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Hollow mesoporous silica nanoparticles facilitated drug delivery via cascade pH stimuli in tumor microenvironment for tumor therapy

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ABSTRACT

To efficiently deliver anti-cancer drug to tumor site and reduce its toxic side effects on normal tissues, a polyethylene glycol (PEG) shielding and tumor microenvironment triggering cascade pH-responsive hollow mesoporous silica nanoparticles (HMSNs) drug delivery system was fabricated. 3-(3, 4-dihydroxyphenyl) propionic acid (DHPA) functionalized beta-cyclodextrin (β-CD) was grafted onto the surfaces of HMSNs via boronic acid-catechol ester bonds. Then, PEG conjugated adamantane (Ada) was anchored on HMSNs-β-CD nanocarrier via host-gust interaction. Various techniques proved the successful fabrication of the system. The *in vitro* tests confirmed that the system was biocompatible. After the system permeating into tumor via enhanced permeability and retention (EPR) effect, the benzoic-imine bonds between the PEG and Ada were cleaved under weak acid condition in tumor microenvironment (pH 6.8), while the dissociated PEG protective layer facilitating cellular uptake of HMSNs system. Subsequently, the boronic acid-catechol ester bonds linkers further hydrolyzed under even low endosomal pH (4.5–6.5) condition for intracellular drug delivery, leading to efficient cell apoptosis. The *in vivo* results demonstrated that drug loaded HMSNs significantly inhibited tumor growth while only with minimal toxic side effects. The strategy provides new insight into the development of new generation of drug delivery carriers triggering by tumor microenvironment.

1. Introduction

Malignant tumors are worldwide threats to human health [1]. Traditional strategies (radiation therapy *etc.*) treat tumors and normal tissues simultaneously, leading to poor therapeutic effect on tumors and severe toxic side effects on normal tissues [2,3]. Thus, enormous drug carriers were exploited with the development of nanotechnology. Mesoporous silica nanoparticles (MSNs), one of the most promising drug carriers, have attracted extensive concerns owing to its remarkable properties, including highly ordered channels, large surface areas, high pore volume, and massive surface functional groups [4–7]. Thus, a variety of silicon-based composite systems including silica coated upconversion nanoparticles [8], silica nanoparticles mixed with lanthanide [9], dye-doped silica nanoparticles [9] *etc.* were developed for biological imaging, diagnosis and treatment. Meanwhile, silicon quantum dots were also used as nanoprobes for bioimaging [10]. Moreover, silicon nanoparticles have been approved by the US Food and Drug Administration (FDA) for biomedical applications [11].

Except for all advantages of MSNs, HMSNs have enhanced drug storage volume due to the central cavity in structure. Accordingly, to achieve desired therapeutic effect only needs lower amount of HMSNs when comparing with that of MSNs. It thus reduces the potential accumulation of foreign materials in a host in respect to potential biosafety. Therefore, many researchers have devoted to the development of HMSNs drug delivery systems [12–15]. For instance, multifunctional HMSNs were exploited for redox-triggering drug delivery [14]. In another study, α-cyclodextrin molecules were anchored onto the surfaces of HMSNs to achieve pH- triggering drug delivery [15]. Nevertheless, some issues need to be further investigated.

After nanocarriers entering into a host, non-specific plasma protein adsorption and cellular uptake would occur, which leading to rapid clearance from the host via blood circulation. Previous studies confirmed that poly(ethylene glycol) (PEG) motif of PEG-functionalized nanocarriers formed aqueous layers on nanocarriers surfaces, suppressing protein adsorption and recognition

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by reticuloendothelial system for clearance. It thus prolonged the circulation of nanocarriers and then passively targeted tumor site via enhanced permeability and retention (EPR) effect [16,17]. However, the formed PEG layers on nanocarriers surfaces could also inhibit the interactions between the nanocarriers and tumor cells, which hindering the tumor cells' uptake when the nanocarriers reach the tumor site. It would significantly reduce therapeutic efficiency against tumor. The phenomenon was known as 'PEG dilemma' [18,19]. A desirable drug nanocarrier needs to avoid non-specific cellular uptake, to deliver drug to tumor site when it reaches tumor site, and to be triggered by signals from tumor microenvironment for drug delivery. This requires a tumor activating mechanism.

Thus, it is clear that the PEG protective layer of a drug carrier should be removed at tumor site, so as to facilitate phagocytosis by tumor cells. Previously, PEG-sheddable polyplex micelles were developed as gene carriers via matrix metalloproteinase (MMP) cleavable linker, which displayed high cellular uptake, improved endosomal escape and high-efficiency gene transfection in the presence of MMP 2 [20]. However, the MMPs enzyme response process is generally slow.

It is well known that tumor microenvironment is weak acidic with pH value of around 6.5–7.0, lowering than that of normal tissues (pH 7.4) [21,22]. Thus, the pH difference between tumor microenvironment and normal tissues could be employed as a triggering signal for the design of drug delivery systems. In a previous study, a pH-deshielding PEG nanocarrier based on MSNs was developed and triggered by tumor weak acid microenvironment for drug delivery [22]. However, the anticancer drug mostly released to tumor tissue instead of tumor cells, restricting its therapeutic effect. On the other hand, an endosome/lysosome would be formed in cells that encapsulating the drug carrier, once it was uptaken by tumor cells. The tumor endosome/lysosome environment is severe acidic, with pH value of 4.5–5.5 [23,24]. How to utilize the pH signal in endosome/lysosome for triggering intracellular drug delivery is important for improving therapeutic effects [25–28]. Although previous studies reported pH-responsive MSNs systems, however, only using single pH trigger with relatively low pH value [29,30]. Hence, it is reasonable to design cascade pH responsive drug delivery system for tumor therapy, by using tumor microenvironment pH (6.5–7.0) signal to facilitate cancer cell phagocytosis and endosome/lysosome pH (4.5–5.5) signal to trigger intracellular drug delivery. Nevertheless, the related study was not reported so far.

