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Morphology control of polylactide microspheres enclosing irinotecan hydrochloride with polylactide based polymer surfactant for reduction of initial burst

Satoru Nishino [a], Akio Kishida [b], Hidekazu Yoshizawa [c,*]

[a] *Department of Chemical Engineering, Nara National College of Technology, 22 Yata, Yamatokoriyama, Nara 639-1080, Japan*
[b] *Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan*
[c] *Department of Material and Energy Science, Graduate School of Environmental, Science, Okayama University, 3-1-1 Tsushima-naka, Okayama 700-8530, Japan*

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Abstract

We introduced newly biodegradable polymer surfactant to poly(D,L-lactide) microspheres for exhibiting precisely controlled release rate of antitumor agent. We newly designed biodegradable polymer surfactant, poly(ethyleneoxide monooleate)-block-poly(D,L-lactide); (MOPEO-PLA), which has a biocompatible, oil-soluble and compatible with polylactide matrices. Polylactide (PLA) microspheres with MOPEO-PLA enclosing Irinotecan hydrochloride (CPT-11) was successfully fabricated by solvent evaporation method via O/O emulsion. The surface morphology was strongly affected to MOPEO-PLA content in PLA microspheres; the wrinkles on microsphere surface were decreased with increasing MOPEO-PLA content. It was found from DSC scans that Tg of PLA drastically decreased with introducing MOPEO-PLA, indicating that MOPEO-PLA miscible with PLA. MOPEO-PLA, played as a plasticizer, made the surface smoothed. The rate of release in a short duration was minimum value at adding 20 wt.% of MOPEO-PLA. This result was due to the smaller surface area of PLA microspheres with smoothed surface.

Keywords: Polymer microspheres; Polylactide; Antitumor agent; Surface morphology; Microparticulate drug delivery system

1. Introduction

Biodegradable polymer has been focused on biomedical field such as tissue engineering, bone materials and matrices for drug delivery due to the high-compatibility with living cells (Asano et al., 1989; Chang et al., 1996). Especially, polylactide (PLA), which is aliphatic polymer composed with plant, is preferably applied to the carrier for drug delivery (Ijichi et al., 1997; Yoshizawa et al., 1995, 1996; Tokuda et al., 1995, 1996) because PLA has high strength and processability (Langer, 1997; Jain, 2000). In our previous study, antitumor agent was successfully microencapsulated in PLA microspheres, however, showing undesirable release properties of drug (Yoshizawa et al., 2003). In particular, the initial burst; large quantity of drug released from microspheres in a short time, had not been overcome, in spite of changing the surface morphology of PLA microspheres by optimizing a preparation condition such as temperature and pressure (Nishino et al., 2002; Yoshizawa et al., 2005). We concluded that the designing a newly microencapsulation system is necessary for diversified drug delivery.

Normally, the non-ionic surfactant has been used for emulsification during preparation process of microsphere (Rosa et al., 2000) and it adheres to the microsphere surface. In this study, we try to make use of this surfactant property to the surface modification of microsphere and the uniformly distribution of enclosing drug in the microsphere to obtain precisely controlled release rate. Especially, polymer surfactant is expected for drastically change of microsphere property.

Our research group had been synthesized a functional biodegradable polymer surfactant, poly(ethyleneoxide monooleate)-block-poly(D,L-lactide) (hereafter abbreviated as MOPEO-PLA) and investigated that MOPEO-PLA had a sufficient surface activity as well as hydrophilic MOPEO though a bulky

* Corresponding author. Tel.: +81 86 251 8909; fax: +81 86 251 8909.
E-mail address: yhide@cc.okayama-u.ac.jp (H. Yoshizawa).

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hydrophobic PLA segment combined with MOPEO to become oil-soluble polymer surfactant (Nishino et al., 2005).

It is considered that MOPEO-PLA can plasticize the PLA microsphere by being compatible with PLA microsphere, utilizing for surface morphology control of PLA microsphere. Therefore, in the present study, we attempted to introduce MOPEO-PLA to the PLA microspheres enclosing CPT-11 for the purpose of precise release from PLA microspheres by controlling surface morphology. It was found from our previous study on PLA microspheres enclosing Irinotecan hydrochloride (CPT-11) that the rough surface was produced by interaction PLA with CPT-11 and this surface morphology caused a large amount of initial burst. The objective of this work was to clarify the effect of addition of MOPEO-PLA in PLA microspheres on surface morphology and in vitro release property of CPT-11. Furthermore, the mechanism of surface morphological change of PLA microspheres with MOPEO-PLA was investigated by thermal analysis.

