

3. Results and Discussion
The functionalization of HA onto the surface of CMS was validated by different methods. The FT-IR spectra of HA, CMS, CMS-NH₂, CMS-HA were shown in figure 2. Absorption peaks observed at 1080 cm⁻¹, 800 cm⁻¹ and 960 cm⁻¹ could be attributed to typical vibration bands of siliceous materials (curve B, C, D). Compared with HA and CMS (curve A, B), a new absorption peak at 1516 cm⁻¹ was observed in the sample CMS-NH₂, which can be ascribed to N-H bending vibrations of amino groups (curve C). The appearance absorption band at around 1408 cm⁻¹ can be attributed to the C=O stretching of the carboxyl groups within the HA (curve D), which indicated that HA has been successfully grafted on CMS nanoparticles.

Meanwhile, the particle size and the zeta potential of the nanoparticles were measured by DLS. As indicated in figure 3 and table 1, the zeta potential of CMS, CMS-NH₂ and CMS-HA were -22 mV, +37.5 mV, and -20 mV, respectively. CMS-HA are negatively charged due to the ionizing of the carboxyl groups within HA at pH=7.0 [10]. Moreover, the average size of the CMS-NH₂ and CMS-HA were 151 nm and 508.4 nm, respectively. These results confirmed that HA has been successfully conjugated to CMS-NH₂ by the charge inversion and the growing size.

| Materials      | Size (nm) | PDI      | Zeta (mV) |
|----------------|-----------|----------|-----------|
| CMS-NH₂        | 151 ± 1.3 | 0.101 ± 0.053 | 37.5 ± 2.6 |
| CMS-HA         | 508.4 ± 2.2 | 0.543 ± 0.046 | -20 ± 1.7  |

The morphology of CMS-NH₂ and CMS-HA were analyzed by TEM (figure 4). The spherical morphology and the wormhole arrangement mesoporous of CMS-NH₂ were obviously observed with the diameter of 90 nm in figure 4(a). After grafting HA onto the surface of the CMS, the mean diameter grewed to 100 nm. Though there were no obvious differences in shape and diameter presented between CMS-NH₂ and CMS-HA, it was hard to detect the mesoporous structure of CMS-HA and the clear border could be obviously observed in figure 4(b), which confirmed that HA were
efficiently functionalized on the CMS-NH$_2$. 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 [11].

![Figure 4. TEM of CMS-NH$_2$ (a) and CMS-HA (b).](image)

4. Conclusions
A novel colloidal mesoporous silica nanoparticles modified with hyaluronic acid were successfully synthesized by grafting hyaluronic acid onto the external surface of amino functionalized silica nanoparticles using amide bond as a cross linker. The CMS-HA presented negatively charged and had a relatively uniform spherical morphology with about 100 nm in diameter, which might be more compatible with blood. In addition, the carboxyl groups on the surface of CMS-HA could be combined with various types of functional groups. Therefore, CMS-HA would be a promising nanomaterial for applying in target drug delivery system.

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6. References
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