Microfluidic Fabrication of Monodisperse Polylactide Microcapsules with Tunable Structures through Rapid Precipitation

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ABSTRACT

We describe a versatile and facile route for the continuous production of monodisperse polylactide (PLA) microcapsules with controllable structures. With the combination of microfluidic emulsification, solvent diffusion and internal phase separation, uniform PLA microcapsules with a perfluorooctyl bromide (PFOB) core were successfully obtained by simply diluting monodisperse ethyl acetate (EA)-in-water emulsion with pure water. Rapid extraction of

EA from the droplets into aqueous phase enabled for solidification of the polymer droplets at non-equilibrium state during internal phase separation between concentrated PLA/EA phase and PFOB phase. Higher molecular weight PLA generated structural complexity of the microcapsules, yielding core-shell microcapsules with covered with small PFOB droplets. Removal of the PFOB via freeze-drying gave hollow microcapsules with dimpled surface. The core-shell ratios and the diameter of these microcapsules could be finely tuned by just adjusting concentration of PFOB and flow rates on emulsification, respectively. These biocompatible microcapsules with controllable size and structures are potentially applicable for biomedical fields such as drug delivery carriers of many functional molecules.

1. Introduction

Biodegradable polymeric microparticles have attracted a great deal of attention in applications for medical and pharmaceutical fields such as sustained release carriers for drugs (1), agrochemicals (2), medical imaging agents (3), personal care ingredients, and cell scaffolds for tissue engineering (4). In these ubiquitous applications, among many kinds of biodegradable polymers, polylactide (PLA) and poly(lactide-*co*-glycolide) (PLGA) are the most commonly used in the world since they both have good biocompatibility and high mechanical strength (5, 6).

Generally, these microparticles are produced through ”top-down” emulsification approach, known as emulsion-solvent evaporation technique (7-11). In this technique an oil-in-water (O/W) emulsion is formed by emulsification of a polymer/volatile organic solvent mixture in an aqueous surfactant solution, after which the organic solvent is removed by evaporation, yielding polymeric microparticles dispersed in the media. This technique can also be used to fabricate liquid-filled microcapsules by either adding non-solvent to the organic phase prior to

emulsification (12-14), or preparing water-in-oil-in-water (W/O/W) emulsions used as a template (15-18). These techniques have been shown as a promising way to successfully produce core-shell polymeric microcapsules encapsulating lipophilic or hydrophilic compounds in the core and control their release behaviour.

In terms of microcapsule size, monodisperse microcapsules are preferable in several applications, especially in drug delivery carriers because they can decrease undesirable side effects and exhibit controlled drug release kinetics and encapsulation efficiency. Since monodisperse microcapsules can only be produced from precision emulsion droplets, generation of uniform droplets is of utmost importance process in fabrication of microcapsules using top-down techniques. To date, several methods have been developed to produce monodisperse polymeric microcapsules using flow dynamics such as membrane emulsification (19), microfluidic emulsification (20-25), and jet acoustic excitation (26). Among them, droplet-base microfluidic system is considered as one of the most effective ways to prepare monodisperse microcapsules. Pioneer work by Utada *et al*. has been achieved to fabricate monodisperse W/O/W double emulsion using a coaxial microcapillary fluidic device (27). Since then, there have been many reports regarding fabrication of monodisperse microcapsules using a method combining microfluidics with solvent evaporation. For example, Liu *et al*. have successfully controlled the stability and the size of monodisperse PLGA microcapsules by tuning osmotic pressure between the internal and the external aqueous phase during W/O/W double emulsion formation (28). Amstad *et al*. have developed monodisperse polymersomes with thermo- and photoresponsive by introducing thermoresponsive diblock copolymer, poly(*N*-isopropylacrylamide)-*b*-poly(lactide-*co*-glycolide) and photosensitive gold naoparticles into the shell of the polymersomes (29). For oil-filled microcapsules, Lensen *et al*. have employed to