2. Methods

2.1. Synthesize of MOPEO-PLA and D,L-PLA

MOPEO-PLA was prepared by bulk ring-opening polymerization of D,L-lactide initiated with MOPEO in the presence of catalyst of stannous 2-ethylhexanoate described previously (Nishino et al., 2005). Polymerization was carried out in vacuum-sealed glass ampoule at 403 K for 24 h. The obtained polymer was recrystallized and was stored at 323 K in vacuo after isolation. D,L-PLA was synthesized using lauryl alcohol as an initiator. The weight-averaged molecular weight (Mw) of MOPEO-PLA and D,L-PLA were measured by gel permission chromatography (Tosoh, HLC 8120 GPC) using a polystyrene standards with tetrahydrofuran as the eluent.

2.2. Preparation and characterization of PLA microspheres

The preparation procedure of PLA microspheres was similar to those reported previously (Yoshizawa et al., 2003). Briefly, 10 ml of acetonitrile solution consisted of 1 g of PLA (Mw: 102,039), a variety weight of MOPEO-PLA (Mw: 4990) and 20 mg of CPT-11 was dispersed in silicon oil, forming O/O emulsion system. The solvent was evaporated at 313 K reduced pressure for 3 h and PLA microspheres were collected by filtration, washed with petroleum ether, and then freeze-dried for 24 h. PLA microspheres were prepared twice to confirm the reproducibility.

The enclosing efficiency of CPT-11 in PLA microspheres was measured by high performance liquid chromatography (HPLC) method. The chromatographic system consisted of a UV spectrophotometric detector ($\lambda = 254$ nm, SPD-6A, Shimadzu Co.), a reverse phase column (JUPITER 5u C18 300R, Phenomenex) and a chromatopac (C-R6A, Shimadzu Co.). To separate CPT-11 from the polymer matrix, PLA microspheres of 0.1 g were dissolved in 3 ml of chloroform, and 50 g of methanol were then added. The solution was filtered using a hydrophilic membrane with 0.2-μm pores, and was then injected to the HPLC system.

The morphology of PLA microspheres was observed by scanning electron microscopy (SEM; S-4700, Hitachi Ltd.) at 1 kV. A sputter-coater (E1030 Ion Sputter, Hitachi Ltd.) was used to coat the samples with Au–Pd. The average diameter of microspheres was measured from microphotographs of more than 200 microspheres.

Thermal analysis of PLA microspheres were conducted with differential scanning calorimeter (SSC5200H, Seiko Instruments Inc.). The samples were heated at the rate of 5 K/min under a nitrogen atmosphere. The scans were adopted first heating and the measurements were performed twice for each sample.

In vitro release experiments, PLA microspheres of 0.1 g were put into 30 ml of pH 4 buffer solution, in which CPT-11 was stable because lactone–carboxylate transition in the CPT-11 molecule is occurred in the higher pH range, and then placed in a shaker bath thermostated at 310 K. The amount of CPT-11 released into the buffer solution was detected with a fluorescence spectrophotometer (Ex. 365 nm, Em. 440 nm; F-2500, Hitachi, Ltd.). The measurement dates were the mean values of three. After the release experiment, the amount of CPT-11 remaining in the microspheres was measured by the HPLC method described above.

3. Results and discussion

3.1. Characterization of PLA microspheres

The mean diameter and enclosing efficiency of prepared PLA microspheres were summarized in Table 1. In the experiments in this table, the reproducibility was confirmed. The mean diameter of PLA microspheres with MOPEO-PLA was smaller than that of PLA microspheres without MOPEO-PLA. This was the result of polymer surfactant, MOPEO-PLA, to reduce interfacial tension between acetonitrile solution and silicon oil. To neglect the effect of diameter of PLA microspheres on CPT-11 release property, the diameter of PLA microspheres without MOPEO-PLA was adjusted to be the same as that of PLA microspheres with MOPEO-PLA by controlling mechanical stirring rate during solvent evaporation process.

3.2. Scanning electron microscopic observation

Fig. 1 shows SEM images of PLA microspheres with or without MOPEO-PLA. PLA microspheres enclosing CPT-11 without MOPEO-PLA had rough surface (Fig. 1A). From our previous study, it was clarified that surface morphology of PLA microspheres was changed from smooth to rough depending

Table 1
Average diameter and enclosing efficiency of PLA microspheres

MOPEO-PLA (wt.%)	Drug content (mg/ml)	Stirring rate (rpm)	Diameter (μm)	Enclosing efficiency (%)
0	2.0	125	37.2	93.4
10	2.0	100	36.1	100
20	2.0	100	38.9	100
30	2.0	100	39.5	97.8

