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PLGA–lipid liposphere as a promising platform for oral delivery of proteins

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ABSTRACT

The main challenge in the oral delivery of protein drugs is to enhance their oral bioavailability. Herein, we report the uniform-sized liposphere prepared by premix membrane emulsification combined with $W_1/O/W_2$ double-emulsion method as a potential oral carrier for proteins. The protein-loaded liposphere was composed of a hydrophobic poly (D, L-lactide-*co*-glycolide) (PLGA) core and the lipid molecules self-assembled at the interface of W_1/O and O/W_2. During the preparation, the protein structure was effectively maintained. Compared with PLGA microsphere, the liposphere achieved a higher loading capacity (LC, 20.18%), entrapment efficiency (EE, 90.82%) and a lower initial burst (24.73%). Importantly, the lipospheres also showed high transcytotic efficiency with human microfold cell (M cell) model, leading to a potential enhancement of intestinal absorption. This result, together with the above studies supported that the PLGA–lipid liposphere could be a promising platform for enhancing the proteins oral bioavailability.

1. Introduction

With the rapid development in biotechnology and genetic engineering, an increasing number of potential therapeutic protein drugs have been discovered. For proteins delivery, oral administration is a preferred route, because it is patient-friendly and with limited cross-infection [1–4]. However, most protein molecules are fragile and tend to be degraded in the gastrointestinal (GI) tract before they arrive at the absorbed position. Moreover, a tight junction of the intestinal epithelium provides a physical barrier to limit the oral uptake of the macromolecules such as proteins [3]. Even though a little bit of the proteins are taken up, they still suffer the short half-life in circulation [5].

To solve these problems, polymer based delivery systems have been developed [6–8]. Among them, PLGA microparticles are one of the most attractive candidates [9–11]. Indeed, PLGA with biocompatible natural pathways [12] has become a Food and Drug Administration (FDA)-approved pharmacology excipient [13] and the PLGA microparticles can protect the entrapped proteins from the harsh environment of the GI tract. Nevertheless, PLGA microparticles still face some problems for oral delivery, such as (i) with limited interaction of the mucosal surface [9], the PLGA microparticles are hard to be taken up by the intestine, (ii) the protein in the PLGA microparticle generally shows a burst release in the GI tract, leading to less absorption efficiency, (iii) pristine PLGA microparticles are with relatively poor LC and EE for the hydrophilic proteins [14]. Recently, efforts have been made to develop pegylated PLGA microspheres [14,15] and lipospheres [16,17] to improve the performance of pristine PLGA microparticles. The pegylated PLGA microspheres have been widely discussed to increase the EE and prolong the pharmacodynamic efficacy. The morphology and surface texture of pegylated PLGA microspheres were significantly varied due to the hydrophilic/hydrophobic differences of respect polymers [14], and the osmotic agent was generally required to increase drug loading. Other studies have

Abbreviations: PLGA, poly (D, L-lactide-*co*-glycolide); LC, loading capacity; EE, entrapment efficiency; M cell, microfold cell; GI, gastrointestinal; FDA, Food and Drug Administration; BSA, bovine serum albumin; HSPC, hydrogenated phosphatidylcholine; PVA, polyvinyl alcohol; DAPI, 4, 6-Diamidino-2-phenylindole dihydrochloride; FITC, fluorescein isothiocyanate; DCM, dichloromethane; TEM, transmission electron microscopy; CLSM, confocal laser scanning microscopy; SEM, scanning electron microscopy; CD, circular dichroism; UEA, ulex europaeus agglutinin; P_{tm}, trans-membrane pressure; PDI, polydispersity index.

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used wax and polar lipid combined liposphere as another alternative carrier [16] for delivery of bioactive compounds. The lipospheres potentially achieved desired goals for safe delivery and avoided systemic side effects [17].

In this study, we attempt to develop the PLGA–lipid lipospheres for oral delivery of protein drugs. Compared with the classic lipospheres, the PLGA–lipid lipospheres provided long duration release and the release rate could be controlled by modifying the degradation behavior or molecular weight of the polymer. Premix membrane emulsification method was used to control the particle size and size distribution of lipospheres. Bovine serum albumin (BSA) was used as a model protein and entrapped into the lipospheres with $W_1/O/W_2$ double-emulsion method. In this formulation, PLGA built a biodegradable core and the lipid molecules self-assembled at the interface of W_1/O and O/W_2. In addition, such structure pattern would effectively maintain the protein structure, achieve a high LC and EE, reduce the burst release, and enhance the transcytotic efficiency across the *in vitro* M cell model, which are favorable for improving the protein drugs oral bioavailability.

2. Material and methods

2.1. Material

BSA with purity of 98% was purchased from Sigma–Aldrich (St. Louis, USA). Poly (d,l-lactide-*co*-glycolide) with a monomer ratio of 75:25, viscosity of 0.12–0.20 dl/g, and an acid end group, was obtained from Lakeshore Biomaterials (Birmingham, AL). Hydrogenated phosphatidylcholine (HSPC) with purity more than 98% based on dry weight was obtained from Lipoid LLC (Newark, NJ). 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) was purchased from Avanti Polar Lipids (Alabaster, AL). Polyvinyl alcohol (PVA) and the membranes (pore size: 0.8–30.0 μm) were supplied by Kuraray Co., Ltd. (Tokyo, Japan) and National Engineering Research Center for Biotechnology (Beijing, China), respectively. All other reagents were of analytical grade and commercially available.

