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Investigation of 3-D ordered materials with a high adsorption capacity for BSA and their potential application as an oral vaccine adjuvant

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ABSTRACT

3-D ordered macroporous (3DOM) materials were customized for BSA adsorption and further oral immunization. These carriers have a high adsorption capacity and our customized carrier showed a distinctive double-plateau adsorption behavior. Different BSA release rates (between the two plateaus) could be obtained by adjusting the ratio of the protein adsorbed on the internal surface and the external surface. This suggests that the release pattern was determined by the adsorption state. One benefit is that the same carrier could have different release profiles making it possible to study the relationship between the release behavior and adjuvant effects without any distractions. Compared with free BSA alone, a significantly higher level of serum IgG, IgA induced by BSA/3DOM was observed and the release profile had an effect on the immunity. The IgG1 and IgG2a titers suggesting that both the Th1 and Th2 mediated immune response were induced. Therefore, this research could help in the development of a novel inorganic oral adjuvant and provide a new avenue for the administration of oral vaccine.

1. Introduction

Among various routes of administration, the oral route is most popular because of its non-invasive nature [1]. Such administration offers some advantages such as a low cost, associated with good compliance and a low risk of infection. However, even today, it is still very challenging to design an oral protein delivery system that can provide perfect protection for oral proteins. The damaging effect of oral administration is mainly due to the harsh environment in the gastrointestinal tract that involves different pH values and several proteolytic enzymes which lead to the denaturation and degradation of protein drugs.

Many researchers have developed several ingenious vectors as oral protein delivery system such as liposomes [2] and solid lipid nanoparticles [3]. Some of them have achieved gratifying results. However, there remain a number of problems with this research. In some cases, the structure of these "soft" materials becomes unstable when they are exposed to the complex physical and chemical environment of the gastrointestinal tract. PLA and PLGA nanoparticles, for example, are considered to be good vectors but some negative factors limit their use with oral proteins, i.e., they undergo diffusion or degradation in the harsh environment of the gastrointestinal tract [4]. This phenomenon involving structural instability and premature release of antigens is a major challenge in the development of oral proteins. Ordered inorganic materials have been developed as promising candidates to overcome the stability and leakage issues. In recent years, the porous silica material SBA-15 was used as a drug carrier [5]. In 2006, SBA-15 was used as an adjuvant for the first time [6]. Then some researchers have carried out investigations to prove that such inorganic materials provide protection for proteins and act as high performance adjuvant. However, most of them have limited pore size (usually 2–8 nm), and low protein adsorption capacity [7–10].

An adjuvant is a substance that is added to vaccine formulations and the final product can produce a stronger immune response than the antigen alone [11]. Traditional vaccines consist of attenuated pathogens or whole inactivated micro-organisms. These vaccines have some safety issues and may produce side effects [12]. In order to improve the safety of vaccines, highly purified macromolecules have been developed as vaccines. However, most of them are poorly immunogenic [13] and an adjuvant is needed for their prescription. To date, the development of safer and more efficient adjuvant is still the best strategy for the prevention of many diseases.

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In recent years, 3DOM materials have attracted a lot of attention and these materials are usually prepared by the colloidal crystal template method. By controlling the size of the template, a material with a large external pore size ranging from hundreds of nanometers to several micrometers and a small internal pore size from tens of nanometers to hundreds of nanometers can be easily synthesized. Many valuable applications of 3DOM materials such as biosensors, catalysis, and water-insoluble drugs have been reported, without any reports about their use as an oral protein adjuvant [14–18].

3DOM have several valuable characteristics that make them very suitable as a protein vector. For example, (a) Because of the interconnected spaces and a large bimodal pore system, these materials have a high adsorption capacity. That is, large pores tend to accept large amount of protein molecules [19]. (b) The rigid and stable framework of the inorganic carriers was assumed to provide a barrier between the antigen and the physicochemical effects of the gastrointestinal tract, so that the protein can remain active during its transit through the harsh intestinal environment. (c) Compared with some "soft" materials, such as PLGA nanoparticles, organic solvents are not involved in the protein adsorption procedure and prevents the degradation of the protein.

Because of these advantages, we report here the application of 3DOM materials with a high adsorption capacity for BSA and, for the first time, their potential application as an oral vaccine adjuvant. In the current study, bovine serum albumin (BSA) was selected as a model protein. The dimensions of BSA are approximately 5.5 nm × 5.6 nm × 12 nm. Considering its three-dimensional structure, two kinds of 3DOM materials (3DOM carbon and 3DOM aluminum materials) were customized with an average internal pore of about 50 nm.

The purpose of this paper was to evaluate the protein adsorption capacity of 3DOM materials for BSA and examine its potential application as an oral vaccine adjuvant. It is important to mention that our carrier could have different protein release rates by adjusting the protein adsorption status associated with the adsorption isotherm.

