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Surface modified mesoporous silica nanoparticles as sustained-release gallic acid nano-carriers

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ABSTRACT

Gallic acid (GA) is a natural and non-enzymatic antioxidant with known anticancer property. In the present work, two types of mesoporous silica nanoparticles (MSNs) were evaluated as a nano carrier for sustained release of GA. For this purpose, amine-functionalized (AP-MSNs) and chitosan coated (CS-MSNs) MSNs were synthesized, loaded by GA and then characterized by SEM, XRD, N_2 adsorption isotherms and zeta-potential analysis. Optimum conditions for the loading of GA into the modified MSNs were obtained, and then *in vitro* GA release in simulated gastric fluid (SGF, pHs 1.4) was investigated. Cytotoxicity and cellular uptake of the modified MSNs were also investigated using MCF-7 cells. The encapsulation efficiency and loading capacity were measured 20.77% and 47.06% for AP-MSNs, respectively; while for CS-MSNs, they were obtained as 38.14% and 58.67%, respectively. Results showed that the rate of GA release from CS-MSNs was clearly slower than that from AP-MSNs. Moreover, according to the results, no cytotoxicity effects were observed in both AP-MSNs and CS-MSNs while CS-MSNs showed better killing potency against the MCF-7 cells. The cellular uptake of GA loaded CS-MSNs was also studied by TEM analysis and results showed their high biocompatibility and bioavailability.

1. Introduction

Nowadays, there has been more attention to the application of nanotechnology-based drug delivery systems because such systems can design biocompatible and stable formulations, which deliver drugs to the specific tissue at a definite time by the specific release rate with minimum usage and maximum efficacy [1–3]. Among them, mesoporous silica nanoparticles (MSNs) have shown a great promise as a perfect drug delivery system due to their unique properties like uniform pore size, high surface area, typical honeycomb channeled structure and notable loading and entrapment capacity [4–6]. Many research struggles have been dedicated to modify the chemical properties of MSNs by their surface modification to have high loading and perfect release pattern for drug molecules [7,8]. For instance, MSNs were modified with polymeric dialdehyde dextrin [9], β-cyclodextrin [10], polyethylene glycol [11] and 3-aminopropyl triethoxysilane (APTES) [12]. These surface-modified MSNs have shown better performance in comparison with non-modified MSNs.

Chitosan, the second-most abundant polysaccharide after cellulose, has been widely used to prepare smart drug delivery systems [13,14]. Lv et al. reported a pH-sensitive chitosan-coated MSN for targeted delivery of a ruthenium complex with enhanced anticancer effects [15]. In another work, chitosan was used as a pH-responsive biopolymer shell on the surface of MSNs to form a smart drug delivery system in order to enhance curcumin transport [16].

Gallic acid (GA), as a useful antioxidant with phenolic free radicals, is currently identified as an interesting molecule due to its huge potential for inhibition of chemically induced carcinogenesis development in cancers [17]. During last decades, some efforts have focused on GA loading in conventional MSNs [18]. Although, GA encapsulation within MSNs will increase its shelf life in the body, its loading percent in MSNs is very low. To overcome this problem, the surface of MSNs was functionalized with APTES and the effect of these surface modified MSNs (AP-MSNs) on GA loading capacity and entrapment efficiency was investigated [19]. It was found that drug loading and release profile of AP-MSNs were more suitable than those of MSNs.

To the best of our knowledge, no reports on application of chitosan-coated MSNs (CS-MSNs) in delivery of GA have been reported yet, and also no comparative study to analyze the differences between CS-MSNs and APT-MSNs in controlled release of antioxidants has been carried out so far. Therefore, the purpose of this work was to investigate whether, contrary to previous reports on delivery of GA, the usage of

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CS-MSNs could be of benefit as a more sustained release oral nanocarrier, which could be useful in comparison with typical cancer therapy with faster drug release pattern. To achieve this purpose: 1) AP-MSNs were prepared by a fast grafting method in a non-toxic solvent at room temperature; 2) Using the APTES as a coupling agent on the surface of the modified MSNs, CS-MSNs were prepared through the covalent reaction between chitosan and APTES; 3) Both AP-MSNs and CS-MSNs were loaded with GA and characterized by SEM, XRD, N_2 adsorption isotherms and zeta-potential analysis; 4) Finally, loading capacity, entrapment efficiency and release behavior of GA in the simulated gastric fluid (SGF, pH 1.4) from the both modified MSNs as well as their cytotoxicity effects were investigated and compared.

2. Materials and methods

2.1. Materials

N-cetyltrimethyl ammonium bromide (CTAB), hydrochloric acid (HCl, 37%), acetic acid, sodium hydroxide (NaOH), (3-aminopropyl) triethoxysilane (APTES), acetonitrile, tetraethylorthosilicate (TEOS), ethanol, and mesitylene were obtained from Merck (Germany). Gallic acid, glutaraldehyde (8% aqueous solution), trypsin solution, 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT), chitosan (low moleculare weight, 90% DDA), and PBS were obtained from Sigma-Aldrich Company (USA). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), and penicillin–streptomycin were obtained from Gibco BRL (Gaithersberg, USA). All the solutions were prepared with deionized water. The simulated gastric fluid (SGF, pH 1.4) was prepared as described by Klein [20].

