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Protocols

Fabrication of polyurethane microcapsules with different shapes and their influence on cellular internalization

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ABSTRACT

The shape of particles is recognized as an important parameter to influence their interactions with cells. In this study, spherical and discal polyurethane microcapsules were prepared via an adsorption and crosslinking method on the templates with corresponding shapes in organic solvent. Both types of capsules could be well dispersed in aqueous medium and maintain their original shapes. The internalization behaviors of the microcapsules were investigated by co-incubation with RAW 264.7 and HepG2 cells. Compared with the spherical capsules, the discal microcapsules could be internalized with faster rate and higher amount by both types of cells. Both types of capsules did not show significant cytotoxicity even after co-incubation for 72 h at a high ratio of capsule to cell.

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1. Introduction

Microcapsules have attracted much interest in the biomedical fields. They can act as drug delivery vehicles [1,2], biosensors [3], and bioreactors [4]. Compared with nanosystems, the microcapsules or microparticles may have special advantages in some cases. For example, in the application of pulmonary administration, microparticles can deposit deeper in the alveolar region while smaller particles are exhaled [5]. They also can be more easily internalized by macrophages and passively targeted to the antigen-presenting cells of the immune system [6]. The interaction of micronized capsules or particles with macrophages is very important because they are potential therapeutic targets in inflammatory conditions, atherosclerosis and cancer [7]. Thus, the studies on interactions of diverse microcapsules or particles with different cells may help to design better carriers. It is of high importance to understand the interaction between colloidal particles and cells when they are used in the biomedical fields. The colloidal particles are mainly internalized by cells through the micropinocytosis [8], clathrin mediated endocytosis [9] and so on. Recently, the physical properties such as size [10,11], stiffness [12] as well as shape [13–15] have been recognized as important factors that influence the cellular uptake behaviors of colloidal particles. In particular, the shape of colloidal particles has great impact on endocytosis, phagocytic internalization, and passive targeting as well as their flowing and circulating behaviors in the blood vessel [16]. For example, Rod-shaped polymeric particles showed less phagocytosis and could attach more efficiently to the vascular endothelium layer as compared to spheres [17]. Red blood cell-like microcapsules could get through a narrow capillary with a little deformation, leading to a longer blood circulation time [18]. Bowl-like Microcapsules could enhance cellular uptake and silicon discs revealed a prominent higher accumulation in breast tumor tissue on account of the special shape [19,20].

Except the passive interaction of microcapsules or particles with cells, the active targeting also can be achieved by external manipulation. For example, drug carriers combined with magnetic responsive substances could be successfully steered to the region of interests in vivo, which can improve drug delivery efficiency to the target spot [21]. Multilayer composite magnetic microcapsules with the feasibility for remote navigation and localization by external magnetic field were designed and prepared as targeted delivery carriers under the natural conditions in vivo [22]. Surface enhanced Raman scattering (SERS)-active microparticles were synthesized by layer-by-layer process, which could be used to navigate to a certain cellular region and detect the intracellular composition following cellular uptake assisted by the optical tweezers and Raman spectroscopy [23]. However, the influence of shape on such kind of active targeting and cellular uptake are not investigated.

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The fabrication of particles with specific shape and size is the prerequisite for investigating the influence of particle's shape on the biological process. So far many methods such as particle replication in nonwetting templates (PRINT) method [24], microfluidics [25–27], and thermal–mechanical deformation method [28] have been developed to fabricate colloidal particles with different shapes. The colloidal particles with specific shapes also can be fabricated by using a sacrificial template with corresponding shapes, resulting in rod, cubic or discoidal particles [29,30].

Polyurethanes (PUs) are a family of biodegradable polymeric materials with mechanical flexibility and excellent biocompatibility, and widely used in biomedical field [31,32]. PU microcapsules with the ability of encapsulating diverse functional agents including pesticides, drugs and proteins, and the suitable elasticity to deform and recover were also investigated [33–35]. But the PU capsules with anisotropic shapes have not been fabricated so far, thus their interactions with cells are largely unknown. In this work, polyurethane (PU) microcapsules with spherical and discal shapes are fabricated via an adsorption and crosslinking method on sacrificial templates with corresponding shapes. The spherical and discal templates are firstly prepared, and then dispersed in PU solution. Then the adsorbed PU molecules are cross-linked by hexamethylene diisocyanate (HDI). After template removal, the hollow PU capsules with two different shapes are obtained. (Scheme 1a, b) Then, the two different microcapsules are incubated with HepG2 and RAW 264.7 cells to investigate the influence of shape on capsule-cell interaction. (Scheme 1c)