Herein, we report a novel multifunctional tumor microenvironment cascade pH stimuli triggering drug delivery system based on HMSNs for tumor therapy. HMSNs serve as drug reservoirs for loading model anticancer drug of doxorubicin hydrochloride (DOX). The β-CD was employed as gatekeeper of HMSNs due to its appropriate molecular size and good biocompatibility. Briefly, HMSNs were firstly modified with boronic acid molecules for anchoring β-CD onto HMSNs with pH sensitive boronic acid-catechol ester bonds (pH 5.0), leading to encapsulation of DOX within mesopores of HMSNs; Then PEG was grafted to Ada through weak pH sensitive benzoic-imine bonds (pH 6.8); Finally, PEGylated Ada was fixed to the outer orifices of HMSNs by host-gust interaction (Scheme 1A), since it was easy to operate and no introduction of additional materials. Although the binding force of host-gust interaction is generally weaker than covalent conjugation, however, β-CD and Ada had strong binding affinity due to their good size-match and desired hydrophilic and hydrophobic properties. PEG protective layer could efficiently suppress non-specific protein adsorption and cellular uptake, and thus prolong the blood circulation of the system. After the system reaching tumor site via EPR effect, the tumor microenvironment pH signal (6.8) would break down benzoic-imine bonds to release PEG layer for improving cellular uptake. Then, the endosome/lysosome pH signal (5.0) would further hydrolyze boronic acid-catechol ester bonds and dissociate gatekeeper of β-CD, leading to intracellular drug delivery for inducing of cell apoptosis and inhibition of tumor growth (Scheme 1B). Thus, we hypothesized that DOX loaded HMSNs-β-CD/Ada-PEG system could deliver DOX in response to cascade pH stimuli in tumor microenvironment to induce cell apoptosis *in vitro* and inhibit tumor growth *in vivo*.

2. Materials and methods

2.1. Materials

N-cetyltrimethylammonium bromide (CTAB), doxorubicin hydrochloride (DOX), tetraethylorthosilicate (TEOS) and fluorescein isothiocynate (FITC), p-formylbenzoic acid, N,N-Dicyclohexylcarbodimide (DCC), 4-(dimethyl-amino) pyridine (DMAP), 4-carboxyphenylboronic acid (CBA), methoxy poly(-ethylene glycol) (mPEG) (average MW 2000), 3-(3,4-dihydroxyphenyl)propionic acid (DHPA), 1-adamantanecarbonyl chloride, pyridine, 1-adamantanemethylamine (Ada) and 1, 2-dichloroethane were purchased from Sigma–Aldrich (St. Louis, MO, USA). Mono-(6-diethylenetriamine-6-deoxy) -β-cyclodextrin (β-CD-NH_2) was provided by Shangdong Zhiyuan Bio-Technology Co., Ltd (China).

2.2. Synthesis of HMSNs

Firstly, SiO_2 nanoparticles were synthesized with Stoeber method [14]. Briefly, ammonium hydroxide (10 mL) was dissolved into a mixture solution of ethanol/water (428 mL/60 mL) at 30 °C, and TEOS (10 mL) was then added. After stirring for 2 h, the precipitate was collected by centrifugation and washed with ethanol and distilled water each for 3 times, respectively.

Next, SiO_2@CTAB-SiO_2 core/shell nanoparticles were further synthesized. Hexadecyltrimethylammonium bromide (CTAB, 0.15 g) was dissolved into a mixture solution of water (30 mL) and ethanol (30 mL) and then ammonia (0.55 mL) was added. After stirring for 30 min, distilled water (20 mL) containing SiO_2 nanoparticles (0.1 g) was added. Next, TEOS (0.25 mL) was rapidly added and sustained reaction for 6 h. The precipitate were centrifuged and re-dispersed in deionized water (20 mL).

Finally, we synthesized HMSNs via a selective etching approach [14,31]. The above resulting mixture containing SiO_2@CTAB-SiO_2 nanoparticles was stirred for 10 h, and then sodium carbonate (Na_2CO_3, 0.47 g) was added and reacted at 50 °C for 10 h under stirring. The crude product was then suspended into a mixture of methanol/HCL (50 mL/3 mL) and refluxed at 80 °C for 24 h. The final product was obtained through centrifugation and washed with distilled water for 6 times. The product was denoted as HMSNs.

2.3. Synthesis of functional molecules

2.3.1. Synthesis of Ada-PEG

Firstly, we synthesized methoxy poly(ethylene glycol) benzaldehyde (PEG-CHO) [32]. Briefly, mPEG (8 g) was dissolved into dichloromethane (150 mL), and then DMAP (1.2 g), DCC (8.2 g), and p-formylbenzoic acid (6 g) were added to above solution. The solution was filtered and concentrated after stirring for 24 h. The concentrated filtrate was crystallized with isopropanol (80 mL) at 0 °C for 2 h. The crystal was collected by filtration, and then washed with isopropanol and ethyl ether each for 5 times, respectively. The product was denoted as PEG-CHO.

Then, 1-adamantanemethylamine (Ada) (182 mg, 1.1 equiv) was