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Mechanistic studies for monodisperse exenatide-loaded PLGA microspheres prepared by different methods based on SPG membrane emulsification

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ABSTRACT

Poly(DL-lactic-co-glycolic acid) (PLGA) microspheres have been widely prepared by many methods, including solvent evaporation, solvent extraction and the co-solvent method. However, very few studies have compared the properties of microspheres fabricated by these methods. This is partly because the broad size distribution of the resultant particles severely complicates the analysis and affects the reliability of the comparison. To this end, uniform-sized PLGA microspheres have been prepared by Shirasu porous glass premix membrane emulsification and used to encapsulate exenatide, a drug for treating Type 2 diabetes. Based on this technique, the influences on the properties of microspheres fabricated by the aforementioned three methods were intensively investigated, including in vitro release, degradation and pharmacology. We found that these microspheres presented totally different release behaviors in vitro and in vivo, but exhibited a similar trend of PLGA degradation. Moreover, the internal structural evolution visually demonstrated these release behaviors. We selected for further examination the microsphere prepared by solvent evaporation because of its constant release rate, and explored its pharmacodynamics, histology, etc., in more detail. This microsphere when injected once showed equivalent efficacy to that of twice-daily injections of exenatide with no inflammatory response.

1. Introduction

In the last few decades, microspheres based on biodegradable polymers such as poly(DL-lactic-co-glycolic acid) (PLGA) for sustained delivery of protein/peptide have been widely studied [1–3]. Some peptides, such as Lupron Depot® (leuprolide acetate) and Sandostatin LAR® (octreotide acetate), have been incorporated into PLGA microspheres and commercialized as sustained-release systems [4]. Solvent evaporation, solvent extraction and co-solvent methods are the most popular approaches used to prepare the microspheres, because these processes are simple and convenient to control [5]. In the first two methods, an aqueous solution dissolving the drug is emulsified with an organic solution (oil phase) containing polymer. Methylene chloride (MC) is frequently used as the organic solvent in the evaporation method [6], and ethyl acetate (EA) is often used in the extraction method. For the co-solvent method, the drug is dissolved directly in two miscible organic solvents—typically MC and alcohol (methanol and ethanol)—without aqueous solution [7]. The use of different organic solvents results in microspheres with various characteristics. For instance, solvent removal rate affects the solidification, which is critical in determining the morphology, surface area and other aspects of microspheres [8]. Thus, discrepancies of the resultant particles in terms of, for example, release behavior and pharmacology will be generated. Studying this discrepancy can provide more important and general insights into the mechanisms on microsphere degradation and drug release in vitro/vivo, which is a key issue in developing long-term release systems. Although much effort has been devoted to microsphere preparations, relevant information about this discrepancy is scarce. This is partly because the broad size distribution resulting from conventional mechanical stirring will result in particles with poor reproducibility with respect to release

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behavior, drug efficacy, etc. [9–12]. Therefore, to control particle size, narrow down the size distribution and realize mass production, Shirasu porous glass (SPG) premix membrane emulsification has been employed [13].

Exenatide (synthetic exendin-4), a therapy for Type 2 diabetes mellitus (T2DM), was used as a model peptide in this study. It possesses 39 amino acids (H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2), shares about 53% homology with mammalian gut hormone (GLP-1), and also possesses glucoregulatory actions including enhancement of insulin secretion, reduction of food intake, deceleration of gastric emptying and improvement of β-cell function [14,15].

Herein, based on SPG premix membrane emulsification, monodisperse exenatide-loaded PLGA microspheres were prepared by solvent evaporation, solvent extraction and co-solvent methods, respectively. Systematic research into the effect of preparation methods on the properties of microspheres was performed, including release behaviors, molecular weight (M_w) degradation, structural evolution, etc. Then, pharmacological aspects of the optical formulation were investigated, such as pharmacodynamics, immunohistochemistry analysis and inflammatory response.

2. Materials and methods

2.1. Materials

PLGA with a molar ratio of D,Llactide/glycolide 75/25 (Mw 13 kDa) was purchased from Lakeshore Biomaterials (Birmingham, AL, USA). Exenatide was provided by Hybio Pharmaceutical Co. Ltd. (Shenzhen, PR China). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Tokyo, Japan). SPG membranes were purchased from SPG Technology Co. Ltd. (Miyazaki, Japan). The SPG premix membrane emulsification equipment (FMEM-500M) was designed by National Engineering Research Center for Biotechnology (NERCB, Beijing, PR China). Acetonitrile and trifluoroacetic acid (TFA) (both HPLC grade) were purchased from Dikma Co. Ltd. (Lake Forest, USA). All other reagents were analytical grade.

