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Protocols

Biomimetic synthesis of sericin and silica hybrid colloidosomes for stimuli-responsive anti-cancer drug delivery systems

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ABSTRACT

Colloidosomes are becoming popular due to their significant flexibility with respect to microcapsule functionality. This study reports a facile approach for synthesizing silica colloidosomes by using sericin microcapsule as the matrix in an environment-friendly method. The silica colloid arrangement on the sericin microcapsules are orchestrated by altering the reaction parameters. Doxorubicin (DOX), used as a hydrophilic anti-cancer drug model, is encapsulated into the colloidosomes in a mild aqueous solution and becomes stimuli-responsive to different external environments, including pH values, protease, and ionic strength are also observed. Colloidosomes with sub-monolayers, close-packed monolayers, and close-packed multi-layered SiO_2 colloid shells can be fabricated under the optimized reaction conditions. A flexible DOX release from colloidosomes can be obtained *via* modulating the SiO_2 colloid layer arrangement and thickness. The close-packed and multi-layered SiO_2 colloid shells can best protect the colloidosomes and delay the rapid cargo release. MG-63 cells are killed when doxorubicin is released from the microcapsules due to degradation in the microenvironment of cancer cells. The drug release period is prolonged as SiO_2 shell thickness and integrity increase. This work suggests that the hybrid colloidosomes can be effective in a bioactive molecule delivery system.

1. Introduction

Colloidosomes are hollow microcapsules with densely packed colloidal particle walls [1–4]. The functionalities and physical properties of colloidosomes, such as permeability, selectivity, and biocompatibility, can be precisely controlled using suitable colloidal particles and processing conditions [1,2,4–6]. Compared with the shortcomings of direct drug delivery systems, hollow colloidosomes possess a perfect alternative approach that is highly efficient for encapsulating drug. They control drug delivery efficiently at the target site through response to external stimuli and prevent the drug degradation in the bloodstream, prolong circulation time, and improve drug bioavailability [7–10]. These properties make colloidosomes promising vehicles in drug delivery systems and seem reliable in delivering potent drugs to action sites precisely and timely, enhance accumulation in tumor sites, decrease adverse effects, and improve drug tolerance [11–14]. In recent years, many methods have been developed to synthesize colloidosomes, and they are commonly based on the self-assembly of colloidal particles at water/oil interface in immiscible liquids. Silica sol and polystyrene latex are effective building blocks for colloidosome assembly. A shell reinforcement mechanism is necessary for preparing colloidosomes to convert individual assembly units into robust microcapsules and retain their complete structure after the oil–water template removal. Moreover, stimulus-responsive colloidosomes have been developed for inverting or demulsifying the obtained colloidosomes in response to changes in pH or temperature in solutions. The convenient approach offered significant flexibility in microcapsule functionality, which are the main requirements in the fields [15,16].

Silk protein sericin, obtained from silkworm cocoons and once considered a waste product in silk production, is becoming pop-

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ular due to its biomedical and biotechnological applications, as well as its excellent water solubility and biocompatibility. Sericin is earlier regarded as a waste product of the silk industry [17–20]. Recently, smart sericin microcapsules were fabricated through protein self-assembly in water solutions without organic solvents or surfactants in the laboratory [21,22]. Sericin microcapsules exhibit good biocompatibility and multifunctional and tunable properties. However, they show poor stability in harsh environments [21,22]. Colloidal silica microcapsules belong to a prominent class of SiO_2 hollow materials. They are advantageous in drug delivery systems because of their low density, adjustable pore size, high specific surface area, mechanical stability, easy functionalization, low toxicity, and good biocompatibility [23,24]. Based on these, we utilized colloidal SiO_2 nanoparticles as the protection shells for sericin microcapsules to obtain a desirable hybrid colloidosomes. The most important advantage of this approach is that drug degradation in the extreme external environment and bloodstream is prevented because the drug is encapsulated inside the core. SiO_2 colloid shells protect the sericin microcapsules and keep continuous drug release.

In this report, hydrophilic doxorubicin (DOX), an anti-cancer drug model, was encapsulated in the sericin microcapsules through a one-step encapsulation method under mild conditions to avoid toxic organic solvents and complicated synthesis procedures. A biomimetic approach was followed to prepare the hybrid colloidosomes by growing SiO_2 colloids on DOX-encapsulated microcapsule surfaces. The SiO_2 colloid shell morphology and thickness were regulated by controlling the reaction conditions. Altering some of the reaction factors, we synthesize sub-monolayer, close-packed monolayer, and close-packed multilayer shells of SiO_2 nanoparticle hybrid colloidosomes and control DOX release from the core. The SiO_2 colloid shell enhances the mechanical stability o and drug circulation time of the microcapsules. Moreover, polymeric core/shell colloidosome structures enable the drug carriers to counteract extreme environments, such as pH values, protease concentrations, and ionic strengths. We observed MG-63 cells undergo necrosis at different stages upon culturing with different DOX-encapsulated hybrid colloidosome thicknesses.

