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Hybrid nanoparticles for drug delivery and bioimaging: Mesoporous silica nanoparticles functionalized with carboxyl groups and a near-infrared fluorescent dye

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ABSTRACT

The development of a drug delivery system with fluorescent biolabels is important in anti-cancer drug delivery application due to the potential for simultaneous diagnosis and treatment of diseases. Here, we reported the synthesis and multiple functionalization of mesoporous silica nanoparticle (MSN) for bioimaging and controlled drug release. After the functionalization with carboxyl group, the nanoparticles exhibited much better dispersity and stability in aqueous solution than MSN. Furthermore, a substantial doxorubicin (DOX) loading level was achieved and DOX-loaded nanoparticles exhibited noticeable pH-sensitive behavior with accelerated release of DOX in acidic environment. Compared with native DOX–MSN, DOX–MSN/COOH–Cy5 exhibited enhanced intracellular uptake efficacy and stronger effect on killing tumor cells. Meanwhile, it was observed that the MSN/COOH–Cy5 was able to locate in the cytoplasm of MCF-7 cells and could accumulate in tumor tissues for a long period of time. Overall, the functional nanoparticle could potentially be used for simultaneous controlled drug release and near-infrared fluorescent bioimaging.

1. Introduction

Cancer remains a major cause of death in most countries in the world, and the incidence of cancer increases with age [1]. Compared to conventional chemo-therapy, nanotherapeutic systems have several potential advantages for cancer treatment. These include increased stability of anti-cancer drugs in blood, decreased non-specific toxicity, easy modification of particle surface for targeting systems and reduced resistance of P-gp expressing cells [2,3]. The mostly studied drug delivery systems nowadays are polymeric micelles systems [4–7], lipid-based drug delivery systems [8,9], polymeric nanoparticles [10,11], carbon nanotubes [12], etc.

Recently, discoveries based on inorganic nanoparticles have opened up new and exciting possibilities in this field [13–15]. Porous inorganic materials, such as mesoporous silica materials, can be easily loaded with drugs without using any organic solvents or additives under very mild conditions, either during the preparation or by post-adsorption into the vast number of nanopores. Mesoporous silica nanoparticles are solid materials, which are comprised of a honeycomb-like porous structure with hundreds of empty channels (mesopores) that are able to absorb/encapsulate relatively large amounts of bioactive molecules. The unique properties, such as high surface area (>900 $m^2 g^{-1}$), large pore volume (>0.9 $cm^3 g^{-1}$), tunable pore size with a narrow distribution (2–10 nm), and good chemical and thermal stability, make them potentially suitable for various controlled drug delivery applications [16–19]. For those applications, the development of mesoporous materials offers new possibilities for incorporating biologically active agents into the well-arranged structure of silica sample followed by the release of these agents from the matrix to the targeted sites [20–23]. Previously, it was observed that mesoporous silica nanoparticle (MSN) could be effectively endocytosed and was able to escape the endolysosomal entrapment [24–27]. More importantly, MSN was proved to be biocompatible [28,29]. These new developments rendered the possibility of designing a new generation of drug delivery system for intracellular controlled release and imaging applications.

Despite the great progresses in using MSN as drug delivery vehicles, a precise control over the release kinetics of the loaded drug by decorating the pore surface with functional groups is challenging [30], and often, an initial burst effect is observed during release. Besides, the distribution behavior of MSN *in vivo* is still unclear. So, more researches are needed to optimize their abilities to release their therapeutic payload in a controlled manner and to

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monitor their cellular and *in vivo* distribution. In this work, MSN functionalized with carboxyl groups and a near-infrared fluorescent dye (MSN/COOH–Cy5) was prepared. The advantages associated with the functionalization of carboxyl groups include excellent aqueous dispersibility and high stability at physiological conditions, high loading capacity for the positively charged model drug doxorubicin (DOX), desirable release acceleration at low pH, enhanced cellular uptake in cancer (MCF-7) cells, and stronger effect on killing tumor cells. More significantly, the conjugation of a near-infrared (NIR) fluorescent dye to the surface of MSN/COOH makes the delivery system suitable for simultaneous bioimaging and controlled drug release, as NIR fluorescence has large penetration depth and tissues (or cells) emit low auto-fluorescence in this region. Recently, MSN labeled with a NIR fluorescence was synthesized by adding Cy5.5-silane conjugate into the synthesis solution to co-condense into the particles [31]. In our work, Cy5-hydrazide was reacted with the carboxyl group on the surface of MSN/COOH via EDC/NHS coupling method to fabricate fluorescent MSN/COOH. Compared with co-condensation, post-synthesis modification does not compromise the framework structure of the parent mesoporous materials.

In addition, the cellular interactions with the hybrid nanoparticle system, cytotoxicity, and *in vivo* imaging were examined in this paper to establish the efficacy of the innovative approach for bioimaging. The results reported here support the potential of carboxyl-functionalized MSN as a nanocarrier for simultaneous controlled drug release and near-infrared fluorescent bioimaging.

2. Experimental

2.1. Materials

Cetyltrimethyl ammonium bromide (CTAB, 98%), tetraethyl orthosilicate (TEOS, 99%), 2-cyanopropyltriethoxysilane (CPTES, 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), and 2-N-morpholino-ethanesulfonic acid (MES) were purchased from Sigma–Aldrich. Cy5-hydrazide was purchased from Lumiprobe. Doxorubicin hydrochloride (DOX) was purchased from Dalian Meilun Biology Technology Co. Ltd. (Dalian, China). Water was purified by distillation and deionization (MilliQ Plus). All other chemicals were analytical grade.