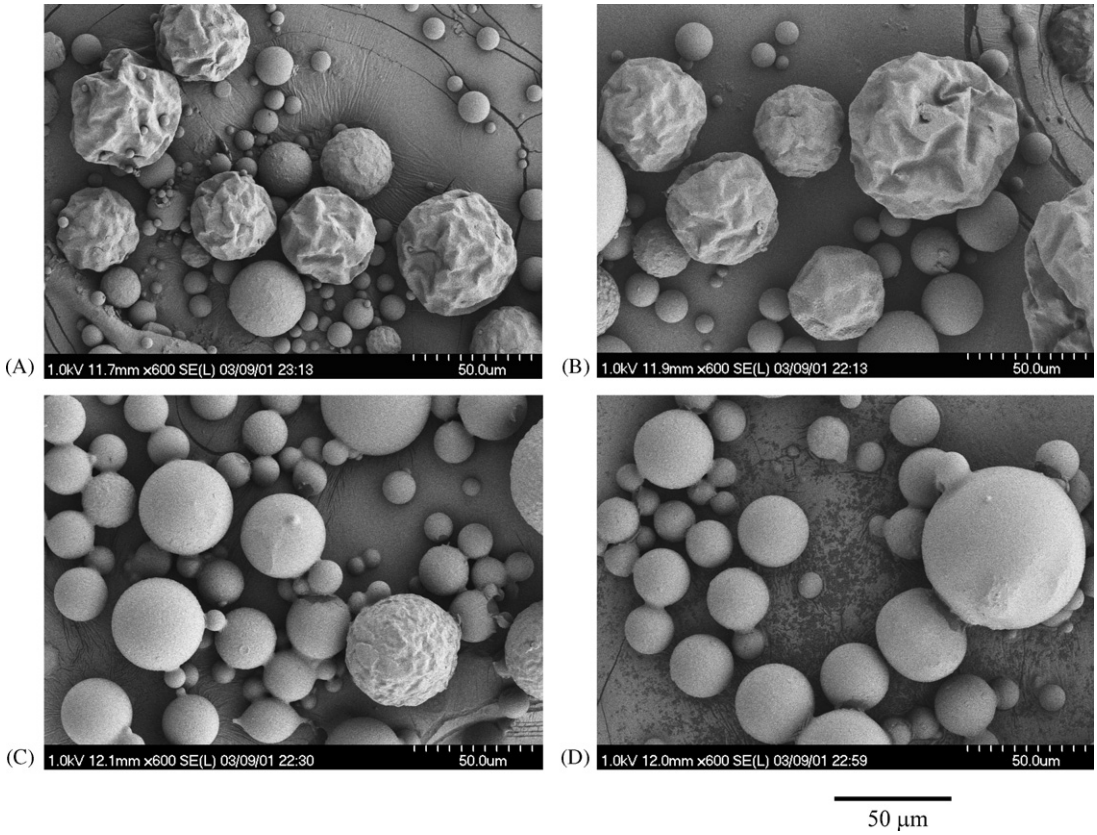

Fig. 1. SEM photographs of PLA microspheres enclosing CPT-11. (photo A) Without MOPEO-PLA, (photo B) with 10 wt.% of MOPEO-PLA, (photo C) with 20 wt.% of MOPEO-PLA and (photo D) with 30 wt.% of MOPEO-PLA.

on encapsulated amount of CPT-11. It is obvious from this figure that the higher MOPEO-PLA content in PLA microspheres, the less degree of winkles of surface morphology becomes. Especially, almost smooth surface was produced in case that MOPEO-PLA content was 30 wt.%.

3.3. Differential scanning calorimetric studies

The morphological change of PLA microspheres with a CPT-11 content greater than 2.0 mg/ml was induced from the lowering of glass transition temperature (Tg) of polymer matrix by interaction between the polymer matrix and CPT-11 (Yoshizawa et al., 2003). In our additional experiment, the surface morphology was changed from rough to smooth by changing the solvent evaporation temperature, much lower and higher than Tg of polymer matrix (Yoshizawa et al., 2005). Therefore, the relationship between Tg of polymer matrix and the solvent evaporation temperature becomes important parameter to determine the surface morphology of PLA microspheres. In this case of PLA and MOPEO-PLA blended microspheres, it is considered that the Tg of polymer matrix affects to the surface morphology of PLAmicrospheres with CPT-11 because the solvent evaporation temperature was constant in this case.

