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Facile synthesis of the lipid bilayer coated mesoporous silica nanocomposites and their application in drug delivery

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ABSTRACT

The lipid bilayer coated mesoporous silica nanocomposites (LMSNs) were synthesized with aim to obtain better performance in the application of drug delivery. Phospholipids composed of soybean lecithin and DSPE-PEG 2000 were pre-prepared into liposomes, then they were allowed to fuse onto the mesoporous silica nanoparticles (MSNs) forming a surrounding lipid bilayer. The obtained LMSNs had an average particle size of 295 nm, zeta potential of −1.0 mV and a good dispersing stability in saline buffers. Facilitated by the affinity of the lipid bilayer with cell membrane, the internalization of LMSNs by cells was markedly increased. In addition, compared with bare MSNs, the cytotoxicity, hemolysis percentage and nonspecific BSA absorption of LMSNs were significantly reduced, making them become more reliable carriers for drug delivery. When encapsulating a model drug, doxorubicin (DOX) into LMSNs, the loading efficiency can reach as high as 16%. The obtained LMSNs-DOX exhibited a pH-responsive release behavior and the presence of the lipid bilayer did not significantly retard the release of DOX. Furthermore, LMSNs greatly enhanced the cellular accumulation and cytotoxicity of DOX toward the MCF-7 cells. In summary, the lipid bilayer coating was a simple and facile strategy to functionalize MSNs, and the obtained LMSNs exhibiting good biocompatibility were promising nanocarriers in improving the cellular uptake and therapeutic efficacy of anticancer drugs.

1. Introduction

In recent decades, a variety of nanostructured materials, such as liposomes, micelles and organic/inorganic nanoparticles have attracted great interest as intelligent drug delivery systems and showed promising prospects in a broad range of therapeutic applications [1–4]. Among these, mesoporous silica nanoparticles (MSNs) are particularly attractive due to their unique properties, including a large surface area and pore volume to load drug with high efficiency [5,6]; a uniform and tunable pore size to accommodate molecules with various steric hindrance and an excellent physiochemical stability to protect the encapsulated drugs from degradation by endogenous enzymes [7].

Recently, MSNs have been widely employed as nanocarriers to achieve stimuli-responsive drug release and targeted drug delivery [8–10]. However, the application of MSNs as drug carriers has been limited by some of their properties. Such as easy aggregation in saline buffers, rapid clearance by the reticuloendothelial system (RES) and the risk of inducing hemolysis when given intravenously, a common route of administration [11]. Therefore it is necessary to functionalize MSNs in order to counteract the above disadvantages. Surface modification with polymers, dendrimers [12] or natural materials through chemical reaction or electrostatic attraction (such as layer by layer technique) [13] was reported to be an effective way to change the properties of nanoparticle for various applications. However, previous studies devoting to improve the biocompatibility of MSNs as drug carriers by surface modification were limited and rare, including using polymers such as polyethylene glycol (PEG) or poly(N-vinylcaprolactam-co-methacrylic acid) (P(VCL-s-s-MAA)) [14]. But the functionalization process was complicated and needed to be optimized. In addition, there has

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always been a contradiction in the priority of order between the drug loading and surface functionalization process. If the functionalization comes first, it may cause a reduction in the surface area and pore volume which will adversely affect the drug loading efficiency of MSNs. While if the drug loading is a priority, there will be an inevitable loss of the loaded drugs during the long time chemical reaction and repeated purifying process which are necessary for the successful functionalization. Therefore, it is essential to find a simple and effective approach to modify the MSNs and overcome the above drawbacks of them without adversely affecting the drug loading capacity. Furthermore, as a drug carrier, it is also of great importance to improve the cellular accumulation of the incorporated drugs within the targeted cells to reach effective therapeutic level.

Inspired by the supported lipid bilayer which mimics the cell membrane, the lipid bilayer coating can be an innovative functionalization on MSNs by fusion on the surface of them. The preparation method was based on the previous findings by Mornet et al., in which the pre-prepared liposomes can first adhere to MSNs, then undergo gradual deformation, finally rupture and spread all over the nanoparticles to form a continuous coverage [15]. Reasons for using the lipid bilayer coating on mesoporous silica nanoparticles (LMSNs) are as follows: 1) lipids are well-recognized to be highly biocompatible; 2) the lipid bilayer has a strong affinity with the cell membrane which is advantageous for the subsequent internalization by cells; 3) the PEG modified lipid bilayer can prolong the circulation time and reduce the immunogenicity of MSNs, thereby facilitating the accumulation of drug-loaded carriers at the tumor site via the enhanced permeability and retention (EPR) effect. Moreover, compared with other functionalization methods, the lipid bilayer coating is rather simple and facile. Since it can be completed within a short period of time, the loss of the loaded drugs can thereby be markedly reduced. This integrated drug delivery system is expected to inherit the merits from both MSNs and liposomes and exhibit characteristics superior to those of any of its single component. In the nanocomposites, MSNs acting as a drug reservoir can accommodate various drug molecules with a high payload and serve as a supporting skeleton to stabilize the lipid bilayer. While the lipid bilayer coating can in turn serve to improve the dispersing stability and biocompatibility of MSNs and make them more applicable as drug carriers by mitigating their disadvantages. In several other studies, the lipid bilayer present on MSNs was designed as a “gatekeepers” to control drug release or an intermediate for further conjugation of various targeting ligands. There are rarely any literature studied the effect of mere lipid bilayer coating on the properties of MSNs as drug carriers [16–18].