2.2. Synthesis of mesoporous silica nanoparticle (MSN)

MSN was synthesized following a literature procedure [32]. In brief, cetyltrimethyl ammonium bromide (CTAB, 1.2 g) was dissolved in a solution containing water (180 mL), EG (20 mL) and ammonia aqueous solution (5.5 mL, 25%). After vigorous stirring for about 30 min at 60 °C, TEOS (2.4 mL) was rapidly added to the mixture. The resulting mixture was stirred for 2 h at 60 °C and then was stored at 60 °C for 24 h. Samples were washed with deionized water and ethanol several times and collected by centrifugation at 20,000 rpm for 20 min. Then, the surfactant templates were removed by extraction in acidic ethanol (about 9 mL HCl in 100 mL ethanol at 65 °C for 24 h) and separated by centrifugation at 20,000 rpm to obtain the product. The synthesized nanoparticles were freeze-dried for further use.

2.3. Synthesis of carboxyl-functionalized MSN (MSN/COOH)

The pore surface of the MSN was functionalized by co-condensation. In a typical synthesis, 1.2 g CTAB was dissolved in a solution containing water (180 mL) and ammonia aqueous solution (5.5 mL, 25%). After vigorous stirring for about 30 min at 60 °C, 2.0 mL TEOS and 0.4 mL 2-cyanopropyltriethoxysilane were rapidly added to the mixture. The resulting mixture was stirred for another 2 h at 60 °C and was then kept statically at the same temperature for 24 h. Samples were collected by centrifugation at 20,000 rpm for 20 min, washed, and re-dispersed with deionized water and ethanol several times. Then, the dried product was treated with 9 mol L^{-1} H_2SO_4 solution at 100 °C to produce carboxyl-functionalized MSN. At last, the surfactant templates were removed by extraction in acidic ethanol (about 9 mL HCl in 100 mL ethanol at 65 °C for 24 h) and separated by centrifugation at 20,000 rpm to obtain the product. The synthesized nanoparticles were freeze-dried for further use.

2.4. Synthesis of fluorescent MSN/COOH (MSN/COOH–Cy5)

Cy5-hydrazide was reacted with the carboxyl group on the surface of MSN/COOH via EDC/NHS coupling method to fabricate fluorescent MSN/COOH for imaging. A total of 30 mg of MSN/COOH was resuspended in 5 mL MES saline buffer (0.1 mol L^{-1}, pH 5.5) and activated using EDAC (19.1 mg) and sulfo-NHS (21.7 mg) for 1 h. MES buffer was used to slow down hydrolysis of the NHS esters on the MSN/COOH, as it lacks amino and carboxyl groups, which could compete in the reaction. The NPs were then centrifuged to remove excess EDAC/NHS and the water-soluble isourea byproduct. Activated NPs were resuspended in 5 mL MES saline buffer and reacted with Cy5-hydrazide (0.3 mg) for 24 h. The NPs were then centrifuged and washed with deionized water for three times to remove any unbound Cy5-hydrazide and freeze-dried for further use.

2.5. Measurement of MSN/COOH–Cy5

The morphology and structure of MSN/COOH–Cy5 samples were characterized via transmission electron microscopy (TEM). TEM micrographs were obtained on a JEOL JEM-1200EX microscope (Tokyo, Japan). The size distributions and zeta potentials of MSN and MSN/COOH induced by different pH values of aqueous solutions were measured using a Malvern Zetasizer Nano-S90 (England) particle size and zeta potential analyzer. Powder X-ray diffraction (XRD) patterns of surfactant-free MSN/COOH was recorded on a Bruker D4 X-ray diffractometer (Germany) with Ni-filtered Cu Ka radiation (40 kV, 40 mA) at a scanning rate of 0.4° min^{-1} over the range of 0.7°–8.0° with a step width of 0.002°. The pore characteristics of the samples were studied by determining the nitrogen adsorption using a Quantachrome Autosorb-1-C surface area and pore size analyzer (Florida, USA). Approximately 50 mg of the samples was placed into the sample cell, weighed, and outgassed under vacuum at 150 °C for 24 h prior to sample measurement. FTIR spectra were recorded using a JASCO FT/IR-4100 spectrometer (Tokyo, Japan) in the range between 4000 cm^{-1} and 500 cm^{-1}. UV–Vis absorption spectra were performed using a Persee TU-1800PC spectrophotometer (Beijing, China) in the range between 1000 nm and 400 nm.

2.6. Encapsulation and release of model drug doxorubicin (DOX)

To evaluate the drug loading and release properties, doxorubicin (DOX) was used as a model agent. Typically, 10 mg of MSN/COOH–Cy5 samples was immersed in buffered saline (5 mL, pH = 6.5) containing 5 mg of DOX. After stirring for 24 h under light-sealed conditions, the mixture was centrifuged and the supernatant was removed. The DOX-loaded MSN/COOH–Cy5 samples were washed twice with PBS solution to remove DOX that was adsorbed on the surface but not inside the pores. The DOX concentration in original solution and supernatant solution were determined via UV–Vis spectroscopy.