2.2. Preparation of microspheres

Microspheres prepared by solvent evaporation, solvent extraction and the co-solvent method are abbreviated as EVM, EXM and COM, respectively.

Before preparation, SPG membrane 50.2 μm in size was installed in the equipment (Fig. 1a). For preparation of EVM (Fig. 1b), 1 ml exenatide aqueous solution (3%, w/v, W_1) was emulsified with 8 ml organic solvent (MC, O) containing PLGA (10%, w/v) by homogenization (T18, IKA, Germany) at 18,000 rpm for 60 s to form W_1/O. Next, the W_1/O was stirred at 250 rpm for 1 min with external aqueous phase (W_2) containing PVA (2%, w/v) and NaCl (0.5%, w/v) to form coarse $W_1/O/W_2$ emulsions. These were then poured into a premix reservoir and extruded through the SPG membrane by N_2 pressure at 5 kPa to achieve uniform-sized droplets. After that, they were solidified at room temperature at 250 rpm for 5 h. Finally, the microspheres were collected and washed with distilled water five times by centrifugation for 3 min at 300 g. The washed microspheres were stored in −70 °C overnight, then lyophilized, and obtained after 48 h. The conditions for lyophilization were as follows: ice condenser −80 °C; vacuum −31 °C, 0.34 mbar.

For preparation of EXM (Fig. 1b), EA was used as oil phase (O). The preparation process was the same as above. In addition, the uniform-sized droplets achieved by extrusion through SPG membrane were poured quickly into solidification solution with a large volume (1.6 l containing 0.9% (w/v) NaCl) under magnetic stirring at 250 rpm for 4 h to solidify the microspheres. The microspheres were obtained in the same way as above

For preparation of COM (Fig. 1b), the exenatide powder was dissolved in mixed organic solvent (volume ratio of MC and methanol 6:2) containing PLGA (10%, w/v). The other steps were same as those for EVM.

2.3. Characterization of microspheres

2.3.1. Surface morphology observation and size distribution measurement

The shape and surface morphology of PLGA microspheres were observed by scanning electron microscopy (SEM) with a JSM-6700F (JEOL, Japan).

The particle size and size distribution was measured with a Mastersizer 2000 (Malvern, UK). The size distribution was referred as the Span value and was calculated as follows:

$$\text{Span} = \frac{D_{v,90\%} - D_{v,10\%}}{D_{v,50\%}},$$

where $D_{v,90\%}$, $D_{v,50\%}$ and $D_{v,10\%}$ are volume size diameters at 90%, 50% and 10% of the cumulative volume, respectively. The smaller the Span value, the narrower the size distribution.

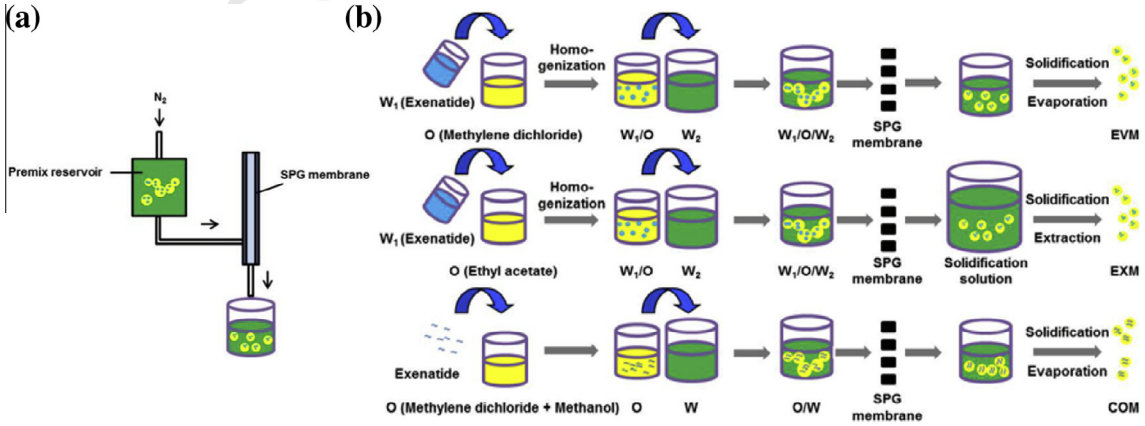

Fig. 1. (a) Schematic depiction of the SPG premix membrane emulsification equipment; (b) schemes of the EVM, EXM and COM preparation.

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