Fig. 2 shows the effect of MOPEO-PLA content in PLA microspheres on Tg of PLA and MOPEO-PLA blended microspheres. The Tg was taken as the node of baseline and tangent line with endothermic peak. From this figure, Tg of PLA drastically decreased with increasing MOPEO-PLA content in PLA microspheres, indicating that some degree of interaction exists between PLA and MOPEO-PLA. Therefore, it was found that the surface morphology was smooth when the Tg of microspheres was less than 310 K, which was solvent evaporation temperature. In the initial stage of solvent evaporation process,

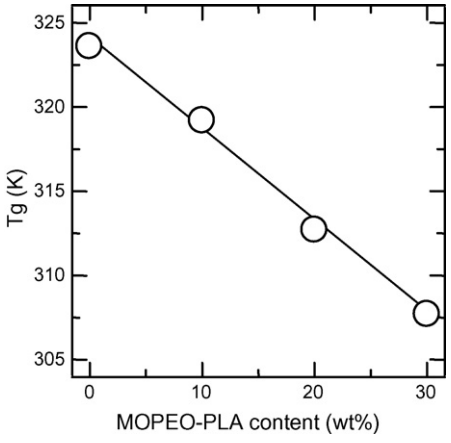

Fig. 2. Effect of MOPEO-PLA content in PLA microspheres on Tg of PLA and MOPEO-PLA blended microspheres.

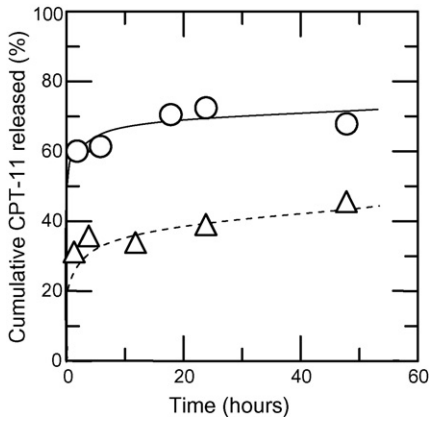

Fig. 3. Release profiles of CPT-11 from PLA microspheres without MOPEO-PLA and with 20 wt.% of MOPEO-PLA. MOPEO-PLA content was (◯) 0 wt.% and (△) 20 wt.%.

the surface of dispersed droplets gradually solidified with evaporation of solvent and form a polymer film. In case of PLA microspheres with high content of MOPEO-PLA, the polymer film was softened by interaction of PLA and MOPEO-PLA. In the process of forming a polymer microsphere with shrinkage of dispersed droplet by removal of solvent in dispersed droplet, the flexible polymer film was solidified to form a smoothed-surface. This is the reason why the surface morphology of PLA microspheres with high content of MOPEO-PLA was smooth. It is considered that MOPEO-PLA had a good compatibility with the matrix of PLA microspheres and performed the plasticizer role in forming the smoothed-surface of PLA microspheres.

3.4. In vitro release test

Fig. 3 illustrates the release profiles of CPT-11 from PLA microspheres without MOPEO-PLA and with 20 wt.% of MOPEO-PLA. The release of CPT-11 after initial burst was depressed in both cases. This tendency was due to that release from the core part of microspheres became very slow, because polymer used as microsphere matrix had higher molecule weight and a dense structure (Wada et al., 1988). Fig. 4 shows the effect of MOPEO-PLA content in PLA microspheres on initial burst. The initial burst was defined by extrapolating a linear portion of the release curve to *Y*-axis in Higuchi plot. The initial burst of CPT-11 from PLA microspheres without MOPEO-PLA was approximately 60%, this significant initial burst was contributed to the rough surface morphology having a large surface area (Yoshizawa et al., 2003). The initial burst was achieved to minimum when MOPEO-PLA content was 20 wt.%. As can be seen from Fig. 1, wrinkles on the surface of microspheres were disappeared with increasing of MOPEO-PLA content. That is, the more the MOPEO-PLA content was, the smaller the surface area of microspheres was. This reduction of surface area led to suppress the initial burst. However, largely initial burst was observed at 30 wt.% of MOPEO-PLA content although the microspheres had a smooth surface. This was attributable to the enhancing of water penetration into PLA microspheres because many hydrophilic PEO segment located in surface and inside the PLA microspheres in case of adding 30 wt.% of MOPEO-PLA.

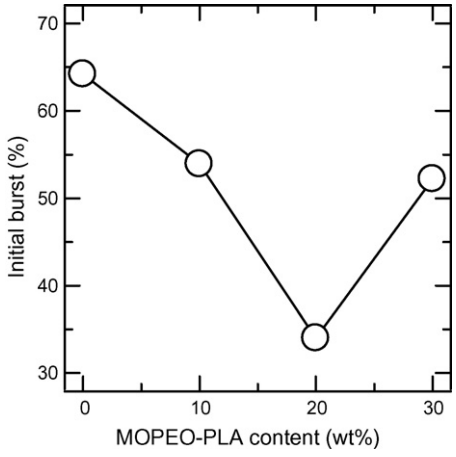

Fig. 4. Initial burst of CPT-11 from PLA microspheres with various content of MOPEO-PLA.

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