2. Materials and methods

2.1. Materials and animals

Aluminum isopropoxide was purchased from Aladdin (Shanghai, China). Bovine serum albumin (BSA) and Freund's complete adjuvant (FCA) were obtained from Sigma–Aldrich (USA). BCA protein assay kit was obtained from Kangwei Company (Shanghai, China). Goat anti-mouse lgG-HRP was purchased from Zhongshanjinqiao Company (Beijing, China). Goat anti-mouse IgG1-HRP and IgG 2a-HRP were purchased from Proteintech (USA). Goat anti-mouse lgA-HRP was purchased from Santa Cruz (USA). All other chemicals were analytical grade as required without further purification. The animals were handled under ethical conditions, according to the committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University. The female BALB/c mice (18–22 g) were provided by the Lab Animal Center of Academy of Military Medical Sciences of the PLA (Beijing, China).

2.2. Preparation of monodisperse PS spheres

These monodisperse spheres were synthesized using a soap-free emulsion polymerization as described in the literature with some modifications [20]. Briefly, 50 ml styrene (St) was washed with 500 ml 0.5 M NaOH solution for five times in a separatory funnel. Then, 40 ml St monomer and 400 ml double distilled water were added to a stoppered conical flask and transferred to a water bath with the temperature of about 50 °C. The mixture was magnetically stirred and 0.6 g polyvinylpyrrolidone (PVP) K30 was added and the temperature of the system was raised to 60 °C. After addition of 50 ml 0.16 M potassium persulfate (KPS), nitrogen was introduced to afford an inert atmosphere and the reaction temperature was raised to 70 °C. The polymerization reaction was maintained at 70 °C for 24 h. After that, the system was cooled down at room temperate and the suspension was homogenized using an ATS homogenizer (ATS Engineer Inc., Shanghai, China) then centrifuged at 6000 rpm for 40 min (TDL-6A, Feiyier, Shanghai, China). The final white polymeric material was collected after drying at 40 °C for 12 h.

2.3. Synthesis of 3DOM materials

Both 3DOM carbon materials (3DOMC) and 3DOM aluminum materials (3DOMA) were prepared using PS spheres as templates. The synthetic procedure was described in the literature with some modifications [21]. In brief, the powder of PS spheres was transferred to a Buchner funnel with vacuum environment and wetted with ethanol. In order to synthesize 3DOMC, the precursor of carbon was obtained by diluting 10 ml H_2SO_4 in 100 ml double distilled water then adding in 13 g sucrose. After the sucrose dissolved, the liquid was added slowly to the PS sphere layer under vacuum to make it infiltrate into the PS spheres completely. Then the products were carbonized with the temperature of about 60 °C and 120 °C, respectively. Then, the spheres were removed by calcinated at 700 °C under vacuum. After that, this pristine carbon could undergo a further surface functionalization. For a typical treatment, 0.5 g carbon was added to a round-bottomed flask which containing 30 ml 1 M ammonium persulfate (APS) solution (dissolved in 2 M H_2SO_4). The mixture was transferred to a water bath with a reflux device and mild stirred for 6 h. Then the resulting product was filtered and carefully washed three times with distilled water and ethanol, respectively. The final product was obtained after drying at 40 °C for 12 h. The functionalized carbon materials were referred to as S-3DOMC.

The preparation of 3DOM aluminum material was similar to that of 3DOM carbon material. The precursor of aluminum was obtained by mixing 15 ml ethanol with 2.4 ml nitric acid, and then 6 g Aluminum isopropoxide was added in and stirred for 3 h at room temperature. After that, the liquid was added to the Buchner funnel under vacuum ensuring that the layer of the PS spheres was completely infiltrated. Then, the resulting products were dried in an oven for 24 h. At last, the brown powder was obtained by calcinations at 550 °C for 6 h.

2.4. Protein adsorption

The obtained 3DOM materials were crushed to pass a 60-mesh sieve. The adsorption equilibrium method was used for BSA adsorption. In detail, a certain amount of BSA was dissolved in 7 ml 20 mM phosphate buffer solution (pH 7.4) in a vial with a stopper, then an aliquot of this liquid was mixed with 3DOM materials and mild stirring for 24 h at 4 °C. After that, the suspension was centrifuged at 8000 rpm for 5 min and the supernatant liquid with non-encapsulated BSA was collected and measured by the BCA assay. The amount of BSA that encapsulated in 3DOM materials was determined by subtracting the protein in supernatant liquid from the total amount of the protein.

2.5. SEM characterization, nitrogen adsorption and zeta potential characterization

The morphology of the PS spheres, S-3DOMC and 3DOMA materials was characterized using a scanning electron microscopy