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Thermo-responsive mesoporous silica/lipid bilayer hybrid nanoparticles for doxorubicin on-demand delivery and reduced premature release

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ABSTRACT

Hybrid nanocarriers based on mesoporous silica nanoparticles (MSNs) and supported lipid bilayer (SLB) have been studied as drug delivery system. It still remains challenges to develop these nanocarriers (SLB-MSNs) with on-demand drug release profile for chemotherapy. Here, we reported the biocompatible SLB-MSNs with high drug loading, which could release doxorubicin (DOX) in response to hyperthermia and reduce premature release. After synthesis of MSNs *via* a sol-gel procedure, the thermo-responsive SLB was deposited on the MSNs by sonication to completely seal the mesopores. The obtained SLB-MSNs consisted of 50 nm-sized MSN cores and 6.3 nm-thick SLB shells. Due to the big surface and pore volume of MSNs, the high drug loading content ($7.30 \pm 0.02\%$) and encapsulation efficiency ($91.16 \pm 0.28\%$) were achieved. The SLB blocking the mesopores reduced 50% of premature release and achieved on-demand release in a thermo-responsive manner. Moreover, SLB-MSNs showed good hemocompatibility at any tested concentration (25–700 μg/mL), while bare MSNs caused 100% of hemolysis at concentration larger than 325 μg/mL. In addition, *in vitro* U251 cell uptake experiment demonstrated that compared with uncapped MSNs, SLB-MSNs could prevent untargeted cellular uptake of DOX owing to reduced premature release and steric hindrance of PEG, which would be beneficial to minimize toxicity for healthy tissues. These results indicated that SLB-MSNs with thermo-responsive release capacity possessed great potential in future synergistic thermo-chemotherapy.

1. Introduction

The application of nanoparticles-based delivery system for tumor therapy has received considerable interesting in the past decades. Due to the enhanced permeability and retention (EPR) effect, intravenously injected nanoparticles can passively accumulate into the tumor tissue [1,2]. Moreover, surface modifications to the nanoparticles by using hydrophilic protein-repellent polymers (e.g. PEG) can enhance biocompatibility and prolong circulation time, leading to increased possibility for passive targeting [3,4]. Furthermore, attachment of targeting head groups like peptides, antibody, and small molecules to surface of nanoparticles can result in active targeting, which could enhance tumor penetrating and cancer cellular endocytosis [5,6]. Therefore, numerous kinds of nanoparticles have been developed to deliver chemotherapy agents for improved efficacy and reduced toxicity toward cancer therapy.

Among these nanoparticles, liposomes represent one of the most successful nanocarriers for passive and active targeted delivery, and they are now approved by FDA for cancer therapy with considerable clinical acceptance [7]. The advantages of liposomes mainly include ease of surface modification, high biocompatibility and biodegradability, prolonged circulation time, targeting capacity, and improved biodistribution [4]. Despite of above merits, liposomes as a type of soft matter nanocarrier yet suffer from some drawbacks such as limited stability, low drug loading content, and payload leakage [8]. By contrast, mesoporous silica nanoparticles (MSNs) as rigid nanoparticles can eliminate such defects of soft liposomes. They have good colloidal stability and well controlled size and shape [9]. Particularly, the unique mesopore structures of MSNs afford vast internal surface area and big pore volume, which are responsible for high drug loading [10]. However, some disadvantages limit the wide application of MSNs as a potential drug delivery system. Generally, the drug loading in MSNs depends on

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electrostatic interactions and/or physical absorption [11]. Without tailored mesopore capping, drugs loaded in the accessible mesopores through electrostatic attraction or adsorption could freely leach out into the body fluid *via* ions/molecules exchange mechanism, leading to premature release [12,13]. Furthermore, bare MSNs without coating will display surface silanol, which could non-specifically interact with various cells, especially resulting in poor hemocompatibility [14]. The above drawbacks of MSNs are likely to be addressed by coating MSNs with liposomes.

Recently, the kind of inorganic-organic hybrid nanoparticles, characteristic of coating MSNs with liposomes, has been reported [13,15–17]. When supported lipid bilayer (SLB) shells are deposited on the MSNs cores, the hybrid nanoparticles (SLB-MSNs) were formed[10]. Besides the independent merits from MSNs and liposomes, SLB-MSNs present some outstanding properties stemming from synergism of SLB and MSNs. Firstly, the adhesion energy between MSNs and SLB suppresses large-scale fluctuation of SLB to stably seal MSNs, preventing premature release [10]. Secondly, the MSNs and SLB with different polarities can simultaneously accommodate hydrophilic and hydrophobic drugs in the same nanocarrier, which is beneficial for combination chemotherapy [18]. Lastly, the synergistic action from the composite structure contributes to construct stimuli-responsive delivery system to achieve on-demand release at tumor site [19,20].

Regarding tumor-specific stimuli, local hyperthermia is considered as one of the most promising physical stimuli for site-selective drug delivery. It is well known that local hyperthermia can be conveniently created through the entire tumors by several means including ultrasound, magnetic field, light, and radiofrequency [21,22]. Additionally, it has been well developed to integrate energy absorbing materials (like iron oxide and gold nanoparticles) into nanocarriers to elevate the local temperature of tumors [23,24]. Importantly, local hyperthermia therapy with minimal invasion can induce cancer cellular apoptosis and necrosis so as to synergistically improve the effects of chemotherapy [25]. Thus developing the thermo-responsive drug delivery systems should be important to anticancer therapy. In the context of SLB-MSNs, previous studies have demonstrated that SLB-MSNs were capable of releasing payload in response to internal stimuli (pH and redox) in tumor microenvironment [26,27]. Nevertheless, few examples of the thermo-responsive SLB-MSNs delivering chemotherapy agent to tumors have been reported.

