Colloids and Surfaces B: Biointerfaces 136 (2015) 470–478

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Synthesis of multifunctional bovine serum albumin microcapsules by the sonochemical method for targeted drug delivery and controlled drug release

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ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 2 September 2015
Accepted 27 September 2015
Available online 1 October 2015

Keywords:
Multifunctional
Microcapsule
Sonochemical
Targeted
Release

ABSTRACT

Multifunctional folic acid conjugated BSA@Fe_3O_4 microcapsules (FA-BSA@Fe_3O_4 MCs) have been prepared successfully based on the sonochemical method. The as-synthesized FA-BSA@Fe_3O_4 MCs have a suitable size range for biomedical applications, and a high loading capacity for water-insoluble drugs. Furthermore, FA-BSA@Fe_3O_4 MCs show excellent magnetic properties. Cytotoxicity tests indicate that FA-BSA@Fe_3O_4 MCs are non-toxic. Cellular uptake and flow cytometric assay illustrate together that FA-BSA@Fe_3O_4 MCs can target tumor cells selectively through molecular targeted endocytosis. As carriers for water-insoluble drugs, FA-BSA@Fe_3O_4 MCs are also proved to possess superior redox- and thermo-dual responsiveness for controlled drug release.

1. Introduction

For many water-insoluble drugs, a megadose can usually achieve a good therapeutic effect, but it in turn may cause serious side effects, especially cellular multi-drug resistance (MDR) [1,2]. Therefore, an appropriate carrier is necessary for delivery and controlled release of water-insoluble drugs.

Protein microcapsules (MCs) have potential applications in the treatment of deadly diseases as drug carriers, thus they have received considerable attention [3–5]. So far, many techniques or methods have been designed to fabricate protein MCs, especially bovine serum albumin (BSA) MCs [6–8]. Among these techniques, sonochemical method is a simple physicochemical approach for effectively yielding stable and biocompatible protein MCs [9–11]. Sonochemistry provides the advantage that no additional molecule is needed for the process. Moreover, high-dose water-insoluble drugs can be readily loaded into protein MCs via sonication [12,13]. Although protein MCs prepared by the sonochemical method have been studied for several years, their development as drug carriers is restricted due to the scarcity of specific delivery and controlled drug release.

Iron oxide magnetic nanoparticles (Fe_3O_4 MNPs) have strong superparamagnetism, good chemical stability, superior biocompatibility and low toxicity [14,15], so they are widely applied in targeted drug delivery as magnetic materials [16,17], increasing the local drug concentration and improving the therapeutic efficiency [18–22]. Besides, Fe_3O_4 MNPs under appropriate radio frequencies can be driven to generate heat locally which can contribute to hyperthermia treatment and further optimization of the pharmacokinetics of drugs [23,24]. Alternatively, folic acid (FA) is often identified as a promising targeting ligand [25,26]. Folate receptors (FRs) can be overexpressed at elevated levels as a tumor marker [27–29], hence FA molecules can bind specifically to the FR-expressing tumor cells, presumably through FR-mediated endocytosis [30,31]. In addition, FA is attractive partly because of its ability to conjugate with various molecules, low cost, and high stability [32,33]. Therefore, protein MCs with targeting materials (Fe_3O_4 MNPs or FA molecules) shall be a nice choice to develop the vehicles for specific delivery.

In addition to the targeting modification of protein MCs, to endow protein MCs with a thermosensitive ability is valuable for achieving the controlled drug release. Among various thermosen-

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http://dx.doi.org/10.1016/j.colsurfb.2015.09.056
0927-7765/

sitive materials, 12-hydroxystearic acid (12-HSA) extracted from castor oil is usually chosen as a gelator because of its biocompatibility and commercial availability [34]. Moreover, the thermosensitive behaviour of 12-HSA-mediated gels is thermoreversible [35,36]. If a 12-HSA-mediated gel is introduced into protein MCs, the as-synthesized protein MCs will be thermosensitive and can be used for drug delivery and controlled drug release.

Based on sonochemical synthesis of protein MCs, we designed and prepared a novel magnetic and molecular dual-targeted, redox- and thermo- dual-responsive smart carrier (FA-BSA@Fe_3O_4 MCs). For FA-BSA@Fe_3O_4 MCs, capsule membranes with targeting materials (Fe_3O_4 MNPs and FA molecules) were biocompatible and available for specific delivery, and the inner thermosensitive oil cores played an important role in loading water-insoluble drugs and controlled drug release. Certainly, the inherent redox-responsive ability dependent on the cross-linking disulfide bonds among the BSA molecules could not be ignored [37,38]. So far we hoped that FA-BSA@Fe_3O_4 MCs would be a promising candidate as smart drug carriers.

2. Material and methods

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, >99%), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, >99%) and soybean oil were purchased from Tianjin Guangfu Chemical Reagents Company (Tianjin, China). Aqueous ammonia (NH_4OH, 25%), dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF) and sodium citrate ($C_6H_5Na_3O_7$, >99.0%) were purchased from Beijing Chemical Reagent Company (Beijing, China). Bovine serum albumin (BSA), *N*-hydroxysuccinamide (NHS) and 1-ethyl-3-(3-dimethylamine propyl) carbodiimide hydrochloride (EDC) were purchased from Shanghai Boao Biochemical Technology (Shanghai, China). Folic acid (FA), DL-dithiothreitol (DTT), 12-hydroxystearic acid (12-HSA) and coumarin 6 (C6) were purchased from Sinopharm Chemical Reagent Limited Corporation (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte trazolium bromide (MTT) was purchased from Sigma–Aldrich. Phosphate buffer solution (PBS) was prepared by us. All other chemicals were of analytical grade and were used without further purification.

