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Nano-functionalization of protein microspheres

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ABSTRACT

Protein microspheres are promising building blocks for the assembly of complex functional materials. Here we demonstrate a set of three techniques that add functionality to the surface of protein microspheres. In the first technique, a positive surface charge on the protein spheres is deposited by electrostatic adsorption. Negatively charged silica and gold nanoparticle colloids can then electrostatically bind reversibly to the microsphere surface. In the second technique, nanoparticles are covalently anchored to the protein shell using a simple one-pot process. The strong covalent bond between sulfur groups in cysteine in the protein shell irreversibly binds to the gold nanoparticles. In the third technique, surface morphology of the protein microsphere is tuned through hydrodynamic instability at the water–oil interface. This is accomplished through the degree of solubility of the oil phase in water. Taken together these three techniques form a platform to create nano-functionalized protein microspheres, which can then be used as building blocks for the assembly of more complex macroscopic materials.

1. Introduction

A goal of modern materials science is to create complex functionalities in new materials. In nature, living organisms are based on the biological cell—a highly complex microsphere that can sense, transport and process matter, energy, and information [1]. The cell interacts with its surroundings through nano-scale functional surface structures that act as receptors, channels, binding sites, and communication sites [2]. To mimic this basic design principle nano-functionalization of the surface of micro-sized spherical particles will be necessary.

Protein microspheres with their hollow spherical shape and microscale size offer an excellent opportunity to mimic surface functionalization of cells. In 1990, Suslick and Grinstaff developed a synthesis technique for protein microspheres using ultrasonic irradiation of solutions [3]. Ultrasonic emulsification of oil droplets into the protein aqueous solution occurs first. Next, the protein molecules cross-link on the surface of oil droplets through disulfide bonds initiated by superoxide radicals produced from ultrasonic energy resulting in oil-filled, protein shells. Protein microspheres have several advantages as building blocks for more complex materials. First, the ultrasonic process can create protein microspheres from a wide variety of proteins and with a controllable range of sizes. Second, the intermediate size enables extensive functionalization at the nanoscale of individual microspheres and subsequently the rapid assembly into macro-scale materials. For assembling complex materials it will be necessary to create techniques to introduce more functionality on to the surface of the microspheres.

In this paper, we introduce three nano-scale surface functionalization techniques. Nanoparticles are attached to the surface of the protein microsphere through two different methods. First, the nanoparticles are reversibly attached electrostatically through surface charge modification. This technique is versatile and allows binding of a wide variety of charged nanoparticles and macromolecules. It is demonstrated here for both silica and gold nanoparticles. Second, a one-pot synthesis technique is used to covalently anchor gold nanoparticles directly to the protein shell through metal–sulfur bonds. Third, a technique is described to tune the surface morphology of the protein shell through selection of an appropriate oil phase. These techniques enable functionality to be integrated into the microspheres and provide the opportunity to assemble more complex materials.

2. Materials and methods

2.1. Materials

The following materials were purchased from Sigma–Aldrich: Bovine serum albumin (BSA, Cat. No. A2153), PDADMAC (polydiallyldimethylammonium chloride, 20 wt% in water, Cat. No. 409014), silica nanoparticle (LUDOX® TM-50 colloidal silica, 22 nm

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in diameter, Cat. No. 420778), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, Cat. No. 520918), trisodium citrate dehydrate (Cat. No. S4641), chloroform (Cat. No. C2432), diethyl ether (Cat. No. 309966), ethyl acetate (Cat. No. 270989), *sec*-butanol (Cat. No. B85919), and glutaraldehyde (Cat. No. G5882). *n*-Dodecane (Cat. No. 10500-00) was purchased from Kanto Chemical, toluene was obtained from Daejung Chemicals & Metals (Cat. No. 8541-4400), and *n*-pentanol (Cat. No. P0055) was purchased from Tokyo Chemical Industry. All chemicals were used without further purification. Ultra-pure water was used in all experimental procedures.

2.2. Synthesis of BSA protein microspheres

The synthesis of the protein microspheres followed the sonochemical method developed by Suslick and Grinstaff [3]. Briefly, 6.7 ml of n-dodecane was layered on the top of 10 ml of 5% (w/v) BSA aqueous solution. A high-intensity ultrasonic horn (Vibra-Cell VC750, Sonics and Materials) was introduced at the water/oil interface for 3 min at 75% amplitude (560 W). The reaction container temperature was maintained below 30 °C by a cooling bath. After synthesis, the milky-colored BSA microspheres were washed with water 5 times by centrifugation (1000 × *g*, 10 min). Complete removal of residual protein was confirmed by UV–vis spectroscopy through characteristic absorption peaks near 200 nm [4].