In this work, we integrated the advantages of liposomes and MSNs to fabricate a thermo-responsive inorganic-organic hybrid nanocarrier with high biocompatibility, which could effectively reduce leakage of loaded antitumor drug (doxorubicin, DOX) at physiological temperature, but release the payload at a hyperthermia temperature. Uniform MSNs as core for drug loading were synthesized by a sol-gel procedure. After drug loading into MSNs, thermo-responsive phospholipids bilayer as mesopore gatekeeper was supported on the MSNs core by simple sonication to form the hybrid nanoparticles (Scheme 1). Moreover, the morphology, infrared spectra, drug loading, thermo-responsive release, hemocompatibility, and the cellular uptake of DOX were also evaluated. It was demonstrated that this thermo-responsive SLB-MSNs delivery platform should be promising in the synergistic thermo-chemotherapy for tumors.

2. Materials and methods

2.1. Materials

Hexadecyl trimethyl ammonium chloride (CTAC), tetraethyl orthosilicate (TEOS), sodium chloride (NaCl), hydrochloric acid (36.5−38%, HCl), cholesterol (Chol), and ethanol were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Triethanol amine (TEA), triton X-100 (TX100) were supplied by Aladin Chemical Co. Ltd (Shanghai, China). 1,2-Distearoyl-sn-*glycero*-3-phosphocholine (DSPC), 1,2-distearoyl-sn-*glycero*-3-phosphoethanolamine-N-[methoxy-(polyethylene glycol)-2000] (DSPE-PEG2000), 1,2-dipalmitoyl-sn-*glycero*-3-phosphocholine (DPPC) were obtained from Advanced Vehicle Technology Pharmaceutical Co. Ltd (Shanghai, China). A hydrochloride salt of doxorubicin was purchased from Zhejiang Hisun Pharmaceutical Co. Ltd (Taizhou, China). All the other chemical reagents were analytical grade and used as received.

U251 cells were obtained from Shanghai Cell Bank, Chinese Academy of Medical Sciences. The cells were maintained in RPMI 1640 medium, and supplemented with 10% (v/v) FBS, 100 Unit/mL penicillin and 100 mg/mL streptomycin sulfate at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Synthesis of MSNs and DOX loading

Synthesis of MSNs was described in our previous report [12]. One g of CTAC, 40 mg of TEA, and 40 mL of water were mixed and stirred intensively for 1 h in an oil bath at 95 °C. Then, 3.0 mL TEOS was added drop wise into the above solution. After another 1 h of stirring, the synthesized MSNs were separated by centrifugation. To remove the template in the MSNs, nanoparticles were extracted by refluxing with a solution containing hydrochloric acid and ethanol (1:9, v/v) for 6 h at 78 °C.

To prepare the DOX loaded MSNs, DOX was dissolved in PBS (pH 7.4, 10 mM) to be 0.4 mg/mL. Next, 25 mg of MSNs were dispersed in 5 mL of DOX solution by ultrasonic and the mixture was shaken in dark on a reciprocating shaking-table for 8 h.

2.3. Assembly of SLB-MSNs

The thermo-responsive SLB was composed of DPPC/DSPC/Chol/DSPE-PEG2000 at a molar ratio of 65:5:25:5. These components with a total lipid mass of 30 mg were dissolved in chloroform at 5 mg/mL in a round bottom flask. A thin lipid film was formed on the bottom of flask after the organic solvent was removed by a rotary evaporator. Then, 25 mg of MSNs dispersed in 5 mL solution were added on top of the lipid film. Next, this lipid film was hydrated for 15 min at 37 °C, and the suspension was treated with bath sonication for 10 min to fuse the lipid bilayers and MSNs. The resulting SLB-MSNs were separated by centrifugation and redispersed in PBS.

To determine drug loading content (LC%) and encapsulation efficiency (EE%), the loaded MSNs in DOX solution were added on top of lipid film and treated according to the same way described previously. The DOX loaded SLB-MSNs were separated by centrifugation and the fluorescence of DOX in supernant was measured by a fluorescence spectrophotometer (Hitachi, F4600, JP) to calculate the concentration of free drug. We defined LC% = (the amount of loaded DOX/the amount of MSNs) × 100%, and EE% = (the amount of loaded DOX/the total amount of DOX added) × 100%.

2.4. *Drug* in vitro *release study*

To determine the *in vitro* release behavior of DOX from nanoparticles under different conditions, 0.82 mL of nanoparticles suspension (containing about 300 μg of DOX) was dialyzed against release medium with a dialysis bag (molecular weight cutoff of 3000 Da). The release medium was 60 mL of 150 mM NaCl solution (pH 5.0 and 7.4, respectively) at 37 °C and shaken at 120 rpm in dark. At desired time intervals, 0.5 mL of release medium was taken out to quantify the concentration of DOX released by fluorescence spec-