2.2. Preparation of MSNs

Synthesis of the MSNs was performed according to the previous work [18]. Briefly, 3.5 mL of the NaOH (2.0 M) was added to the CTAB solution containing 1 g of CTAB in water (480 mL) at 80 °C. Then, 5 mL of the TEOS was slowly added at a rate of 1 mL/min. The mixture was strongly stirred for 2 h at 80 °C. White precipitate was filtered by 0.45 μm polypropylene filter, washed with water/ethanol solution five times and then dried under vacuum. The CTAB was removed via calcination for 4 h at 540 °C and a heating rate of 1 °C/min.

2.3. Preparation of AP-MSNs

The synthesized MSNs (0.5 g) were dispersed in ethanol (50 mL) by an ultrasonic bath for 15 min; then, the ethanol pH value was adjusted to 3.5 by acetic acid solution using a magnetic stirrer at 500 rpm. The APTES (0.5 g) was quickly added and stirred at 500 rpm for 3 h at room temperature. The AP-MSNs were separated by centrifugation at 7000 rpm for 5 min, washed three times with ethanol and then dried under vacuum at room temperature overnight.

2.4. Synthesis of CS-MSNs

The CS-MSNs were prepared according to the procedure reported by Gulfam et al. [21] with minor modification. Chitosan (2.0 g) was first dissolved in 200 mL acetic acid solution (5 wt%). After magnetic stirring for 24 h at room temperature, a transparent solution of chitosan (1% w/v) was formed. The MSNs (0.1 g) were dispersed in ethanol (10 mL) with ultrasonic bath for 15 min and the ethanol pH value was adjusted to 3.5 by acetic acid solution. The APTES (0.1 g) was quickly added and stirred at 500 rpm for 3 h at room temperature. The chitosan solution (20 mL) was added and then stirred for 24 h at room temperature. The CS-MSNs were centrifuged at 7000 rpm for 5 min, washed with deionized water and ethanol and then dried under vacuum oven at 25 °C.

2.5. Characterization of prepared MSNs

Q11 XRD patterns were obtained using a multipurpose diffractometer (Philips X' Pert) with a thin film attachment to measure low-angle glazing incidence. A Micromeritics ASAP 2010 analyzer was used for determination of surface area and porosity. Afterwards, the pore size distributions were obtained from the desorption branch using the Barrett–Joyner–Ha lend a (BJH) method, and then pore volumes were measured at P/P0 = 0.2–0.4. Field emission scanning electron microscopy (FE-SEM, Philips XL-30), at a working distance of 8.5–10.5 mm and voltage of 25.0 kV, was used to determine the particle morphology. For this purpose, MSNs were sputter-coated with gold using a Benchtop Turbo III Sputter Coater (Denton Vacuum). In addition, the morphology and structure of the nanoparticles were analyzed using transmission electron microscopy (TEM, Philips CM120, 120 kV, LaB6 emitter, Twin lens: resolution 0.34 nm (point), 0.20 nm (line), Magnification range: 35× - 750,000×). The ultrathin polymerized films (thickness of ca. 50 nm) were prepared using microtoming. Nanoparticles for TEM images were prepared by depositing a drop of suspension onto carbon-coated copper grids.

Zeta potential measurements were performed using a Malvern Zeta sizer Nano (S90, UK) at 25 °C in buffer solutions. The endocytosis of the nanoparticles into the MCF-7 cells was studied with transmission electron microscopy (Philips CM120). HPLC (Yunglin, YL9100) unit equipped with a UV detector used for determination of the released GA into SGF mediums.

2.6. Measurement of loading capacity and entrapment efficiency

Solutions with different concentration of GA in ethanol (10, 20, 30, 40 and 50 mg/mL) were prepared. Then, 10 mg of the AP-MSNs and CS-MSNs were separately added to 2 mL of each solution. The vials were located on a shaker at 155 rpm at room temperature in darkness for 24 and 48 h. Then, the GA-loaded AP-MSNs (AP-MSNs/GA) and GA-loaded CS-MSNs (CS-MSNs/GA) were separated by centrifugation at 7000 rpm for 5 min, washed with ethanol three times, and dried under vacuum at room temperature. The supernatant was removed for HPLC analysis. Drug loading capacity (LC) and entrapment efficiency (EE) were calculated according to the following equations:

$$\text{LC (\%)} = \text{Weight of GA into nanoparticles} / \text{Weight of total nanoparticles} \quad (1)$$

$$\text{EE (\%)} = \text{Weight of GA into nanoparticles} / \text{Weight of feeding GA} \quad (2)$$

2.7. In vitro GA release

The *in vitro* drug release from the AP-MSNs/GA and CS-MSNs/GA was determined in the SGF (pH 1.4). Briefly, 10 mg of the GA-loaded MSNs were suspended into a vial containing 20 mL of buffer solution. For each solution, a blank was prepared with free GA nanoparticles. Then, the vials were put on a shaking water bath at 37 °C and 80 rpm. After predetermined time intervals, the solutions were centrifuged at 20000 rpm for 5 min, 1 mL of the supernatant was occasionally removed and dissolution medium was immediately replaced with a fresh medium. The amount of the released GA in the supernatant was analyzed by the HPLC. All the measurements were performed in triplicate.

2.8. HPLC condition

The released GA was determined by high-performance liquid chromatography (HPLC) (Young Lin-9100, Korea) in a system consisting of a quaternary pump coupled to a UV detector. A conventional reversed-phase Athena C18-WP (Column: 4.6 mm × 250 mm, 100 A°, 5 μm) was used as the stationary phase. The gradient elution program was