2. Results and discussion

2.1. The morphology evolution of hybrid colloidosomes by altering the reaction conditions

According to the Stober reaction, two steps are involved in the silica growth process. One is the tetraethylorthosilicate (TEOS) hydrolysis and the other is the condensation of SiO_2 onto the microcapsule surfaces [25–27].

$$Si(OC_2H_5)_4 + 4H_2O \xrightarrow{NH_3/Alcohol} Si(OH)_4 + 4C_2H_5OH$$

$$Si(OH)_4 \xrightarrow{NH_3/Alcohol} SiO_2 \downarrow + 2H_2O$$

The ζ-potential of the microcapsules was -26.7 ± 1.83 MV. The presence of ammonia negatively charged SiO_2 colloids to stabilize the surface. The electrostatic adsorption was weak because the surface charges of the microcapsules and SiO_2 were negative. The sericin microcapsules had thin and smooth SiO_2 surfaces upon direct TEOS hydrolysis onto the microcapsules without surface any modification (Fig. 1a). Therefore, we used poly (allyl amine hydrochloride) PAH with amine groups as scaffolding for SiO_2 nucleation and growth on the substrate surface.

In principle, the nucleation and growth rates of inorganic nanoparticles are strongly dependent on the environment factors, such as reaction temperature, time, surfactants, and concentration of solutions [28–30]. To improve microcapsule morphology regulation, we further investigated the influence of some reaction parameters. SEM images of resulting hybrid colloidosomes are presented in Figs. 1 and 2.

We first studied the effects of reaction temperature. Sub-monolayer colloid shells on the microcapsules were observed at 25 °C (Fig. 1b). When the temperature was elevated to 50 and 60 °C,

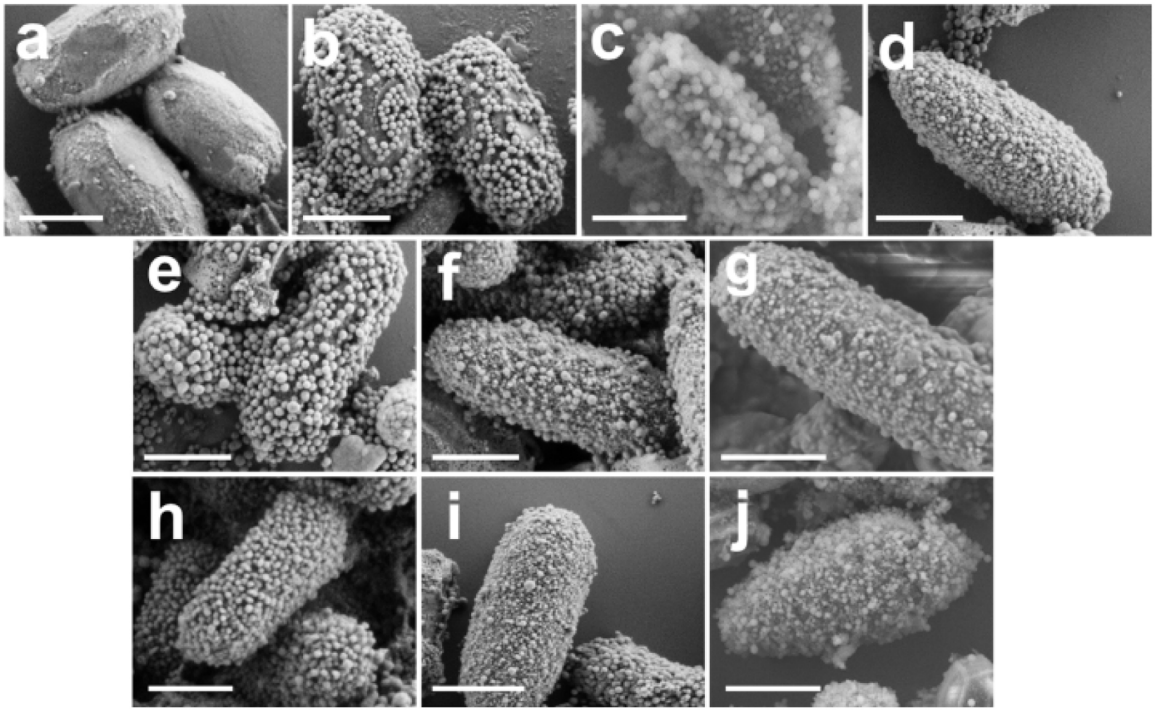

Fig. 1. SEM images of the hybrid colloidosomes with different coating conditions: (a) without poly(allylamine hydrochloride) (PAH), (b)–(d) hydrolysis temperatures of 25, 50, and 60 °C, (e)–(g) hydrolysis durations of 20, 50, and 60 min, and (h)–(j) condensation durations of 2, 8, and 20 h, respectively. The scale bar is 1 μm.