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Fabrication of nonporous and porous cationic PLGA microspheres

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ABSTRACT

A modified water–oil–water double emulsion solvent evaporation method was used to prepare cationic poly (lactic-co-glycolic acid) microspheres. Polyethyleneimine in external water phase was used to stabilize the microspheres and ammonium bicarbonate in internal aqueous phase was adopted to facilitate the formation of pores. It is found that the microspheres with or without pores could be manipulated by easily adjusting the polyethyleneimine concentration. In order to understand the drug delivery potential of the porous microspheres, the model macromolecule agent, fluorescein isothiocyanate–dextran was used. The results show that the porous microspheres obtained by emulsion processing have higher absorption capability compared with non-porous ones. Therefore, the as-obtained porous microspheres could be the suitable platforms for the delivery of bioactive agents.

1. Introduction

A controlled release of therapeutic agents is very important in the area of drug delivery and regenerative medicine. To achieve this goal, a proper delivery system is of particular interest, such as aluminum oxide (Al_2O_3), polymer microspheres, and porous silicon [1,2]. Among a variety of candidates, Poly (lactic-co-glycolic acid) (PLGA) has been used due to their good biocompatibility, low cytotoxicity and excellent biodegradability [3,4]. Particularly, the porous PLGA microspheres are promising for encapsulation and delivery of active compounds such as gases, drugs, enzymes, and proteins [5,6]. A number of synthetic approaches have been employed to obtain porous PLGA microspheres, including the double-emulsion solvent evaporation (DSE) [7], microfluidic system [8,9], and phase separation [10]. Compared to other approaches, the DSE strategy is widely used because of the easy control over microsphere morphology. A typical method is to make use of the various osmogens. When the osmo-regulator (e.g. Hydroxypropyl-β-cyclodextrin or polyethyleneimine (PEI), etc) is encapsulated in the inner of the microspheres, the osmotic pressure gradient differences between the internal and external phases will result in formation of pores [11]. In addition, the pores can also be produced by the effervescent agents (e.g. ammonium bicarbonate (ABC), hydrogen peroxide, etc) because they can be decomposed into gas molecules in the preparation process, [12,13]. Whereas, an alternative solution has been desired to prepare the porous microspheres to load and deliver the hydrophilic macromolecules drug. In this work, based on the modified DSE, the porous PLGA microspheres were prepared by using PEI in external water phase (W_2) and ABC in internal water phase (W_1). The pores can be controllably formed by adjusting the concentration of PEI. In order to investigate the drug loading capability of the porous PLGA microspheres, the fluorescein isothiocyanate–dextran (FITC–DEX) was utilized as the macromolecules model. The results indicate that the porous PLGA microspheres have higher absorption capability for the macromolecules.

2. Experimental

PLGA (50:50, M_W=10,000) was purchased from Shandong Daigang Company. PEI (25 kDa branched) and FITC–DEX (46 kDa) were obtained from Sigma-Aldrich, Inc., USA. The ABC and dichloromethane (DCM) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd, China. All chemicals were analytical grade and used without any other treatment.

PLGA microspheres have been prepared by a DSE method as previously described with modification [14,15]. In this experiment, PEI was added to this solution to keep the particles cationic. Briefly, the internal water phase (W_1) was prepared by adding 2 mL of an ABC (5 mg/mL) solution previously prepared with deionized water (DI). PLGA (500 mg) was initially dissolved in 20 mL DCM (oil phase, O). This W_1/O emulsion was formed by using a probe sonicator (20 kHz, 35%, Sonics & Materials, Newtown, CT, USA) for 100 s. The primary emulsion with 50 mL PEI solution (external water phase, W_2) was then homogenized (T-25

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digital ULTRA-TURRAX® IKA®, Germany) at 6000 rpm for 30 min. Then, 50 mL DI was added and stirred overnight at room temperature to facilitate the removal of the DCM. The final solution was washed three times with DI to remove excess PEI. In order to examine the influence of PEI concentration in W_2, various concentrations (0.5 wt%, 1 wt% and 2 wt%) were investigated.

The FITC–DEX was loaded into the microspheres by a briefly mixing method. Purified microspheres (20 mL, the number of microspheres in suspension was adjusted to 1×10^6/mL) were incubated with a solution of FITC–DEX (5 mL, 1 mg/mL) for 4 h. The suspension was centrifuged (3000 rpm, 10 min) three times and the supernatant was discarded.