2.2. Synthesis of Fe_3O_4 MNPs

Fe_3O_4 MNPs were synthesized through chemical coprecipitation. Along with inlet of nitrogen gas, Fe^{3+} and Fe^{2+} in a molar ratio of 2:1 were dissolved into deionized water (100 mL) under constant stirring. After that, pH of the solution was adjusted rapidly to 10.0 by injecting aqueous ammonia (NH_4OH, 25%). 5 min later, sodium citrate (0.5 mol L^{-1}, 10 mL) was poured into the solution in an 80 °C water bath to stabilize the solution. After stirring for 35 min, the solution was cooled naturally to room temperature, and the particles were collected by high-speed centrifugalization and washed with deionized water repeatedly until pH was approximately 7.0. Finally, Fe_3O_4 MNPs with an average particle size of 20 nm were obtained.

2.3. Synthesis of core–shell BSA@Fe_3O_4 MNPs

Core–shell BSA@Fe_3O_4 MNPs were prepared by BSA molecules binding directly with Fe_3O_4 MNPs. Firstly, BSA (120 mg), EDC (100 mg) and NHS (120 mg) were dissolved in PBS (pH 6.3, 20 mL) and constantly stirred by 500 rpm for 30 min, forming a mixed BSA solution. In the process, EDC/NHS could activate the carboxyl groups of BSA molecules. At the same time, Fe_3O_4 MNPs (60 mg) were dispersed into PBS (pH 6.3, 5 mL) by ultrasound, forming a magnetic fluid. Afterward, the magnetic fluid (5 mL) was injected into the mixed BSA solution (20 mL), and strong mechanical stirring (700 rpm) was carried out in a 25 °C water bath. After 24 h, BSA molecules were immobilized on the surface of Fe_3O_4 MNPs by chemical adsorption and cross-linking to synthesize core–shell composite nanoparticles—BSA@Fe_3O_4 MNPs. Then the core–shell BSA@Fe_3O_4 MNPs were separated via a magnet and washed with deionized water several times to remove the unbound BSA molecules. At last, the core–shell BSA@Fe_3O_4 MNPs (about 50 nm) were dispersed into deionized water (5 mL).

2.4. Sonochemical preparation of thermosensitive BSA@Fe_3O_4 MCs

110 mg of 12-HSA was dissolved into 10 mL of soybean oil to form a thermosensitive oil mixture that was liquid above 37 °C. BSA aqueous solution (1 wt%, 100 μL) was added into BSA@Fe_3O_4 MNPs aqueous solution (2 wt%, 4 mL), forming a mixed solution containing BSA molecules and BSA@Fe_3O_4 MNPs. Then the thermosensitive oil mixture (400 μL) was layered on top of the mixed solution. Subsequently, the probe of an ultrasonicator (GEX 600, Sonics & Materials, New town, CT, USA) was inserted into the oil/water mixed solution, and its tip lay at the oil–water interface. Afterward, an acoustic wave with a frequency at 20 kHz was impinging at a power of 350 W cm^{-2} for 6 min in a pulse mode (2 s/2 s). During the ultrasound treatment, the temperature of the oil/water mixed solution was maintained at 40 °C to keep the oil mixture in a liquid state. After ultrasonication, the oil/water mixed solution turned into a brown suspension. The resultant suspension was cooled naturally at room temperature to turn liquid cores of the product into gel cores. Finally, the thermosensitive BSA@Fe_3O_4 MCs were separated from the suspension by a magnet and then washed with deionized water.

2.5. Immobilization of FA onto the BSA@Fe_3O_4 MCs

FA (2 mg), EDC (12 mg) and NHS (15 mg) were dissolved in DMSO (10 mL) and stirred in dark for 30 min, in which EDC/NHS could activate the carboxyl groups of FA molecules. Then BSA@Fe_3O_4 MCs with gel cores (20 mg/mL, 1 mL) was added into DMSO solution, and the mixed solution was agitated in dark at room temperature. After 24 h, FA conjugated BSA@Fe_3O_4 MCs (FA-BSA@Fe_3O_4 MCs) were harvested using an external magnet and washed several times to remove free FA and finally redispersed in deionized water.

2.6. Cellular uptake

To easily evaluate the cellular uptake of FA-BSA@Fe_3O_4 MCs, a hydrophobic fluorescent dye coumarin 6 (C6) was dispersed in the thermosensitive oil mixture and then loaded into FA-BSA@Fe_3O_4 MCs. For the cellular uptake tests, HeLa cells were seeded and incubated in 6-well plates (1×10^5 cells per well) for 24 h before endocytosis. Then some cells were incubated in serum-free medium with C6-loaded BSA@Fe_3O_4 MCs (2 mL), some cells were incubated in serum-free medium with C6-loaded FA-BSA@Fe_3O_4 MCs (2 mL), and other cells were pretreated with FA (10 mg/mL) for 1 h before they were incubated in serum-free medium with C6-loaded FA-BSA@Fe_3O_4 MCs (2 mL). All the cells were incubated for another 6 h and then harvested. Subsequently, the harvested cells were washed with PBS (pH 7.4) and fixed with ethanol (75%). A549 cells were also incubated in serum-free medium with C6-loaded FA-BSA@Fe_3O_4 MCs and treated by the above processes. The analysis of the cellular uptake was carried out using a confocal laser-scanning microscope (CLSM).