In this study, we innovatively investigated the effect of the lipid bilayer coating on the applicability of MSNs as carriers for intravenous drug delivery. And the superiority of the prepared LMSNs was systematically evaluated by comparing them with bare MSNs in terms of dispersing stability, cellular uptake efficiency and biocompatibility. The prepared LMSNs were then employed to load doxorubicin (DOX), one of the most potent drugs used in clinical chemotherapy. And the in vitro drug release, cellular internalization, and cytotoxicity of LMSNs-DOX were systematically investigated.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS, 98%), hexadecyl-trimethyl ammonium bromine (CTAB, >99%) and ammonium hydroxide (NH_4OH, 25–28%) were obtained from Tianjin Bodi Chemical Holding Co., Ltd (Tianjin, China). Poloxamer 407 (Lutrol® F-127) was obtained as gift sample from BASF (Ludwigshafen, Germany), Soybean lecithin and cholesterol were purchased from LIPOID GmbH (Ludwigshafen, Germany). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] ($mPEG_{2000}$-DSPE) and Rhodamine B PE was obtained from Avanti Polar Lipids (Alabaster, AL). Anticancer drug doxorubicin hydrochloride (DOX) was offered by Zhejiang Hisun Pharmaceutical Co., Ltd (Zhejiang, China). RPMI 1640, fetal bovine serum (FBS) and penicillin/streptomycin solution were attained from GIBCO (BRL, MD, USA). Bovine serum albumin (BSA), fluorescein isothiocyanate (FITC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and trypsin were purchased by Sigma–Aldrich (St. Louis, MO). Fluorescent Hoechst 33258 was obtained from Molecular Probes (Eugene, OR, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Synthesis of mesoporous silica nanoparticles (MSNs)

As reported previously, mesoporous silica nanoparticles were prepared based on the modified Stöber method [19]. In brief, 0.5 g CTAB and 1.0 g triblock copolymer F127 were dissolved in 100 mL distilled water, in which 10 mL 29 wt.% ammonium hydroxide solution and 40 mL ethanol were subsequently added. After complete dissolution, 1.9 mL TEOS was then introduced dropwise to the mixture under intensive stirring, and the reaction was maintained at room temperature for 24 h. The white solid product was collected by centrifugation at 10,000 rpm. For template extraction, the synthesized nanoparticles were re-dispersed in 100 mL absolute ethanol containing 1 g NH_4NO_3, and allowed to reflux at 80 °C overnight with constant stirring to remove the surfactant. The final product was centrifuged, washed with ethanol for 3 times and dried overnight at room temperature in vacuum.

2.3. Preparation of liposomes

Liposomes were prepared by the lipid film hydration method, in the experiment 100 mg soybean lecithin, 10 mg DSPE-PEG 2000 and 20 mg cholesterol were dissolved in 5 mL chloroform and evaporated to form a thin lipid film using a rotary evaporator. Then the lipid film was hydrated in PBS of pH 7.4 at concentration of 10 mg/mL and was then extruded for 15 cycles using a mini extruder (Avanti Polar Lipids, Inc., USA) equipped with progressively decreasing pore-sized polycarbonate membrane of 200 nm and 100 nm. The prepared liposomes for further experiment were stored at 4 °C for no more than a week.

2.4. Development of the lipid bilayer-mesoporous silica nanocomposites (LMSNs)

The LMSNs were prepared by first re-dispersing 20 mg of dried MSNs in 2 mL distilled water under ultrasonic irradiation for 1 min. Then 1 mL of the pre-prepared lipid vesicles was added to the particle suspension, the mixture was well vortexed and left to stand for 1 h at room temperature to allow thorough fusion of liposome on MSNs. Extra liposomes in the supernatant were removed via centrifugation at 10,000 rpm for 10 min. After being washed with PBS for three times, the formed LMSNs were finally dispersed in the PBS solution.

2.5. Drug loading process and loading efficiency measurement

Drug loading was conducted prior to the lipid bilayer coating, 20 mg of the dried MSNs was ultrasonically re-suspended in 2 mL of