The morphology and structure of the microspheres were studied by scanning electron microscope (SEM, FEI Sirion-200, USA) working under acceleration voltage of 1 kV. The mean size distribution of the particles was measured by a dynamic light scattering technique (DLS, Microtrac S3500, USA) after dispersing the formulations in DI (the number concentration of microspheres in suspension was adjusted to 1×10^5/ mL, pH=7.4) at room temperature. The zeta potential measurements were performed on a PALS Zeta instrument (Brookhaven, USA) in DI (the number of microspheres in suspension was adjusted to 1×10^5/ mL, pH=7.4) at 25 °C. The FITC–DEX loaded microspheres were investigated by confocal laser scanning microscopy (CLSM, Olympus FV1000, USA) at the 488 nm wavenumber. Release studies were conducted in 50 mL (5 mg/mL) FITC–DEX loaded porous microspheres at 25 °C with gentle shaking at 100 rpm. The incubation solution was collected at different time intervals, and an equal volume of fresh medium was compensated. The release amount of FITC–DEX was quantitatively measured by UV–vis spectrophotometry (UV-3600, SHIMADZU, Japan) at 451 nm wavelength by analyzing the supernatant. Then, the accumulative ratios of the released FITC–DEX were calculated as a function of time.

3. Results and discussion

The biodegradable nonporous and porous microspheres were prepared by a modified DSE technique, which is illustrated in Fig. 1. During the preparation, W_1 was firstly emulsified into oil phase by probe sonicator, and then the W_1/O simple emulsion was further dispersed into W_2 by the homogenizer. There were quite a number of small W_1/O droplets within every single W_2 globule and were firstly endowed with multi-core morphology. It is well known that double emulsion is an unstable system and the evolution of the solidification process is bound to influence the microspheres morphology. The formation mechanism mainly relies on the solvent diffusion–evaporation from W_1 to W_2 [16].

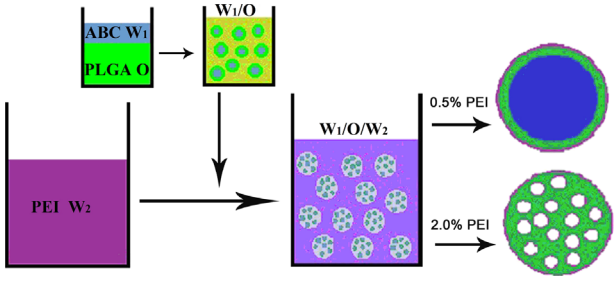

Fig. 1. A proposed scheme for the formation of PLGA microspheres with or without pores under the different PEI concentrations.

Generally, stabilizer concentration in W_2 is a key factor to influence the size and morphology of microspheres. Fig. 2 shows the typical SEM images of microspheres fabricated at 0.5 wt%, 1.0 wt% and 2.0 wt% PEI concentration. A significant difference in surface morphology of microspheres can be achieved by adjusting the PEI concentration in W_2. Fig. 2a (0.5 wt% PEI) indicates that the microspheres have a smooth and non-porous surface structure. When the PEI concentration is 1 wt%, small amount of pores appear on the microspheres (see Fig. 2b). For 2 wt% concentration, larger-sized pores can be observed (Fig. 2c). Therefore, 2.0 wt% PEI concentration was chosen preparation of porous microspheres to be tested for the drug loading demonstration.

For the mechanism of pores formation in the microspheres, there may be two key factors. First, it is the influence of PEI concentration. Normally, the coalescence of emulsion droplets can be caused by inherent thermodynamic instability of an interface between the two immiscible (W_1/O and W_2) phases [17]. When the PEI concentration is 0.5 wt%, the multi-core globules would spontaneously transform and coalescence to single-core ones with no any pores on the surface of the microspheres. When the PEI concentration is 2 wt%, the spontaneous coalescence of the multi-core globules can be avoided because high concentration of PEI in W_2 evolved from the surface of water droplets can make these two metastable emulsion droplets to be collided prior to coalescence. Therefore, high concentration of PEI in W_2 will produce the porous microspheres. Second, generally, the ABC in the W_1 droplets may undergo hydrolysis to produce carbon dioxide [9]. The diffusion rate of the free carbon dioxide gas will be slowed down under the condition of higher PEI concentration resulting in producing open porous morphology throughout the PLGA microspheres. Interconnection between the pores can be observed by SEM image in Fig. 2c and CLSM image in Fig. 3b.