2.3. Electrostatic binding of nanoparticles

For attachment of nanoparticles through electrostatic deposition, 1 wt% of PDADMAC was added to 1 ml 1% (v/v) BSA microspheres solution and magnetically stirred for 1 h. After the adsorption period, the unreacted PDADMAC was removed by the centrifugation (1000 × *g*, 7 min) and washing. Electrostatic adsorption was demonstrated with two different types of nanoparticles, silica and gold. Silica nanoparticles were diluted 50 times to minimize coagulation. Gold nanoparticles were synthesized by trisodium citrate reduction using the method reported in Ref. [5]. The surface charge of the BSA microspheres was measured by Zetasizer Nano ZS (Malvern instruments). The microscopic features of nanoparticles coated BSA microspheres were recorded using FESEM, JSM-6701F (JEOL, Japan) after fixation with glutaraldehyde. Both silica and gold nanoparticles remained stably attached to the protein microspheres in solution for more than 3 months when stored in the refrigerator.

2.4. Covalent binding of gold nanoparticles

Gold nanoparticles were incorporated directly through a one-pot synthesis technique. BSA protein solution, 0.01–5% (w/v) was mixed into the gold nano-colloid. Varying amounts (6.7–1 ml) of *n*-dodecane was layered on the aqueous solutions. The sonochemical synthesis procedure for microspheres was the same as above. Elemental composition was determined by TEM-EDS (JEM 2100F, JEOL), and INCA EDS system (Oxford Instruments). A UV–vis spectrometer (UV-3600 Shimadzu) was used to characterize the gold nanoparticles after modification. The color of the samples was recorded by a digital camera, HMX-H106 (Samsung). The gold nanoparticles remained stably bonded to the protein microspheres in solution for more than 3 months when stored in the refrigerator.

2.5. Surface morphology control

Seven different organic liquids: *n*-dodecane, toluene, chloroform, *n*-pentanol, diethyl ether, ethyl acetate, and *sec*-butanol were tested as the oil phase for sonochemical synthesis. The remaining steps of the synthetic process were the same as the standard method described above. The surface morphology of the products was studied by atomic force microscopy, XE-100 (Park Systems).

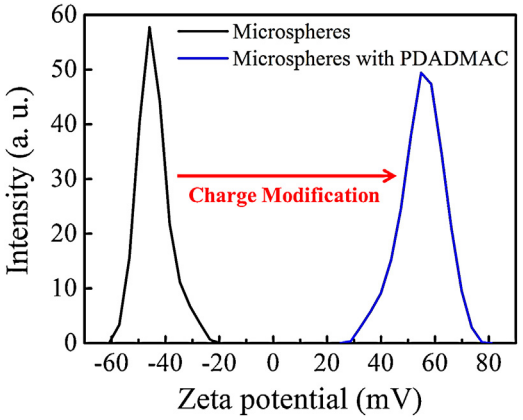

Fig. 1. Zeta-potential (ζ) of native BSA microspheres (black), and BSA microspheres after electrostatic adsorption with PDADMAC (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Results and discussion

3.1. Electrostatic binding of nanoparticles

To use the protein microspheres as a functional unit it is necessary to attach nanoparticles or macromolecules to the surface. BSA protein macromolecules have a net negative charge due to ionization of component amino acids in water at neutral pH [6]. In our experiments the native charge of the microspheres is −49 mV. Positively charged nanoparticles or macromolecules can attach directly to the as synthesized protein microspheres, while for negatively charged species the surface must be modified. Electrostatic adsorption of a cationic polyelectrolyte PDADMAC was used to tune the surface charge of the BSA microspheres [7]. PDADMAC is an excellent choice due to its extended charge-bearing side chains that effectively stabilize larger species like nanoparticles and macromolecules [8]. Fig. 1 shows the surface charge of the BSA microsphere before and after the treatment with PDADMAC. The surface charge of the microspheres is modified from −49 mV to +57 mV with PDADMAC. The large value of the zeta potential and narrow peaks, confirm the excellent stability of the protein microspheres and the uniformity of their charge distribution. No degradation in BSA microspheres was observed during this process.

Modifying the BSA microspheres with positive charge allows any nanoparticle or macromolecule with negative charge to be reversibly attached. Fig. 2(a) and (b) shows the pristine BSA microspheres with smooth and clean surfaces. Stable silica nanoparticles ($\zeta = -41.7$ mV) are coated uniformly on BSA microspheres as shown in Fig. 2(c). Here a high concentration of silica is applied to show that the protein microspheres can be uniformly coated with nanoparticles. The silica nanoparticles themselves can be further functionalized in many ways. Attachment of citrate buffered gold nanoparticles ($\zeta = -42.5$ mV) is shown in Fig. 2(d). Here a low concentration is used and it is noted that each protein microsphere attracts gold nanoparticles, and there is a limited tendency for aggregation.

Electrostatic modification is a simple, versatile and reversible attachment technique. Fig. 3 demonstrates the reversibility of attachment. Gold functionalized microspheres can be dispersed in pure water and then removed from solution while retaining the gold nanoparticles. However, when dispersed in a high concentration salt solution the gold nanoparticles detach and remain

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