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pH-activated doxorubicin release from polyelectrolyte complex layer coated mesoporous silica nanoparticles

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ABSTRACT

Mesoporous silica nanoparticles (MSN) functionalized with doxorubicin (Dox) and coated with a polyelectrolyte complex layer were tested *in vitro* to investigate drug release in cellular environment. The mesoporous silica nanoparticles inner surface was efficiently functionalized with the positively charged antitumoral drug doxorubicin. Polyelectrolyte layer complex was adsorbed on the outer surface of the MSN improving colloidal stability in biological media and forming an electrostatic barrier against the doxorubicin diffusion at biological pH. Dox-loaded silica nanoparticles showed a pH-dependent drug release behavior. Cell uptake of mesoporous silica nanoparticles and drug release dynamic were real-time monitored by laser scanning confocal microscopy. These results suggest that MSN could be exploited as smart carrier with pH-triggered drug releasing properties.

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1. Introduction

The advantages provided by nanotechnology in the battle against cancer were proven extensively in the last years [1,2]. Compared with conventional therapeutic drugs which are nonspecifically distributed in the body, nanomaterials-based drug delivery systems can reduce harmful side effects improving the therapeutic efficacy [3]. Nanoparticles with dimension lower than 200 nm can be preferentially accumulated in the tumor through the enhanced permeability and retention (EPR) effect [4]. This is a clear advantage for nano-based drug delivery and therapeutic agents, because of the possibility to infiltrate the tumor tissue with a variety of different nanosystems including metal nanoparticles [5], magnetic nanoparticles [6], polymeric vectors [7] and multifunctional system composed by combination of the aforementioned nano-objects [8].

Mesoporous silica nanoparticles (MSN) prepared by surfactant templating methods [9] possess attractive features such as well-defined and controllable pore size, large surface area and reactive surfaces for easy functionalization which make them ideal as potential carriers for drugs. Indeed, mesoporous silica nanoparticles were investigated as drug delivery vehicles because of their low toxicity and very high specific surface area with abundant silanol groups on the pore surface. It is known that nanoparticles can accumulate into the liver and spleen after repeated *in vivo* injections [10]. This can cause some side effects in patients and normally limits the amount of drug that can be injected in the body. This is why vectors able to incorporate a high amount of chemioterapic agents are preferable. Jinlou Gu et al. reported that MSN can incorporate 90% in weight of drugs, although with nanoparticles of relatively low dimensions [11]. Moreover, the confinement of the drug into the MSN nanopores provides a physical barrier to enzymatic degradation and premature drug release.

Another key advantage of the mesoporous silica nanoparticles is their biodegradability. Recent experiments using simulated body fluids showed that MSN can be degraded to silicic acid [12]. Lu et al. showed that after 24 h from MSN injection in mice around 95% of silicon was released by urine and feces [13].

Moreover, MSN with dimensions lower than 100 nm undergo endocytosis with higher cellular uptake efficiency. Considering the acidic environment conditions in the tumor area and also the acidic pH of internal cell organelles (pH 4–5 in the lysosomes and pH 6 near the cancer cell membrane) [14,15], pH-sensitive nanoparticles represent an important tool in drug delivery.pH-sensitive polyelectrolyte has been already explored in several applications as carriers for controlled delivery of drugs [16]. These polymers contain acid and basic groups and can undergo structural changes in response to variations of the pH of the environment, which facilitates drug delivery control.

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In this paper we describe a versatile route to synthesize polyelectrolyte-coated MSN loaded with doxorubicin. The novel strategy to conjugate polyelectrolyte complex layer onto MSN described in this paper may leads to a better Dox release control and thereby to a great improvement in cancer therapy efficacy.

Doxorubicin release by the MSN was investigated in *in vitro* cultured cells by laser scanning confocal microscopy (LSCM). The doxorubicin release profiles at pH 5.0 and 7.5 were substantially different demonstrating that electrostatic interaction between entrapped drug molecules and MSN silanol groups plays a significant role in the drug release kinetics.

2. Experimental section

2.1. Materials

Tetraethyl orthosilicate (TEOS, Aldrich, 98%), hexadecyl-trimethylammonium bromide (CTAB, Aldrich), ammonium hydroxide solution (Fluka, 28 wt.% in water), absolute ethanol (99.8%, Carlo Erba) were all used as received.

Poly (allylamine hydrochloride), M_w 4500, poly acrylic acid (M_w 4000) doxorubicin and all other chemicals were purchased from Sigma unless otherwise indicated. All reactions were performed using milli-Q water unless indicated in the text.

2.2. Synthesis of mesoporous silica nanoparticles

Mesoporous silica nanoparticles were prepared according to the method by Qiao et al. [17]. To a 500 ml round bottomed flask were added 128 ml milli-Q water, 22.8 ml ethanol, 5.73 g CTAB, 17.2 ml milli-Q water, 1.25 ml 28% ammonia solution. The reaction contents were stirred at 60 °C for 30 min followed by addition of 14.6 ml TEOS and continued stirring for two hours at 60 °C. The obtained solid was recovered by repeated centrifugation (20 min at 9000 rpm) and sonication (30 min) by washing once with water and three times with ethanol. The solid was dried under reduced pressure (10-1 mbar for 1 h) and finally was calcinated at 550 °C for 6 h to remove the templating surfactant from the pores.