Additionally, the microspheres size was characterized by DLS (in Table 1). For 0.5 wt%, 1 wt%, and 2 wt% PEI concentration, average diameters of PLGA microspheres were 2.7 ± 0.9, 3.8 ± 1.2, and 5.5 ± 2.3 μm, respectively, suggesting that the formation of pores in the microspheres also contributed to the enlarged sizes of PLGA microspheres. ζ- potential measurements of the microspheres, for 0.5 wt%, 1 wt%, and 2 wt% PEI, recorded a positive value of $+41\pm1.0$, $+43\pm1.3$, $+39\pm0.7$ mV, respectively (in Table 1),

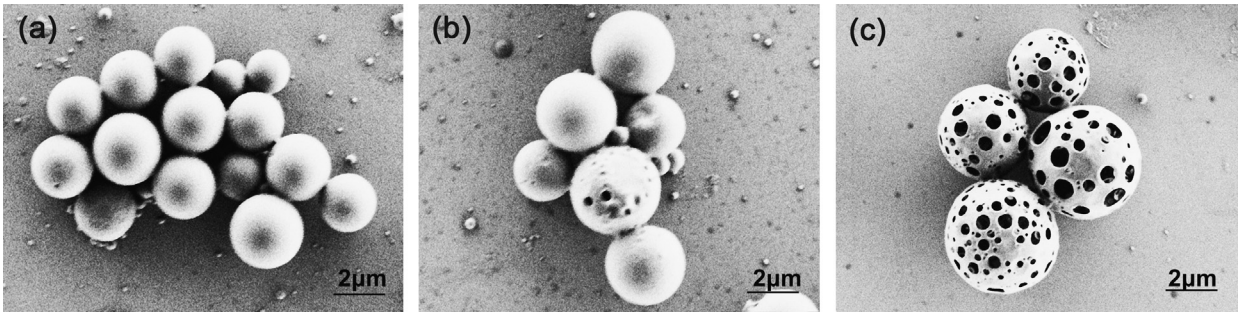

Fig. 2. SEM images of microspheres obtained at (a) 0.5 wt%, (b) 1 wt% and (c) 2 wt% PEI concentration, respectively.

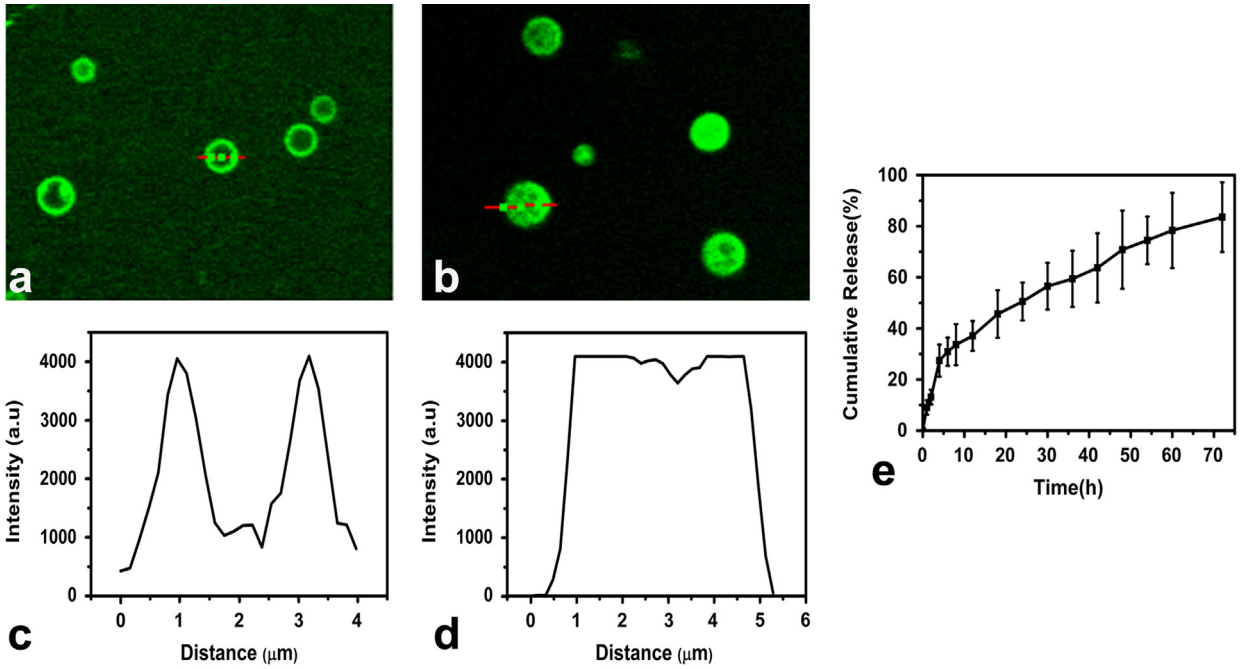

Fig. 3. CLSM images and fluorescence intensity contours of FITC–DEX-loaded non-porous (a and c), porous PLGA microspheres (b and d), and release profiles of FITC–DEX from porous PLGA microspheres (e).

Table 1
Characterization of the as-obtained PLGA microspheres.