2. Experimental section

2.1. Materials

Polyurethane (PU, Mn ~ 58 kDa. The shore hardness is 65A, implying elastic) was obtained from Yantai Wanhua Polyurethane Co., Ltd. China. Hexamethylene diisocyanate (HDI) and stannous octoate were obtained from Acros. Dimethyl formamide (DMF) and toluene were obtained from Sinopharm Chemical Reagent Co., Ltd. Dextran sulfate (DS, Mw ~ 500 kDa), calcium chloride and sodium hydroxide, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO) and rhodamine B isothiocyanate (RBITC) were purchased from Sigma-Aldrich. Manganese sulfate ($MnSO_4$) was obtained from Shanghai Meixing Chemical Factory Co., Ltd. Ammonium hydrogen carbonate (NH_4HCO_3) was purchased from Guangdong Guanghua Chemical Factory Co., Ltd. All chemicals were used as received. The water used in all experiments was prepared in a Millipore Milli-Q Reference purification system. Spherical $MnCO_3$ microparticles were synthesized by mixing $MnSO_4$ and NH_4HCO_3 solutions according to Ref. [36].

2.2. Fabrication of $Ca(OH)_2$ microparticles

To fabricate the discal $Ca(OH)_2$ particles, 100 mL 0.05 M $CaCl_2$ solution with 500 mg DS was completely dissolved, and was rapidly poured into 40 mL 1 M NaOH solution under ultrasonication for 10 s. After 20 min, the discal $Ca(OH)_2$ particles were collected by centrifugation, washed three times with ethanol and finally stored in ethanol.

2.3. Preparation of PU microcapsules with different shapes

The preparation of PU capsules based on different template particles was referred to an adsorption and crosslinking method reported in our previous work with some modification [33]. PU was dissolved in DMF with a concentration of 10 wt%. The spherical $MnCO_3$ particles and discal $Ca(OH)_2$ particles (0.5 g) were incubated in 10 mL PU solution for 24 h, respectively. The particles were washed with DMF and ethanol three times by centrifugation and then dried at 70 °C in an oven for 5 h. The obtained PU-adsorbed $MnCO_3$ particles or discal $Ca(OH)_2$ particles (0.1 g) were dispersed in 15 mL toluene solution containing 0.3 g HDI and 15 mg stannous octoate at 70 °C for 3 h to crosslink the adsorbed PU. After washed three times with ethanol, the particles were incubated in 0.1 M HCl for 1 h to remove $MnCO_3$ or $Ca(OH)_2$ templates. The obtained microcapsules with different shapes were washed three times with water using centrifugation (5500 rpm, 10 min), and finally dispersed in water for subsequent use.

In order to label the capsules with RBITC, the crosslinked PU-adsorbed particles (100 mg) were incubated in RBITC ethanol solution (10 μg/mL, 10 mL) for 24 h under shake. Then the RBITC-labeled microparticles were washed three times with ethanol, followed by incubation in HCl to remove the templates. The RBITC-labeled microcapsules were obtained and dispersed in water.

2.4. Cell culture

Murine macrophage cell RAW 264.7 and human HepG2 cells were obtained from the Cell Bank of Typical Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The RAW 264.7 cells and HepG2 cells were cultured in Dulbecco's modified eagle medium (DMEM, Gibco, USA) consisting of high-glucose, supplemented with 10% fetal bovine serum (FBS, Sijiqing Inc., Hangzhou, China), 100 U/mL penicillin, and 100 μg/mL streptomycin, and cultured at 37 °C in a 5% CO2 humidified environment.

2.5. Cell viability assay

Influence of microcapsules of different shapes on cell viability was investigated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. 3×10^4 cells in 100 μL medium were incubated in a well of 96-well plates overnight. The cells were incubated with microcapsules at different ratios of capsule to cell (10:1, 30:1 and 50:1) for 24 h. Then 20 μL 0.5 mg/mL of MTT in 100 μL fresh medium was added into each well. The cells were further cultured for another 4 h at 37 °C. The dark blue formazan crystals generated by the mitochondria dehydrogenase were then dissolved with 150 μL DMSO, whose absorbance at 570 nm was measured by a microplate reader (Model680, BioRad). The data were normalized to the capsule-free control group. All the experiments were carried out in triplicate.

2.6. Cellular uptake kinetics

The cells were cultured in a 24-well plate overnight, and then incubated with RBITC-labeled microcapsules at a capsule to cell ratio of 20:1 for different time (1, 3, 6 and 12 h). At desired time intervals, the cells were thoroughly washed 3 times with phosphate-buffered saline (PBS) to remove free and loosely adhered microcapsules and harvested by trypsinization. The uptake amount of microcapsules at different time was determined by flow cytometry (FACS Calibur, Becton Dickinson BD).The number of events counted is 10000. The excitation wavelength is 488 nm, and the detection channel is FL2. For the flow cytometry study, the logarithmic fluorescence intensity of untreated cells was set between 10^0 and 10^1. The cells with fluorescence intensity larger than 10^1 are considered to be fluorescent microsphere labeled cells. Through the software Cell Quest Pro, the percentage of RBITC-microcapsule labeled cells to all cells can be calculated. The results were averaged from 3 parallel measurements.

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