2.3. Synthesis of mesoporous silica nanoparticles-doxorubicin conjugates (MSN-Dox)

1 mg of preformed MSN was dispersed in 2.5 ml of 0.15 mg/ml doxorubicin water solution at pH 7.5 under magnetic stirring for 15 h. The MSN-doxorubicin conjugate was then centrifuged at 9000 rpm for 10 min. The supernatant was collected and analyzed by UV–Vis spectroscopy. The loading efficiency was 98.4% and the drug loading capacity (measured as weight of drug in nanoparticles/weight of drug loaded nanoparticles) obtained was 27.2%.

2.4. Synthesis of MSN-Dox/PAH sample

MSN-Dox nanoparticles (0.1 mg) were mixed with an aliquot (2 ml) of water solution with pH adjusted to 9 by NaOH containing 0.1 mg/ml of poly (allylamine hydrochloride) under magnetic mixing for 20 min. The nanoparticles were then centrifuged at 9000 rpm and washed with water three times (sample MSN-Dox/PAH).

2.5. Synthesis of MSN-Dox/PAA sample

MSN-Dox/PAH nanoparticles (0.1 mg) were mixed with 2 ml of water solution containing 0.1 mg/ml of poly (acrylic acid) under magnetic mixing for 20 min. The nanoparticles were then centrifuged at 9000 rpm and washed with water three times (Fig. 1).

2.6. Doxorubicin release from MSN-Dox and MSN-Dox/PAA samples

Drug release analysis was performed at room temperature. MSN-Dox and MSN-Dox/PAA nanoparticles were dispersed in 2.0 ml PBS (10 mM, pH 7.4 and 5.0). At predetermined time intervals, the nanoparticle solutions were centrifuged (9000 rpm, 10 min) and the supernatant was separated from the pellets and analyzed. The total amount of the drug adsorbed onto the nanoparticles (NP) was calculated by using a calibration curve for the doxorubicin. The same curve was used to quantify the amount of Dox released by the NP as function of time at different pH.

2.7. Cell culture

The human lung carcinoma epithelial cell line A549 was cultured in RMPI-1640 medium (Sigma R7509) supplemented with 10% FBS, 4 mM glutamine and incubated at 37 °C in an atmosphere of humidified air with 5% CO_2.

2.8. Cell uptake and drug release investigations of MSN-Dox/PAA

50.000 A549 cells were plated onto 18-mm glass coverslips in a 24-well plate and allowed to grow for 2 days.

For the *in vitro* experiment, the cells were incubated with 200 μl of 0.1 mg/ml MSN-Dox/PAA nanoparticles for 1, 2 and 17 h and then analyzed by laser scanning confocal microscopy.

For doxorubicin localization the excitation wavelength was set to 488 nm and the acquisition range was 550–620 nm.

2.9. MSN characterization

UV–Vis absorption measurements have been performed using an UV–Visible–near infrared spectrophotometer (Cary 5000) in dual beam mode. Photoluminescence spectra were acquired by using a Xe lamp as excitation source coupled to a single grating monochromator, in a spectral range extending from 400 to 750 nm.

TEM images were taken with a JEOL 3010 operating at 300 kV and equipped with a GATAN (Warrendale, PA, USA) multi-scan CCD camera and an Oxford EDS microanalysis detector. TEM specimens were prepared by ultrasonically dispersing the powdered samples in ethanol (approximately 10 mg ml^{-1}) and depositing several drops of the suspension on a holey carbon film supported by a copper grid.

Nitrogen adsorption–desorption measurements were performed at liquid nitrogen temperature (−196 °C) with an ASAP 2010 apparatus of Micromeritics. The analysis procedure is fully automated and operates with the static volumetric technique. The N_2 isotherms were used to determine the specific surface areas through the BET equation (SABET) and the specific pore volume (Vs) calculated at $p/p_0 = 0.98$.

Particle size and ζ-potentials were determined at 25 °C by dynamic light scattering (DLS) in back scattering mode, using a laser particle sizer (Malvern Zetasizer Nano ZS, equipped with a He–Ne laser at 633 nm). For this assay the samples were diluted in milli-Q water to a final concentration of 0.01 mg/ml. Data were analyzed by the Malvern Proprietary Software.

Laser scanning confocal microscopy (LSCM) analysis was carried out using a Leica 200D microscope equipped with an argon laser source. The XPS measurements were carried out using an ESCA200 instrument (Scienta-Gammadata ESCA 200 Uppsala Sweden). Wide scans were acquired in the binding energy (BE) range 1200–0 eV using a 500 eV pass energy while the core lines were acquired at 150 eV pass energy to increase the energy resolution.

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