PEI concentration (wt%)	Mean diameter (μm)	ζ-potential (mV)	Pore size (nm)*	Surface porosity (%)*
0.5	2.7 ± 0.9	+41 ± 1.0	0	0
1	3.8 ± 1.2	+43 ± 1.3	91.3 ± 47.1	2.0 ± 0.3%
2	5.5 ± 2.3	+39 ± 0.7	373.8 ± 137.9	24.1 ± 6.0%

*The pore size and surface porosity (%) were calculated through the image analysis software "Image Pro Plus" based on SEM images. (Surface porosity (%)=(area of pores/total surface area) × 100%.

which indicates that the presence of PEI in W_2 may be useful for the amino groups on the PLGA microsphere surface [18].

The interaction between microspheres and FITC–DEX relies on the hydrogen bond conjugation between the amino groups (PEI) and the OH group (FITC–DEX) [19], which can be realized by simply incubation method. The successful loading of FITC–DEX in the microspheres is confirmed by CLSM in Fig. 3a and b. The different FITC–DEX entrapping ability mainly depends on different intrinsic structure of microspheres. For the non-porous microspheres, FITC–DEX was localized mainly on the surface of microspheres (Fig. 3a and c). And for the porous microspheres, the FITC–DEX was distributed evenly in the matrix of microspheres (Fig. 3b and d) because the porous microspheres maintain interconnectivity between the pores. By calculating the fluorescent intensity of the CLSM images, it suggests that the porous PLGA microspheres have higher fluorescence intensity (Figs. 3c and d). This result implies that the porous microspheres have higher FITC–DEX entrapping efficiency.

To further understand the kinetic release process of FITC–DEX, Fig. 3e demonstrates that the FITC–DEX can be gradually released from porous microspheres. At 72 h, the 80% FITC–DEX can be released, which indicates that the porous microspheres can be used as effective drug delivery carrier.

4. Conclusions

In summary, we demonstrate that PEI can be used as a stabilizer to prepare cationic PLGA microspheres by a modified DSE method. The surface morphology of microspheres can be adjusted simply by varying PEI concentration in W_2. A preliminary application of these microspheres is also performed by entrapping a model hydrophilic macromolecule (i.e. FITC–DEX). The results indicate that the porous microspheres have higher absorption capability compared to non-porous microspheres. We envision that these porous PLGA microspheres have promising applications in tissue engineering, drug delivery and bio-imaging.

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References

[1] Gultepe E, Nagesha D, Sridhar S, Amiji M. Adv Drug Deliv Rev 2010;62:305–15.
[2] Thorat S, Diaspro A, Scarpellini A, Povia M, Salerno M. Materials 2013;6:206–16.
[3] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V. J Control Release 2012;161:505–22.
[4] Acharya S, Sahoo SK. Adv Drug Deliv Rev 2011;63:170–83.
[5] Kim I, Byeon HJ, Kim TH, Lee ES, Oh KT, Shin BS, et al. Biomaterials 2012;33:5574–83.
[6] Desai KG, Schwendeman SP. J Control Release 2013;165:62–74.
[7] Ungaro F, Giovino C, Coletta C, Sorrentino R, Miro A, Quaglia F. Eur J Pharm Sci 2010;41:60–70.
[8] Choi SW, Zhang Y, Yeh YC, Wooten AL, Xia YN. J Mater Chem 2012;22:11442–51.

[9] Kim TK, Yoon JJ, Lee DS, Park TG. Biomaterials 2006;27:152–9.
[10] Blaker JJ, Knowles JC, Day RM. Acta Biomater 2008;4:264–72.
[11] Ungaro F, De Rosa G, Miro A, Quaglia F, La Rotonda MI. Eur J Pharm Sci 2006;28:423–32.
[12] Bae SE, Son JS, Park K, Han DK. J Control Release 2009;133:37–43.
[13] Yang Y, Bajaj N, Xu P, Ohn K, Tsifansky MD, Yeo Y. Biomaterials 2009;30:1947–53.
[14] Yang F, Chen P, He W, Gu N, Zhang X, Fang K, et al. Small 2010;6:1300–5.
[15] He W, Yang F, Wu Y, Wen S, Chen P, Zhang Y, et al. Materials Letters 2011;68:164–7.
[16] Na XM, Gao F, Zhang LY, Su ZG, Ma GH. ACS Macro Lett 2012;1:697–700.
[17] Jiao J, Rhodes DG, Burgess DJ. J Colloid Interface Sci 2002;250:444–50.
[18] Zhou J, Moya S, Ma L, Gao C, Shen J. Macromol Biosci 2009;9:326–35.
[19] Lopez-Marzo A, Pons J, Merkoci A. J Mater Chem 2012;22:15326–35.