Caco-2 and Raji-B cell lines were obtained from Peking University Health Science Center (Beijing, China). DMEM and RPMI 1640 cell cultures were purchased from Gibco (Grand Island, USA). 4, 6-Diamidino-2-phenylindole (DAPI) and fluorescein isothiocyanate (FITC)-conjugated lectin from ulex europaeus were purchased from Invitrogen (Grand Island, USA) and Sigma–Aldrich (St. Louis, USA), respectively.

2.2. Preparation of BSA-loaded PLGA–Wlipid lipospheres

Uniform-sized lipospheres containing BSA as a model protein were prepared by premix membrane emulsification combined with double-emulsion method. Briefly, 1 mL BSA aqueous solution with concentration of 60 mg/mL was used as internal water phase (W_1). Lipids and PLGA with mass ratios ranging from 0% to 100% were co-dissolved in 10 mL dichloromethane (DCM) solution (2.0%, w/v) as oil phase (O). A certain amount of PVA was completely dissolved into distilled water (0.1%, w/v) as external water phase (W_2). The internal water phase was emulsified into oil phase by sonication (Branson, USA) at 25% amplitude for 1 min to obtain W_1/O emulsions. Then, they were further emulsified into external water phase to form coarse emulsions of $W_1/O/W_2$ by gently mechanical agitation. The coarse emulsions were repeatedly passed through the membrane five times at a nitrogen pressure of 200 kPa to achieve uniform-sized $W_1/O/W_2$ emulsions. The emulsions were stirred at ambient environments (4 h) for evaporation of DCM. Finally, the BSA-loaded lipospheres were collected and washed five times with deionized water to remove free proteins and PVA. The lipospheres were lyophilized for future use. The blank lipospheres without loading proteins were prepared by premix membrane emulsification combined with oil/water emulsion method. The experimental parameters were the same as described above except that the oil phase was directly emulsified into external water phase without any BSA containing internal water phase.

2.3. Characterization of the PLGA–lipid liposphere

The structure pattern of the blank liposphere was examined by transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM), respectively. For the TEM experiment, the lipospheres suspension was deposited on a carbon-coated double copper grid and thoroughly dried under ambient conditions. The TEM image was obtained using a JEM-1400 TEM instrument (JEOL, Japan) at an acceleration voltage of 80 kV. Next, to visually observe the lipid molecules at the oil/water interface, we used lissamine rhodamine B-headgroup labeled lipids and a 30-μm pore size of membrane to prepare the fluorescent lipospheres. Then, the CLSM image of these fluorescent lipospheres was obtained using a TCS-SP2 CLSM instrument (Leica, Germany) at an excitation wavelength of 561 nm.

The average particle size and size distribution of the BSA-loaded lipospheres in each preparation were determined by dynamic light scattering (Zetasizer Nano ZS, Malvern, UK). Briefly, 1 mL of particles suspension was added in a sample cell for the measurements. Each experiment was performed in triplicate. A JSM-6700F scanning electron microscopy (SEM, JEOL) was used to assess the monodispersity, shape and surface morphology of the BSA-loaded lipospheres. Accordingly, the samples were thoroughly dried and fixed onto a sample stage for coating with a gold layer under vacuum. The SEM experiment was performed at an acceleration voltage of 5 kV.

2.4. LC, EE and structure stability study of the entrapped BSA

The loading capacity of BSA was determined using the micro-BCA method (Pierce Biotechnology, Rockford, IL). The lyophilized lipospheres were dissolved in 50 mM NaOH solution (100 μg/mL, w/v) and incubated overnight at 4 °C. A microplate procedure with a liner working range of 2–40 μg/mL was used in the experiment. The lipospheres were seeded into 96-microwell plates, and the absorbance was measured at 562 nm using an Infinite M200 microplate reader (Tecan, Switzerland). Blank polymeric liposphere was used as control. LC and EE values were calculated from Eqs. (1 and 2), respectively.

$$\mathrm{LC}(\%) = \frac{W_1}{W_2} \times 100\% \quad (1)$$

$$\mathrm{EE}(\%) = \frac{W_1}{W_3} \times 100\% \quad (2)$$

W_1, W_2 and W_3 represented the mass of encapsulated proteins, the mass of overall particles and the mass of initially added proteins, respectively.

The fluorescence spectra of the native and released proteins were examined to determine whether the tertiary structure of the protein was changed during the preparation and storage. The protein samples were added in a 1 cm path-length cell and excited at 280 nm using an F-4500 FL Spectrophotometer (Hitachi, Japan) at 25 °C. The emission spectra were recorded in the wavelength range of 400 nm. The scan speed was 1200 nm/min and the wavelength slit was 5.0 nm with response time of 0.05 s. In addition, the circular dichroism (CD) spectropolarimetry experiment was performed to evaluate the secondary structure of the protein. The protein solutions (50 μg/mL, w/v) were added in a 1 cm path-length