prepare monodisperse dodecane-filled poly(L-lactide) (PLLA) microcapsules using combination of O/W emulsion-solvent evaporation and internal phase separation (30). However, these methods require toxic organic solvents and much time to form microcapsules, and difficult to tailor microcapsule morphologies. On the emulsion preparation, dichloromethane or chloroform are commonly used as the organic solvent due to a good solvent for biodegradable polyesters and their high volatile properties that facilitate evaporation of the solvent from the emulsion droplets, although the vapour or residue are considered to be harmful to environment and human body. In addition, even when using volatile organic solvents as the dispersed phase, the rate-determining step for the microcapsule formation is solvent evaporation process, which actually takes a few minutes to several hours, depending on the volume of the continuous phase. That is why, the emulsion is required to be stored in a vessel until the solvent evaporates from the system and the microcapsules are formed. Moreover, despite the fact that the morphology of microparticles plays a crucial role in determining the behaviour in the fluid, controlling loading efficiency and release kinetics of the ingredients, tuning the structure of polymeric microparticles at a micrometre scale is still challenging. Hence, developing a simple and environmentally friendly process for production of monodisperse microcapsules with controllable size and tailored architecture is in great demand to expand their functionalities.

Herein, we present a facile straightforward method to continuously produce monodisperse PLA microcapsules with controlled structures by a modified "droplet-to-particle technology" (31) that includes microfluidic emulsification, emulsion-solvent diffusion, and internal phase separation. In our process, for a dispersed organic phase, PLA and perfluorooctyl bromide (PFOB), a non-solvent for PLA, were dissolved in ethyl acetate (EA) that is a non-toxic organic solvent approved by the Food and Drug Administration in the US and has relatively higher

solubility in water (8.3 wt% at 20°C). Monodisperse O/W emulsion droplets produced by using a commercial Y-shaped microfluidic device were directly poured into an excess amount of water, which induced rapid extraction of EA from the droplets and internal phase separation between PFOB phase and concentrated PLA/EA phase. Within a few seconds, PLA precipitated at the surface of each droplet, resulting in forming monodisperse PLA microcapsules encapsulating liquid PFOB. Different from conventional techniques, our approach for forming robust microcapsules takes only a few seconds to precipitate the polymer after onset of solvent diffusion, which has enabled not only to produce uniform microcapsules in a continuous manner, but also to solidify microcapsules at non-equilibrium state and to design their structures. In addition, all components we used in the microcapsule formation are biocompatible, which is great advantageous to applications for biomedical fields. To the best of our knowledge, this is the first report to continuously fabricate monodisperse core-shell PLA microcapsules with tunable size and structures using solvent diffusion from simple O/W emulsion droplets.

2. EXPERIMENTAL SECTION

2.1 Materials

Poly(D,L-lactide) (PLA) and poly(ethylene glycol)-*b*-poly(D,L-lactide) (PEG-*b*-PLA) were synthesized by ring-opening polymerization of D,L-lactide in the presence of tin(II) 2-ethylhexanoate as a catalyst using lauryl alcohol and poly(ethylene glycol) monomethyl ether (PEG, Mn= 4,000, Mw/Mn= 1.06) as an initiator, respectively as previously reported (32). The D,L-lactide was purchased from Purac (Netherland). The PEG was kindly supplied from NOF (Japan). Tin(II) 2-ethylhexanoate, ethyl acetate (EA) and perfluorooctyl bromide (PFOB) were obtained from Wako Pure Chemical Industries, Ltd. (Japan). Porphyrin derivative was kindly

supplied from porphyrin labolatory (Japan). The ultra pure water was produced by a Millipore Milli-Q purification system (EMD Millipore Corporation, USA).

2.2 Preparation of monodisperse PLA microcapsules

A schematic illustration of the preparation procedure of monodisperse PLA microcapsules is shown in Figure 1. The microfluidic device that was used for the microcapsule fabrication consisted of a Y-shaped channel (126 μm- and 136 μm-width channels with 75 μm in depth) made of SUS basement and a glass cover plate, fabricated by Kasen Nozzle Mfg. Co., Ltd, Japan. The continuous aqueous phase and the dispersed organic phase were pumped independently at adjustable flow rates using syringe pumps connected to the device via Teflon tubing. An aqueous solution saturated with EA containing 1 wt% of water-soluble PEG-*b*-PLA (w-PEG-*b*-PLA, Mn= 4,400, Mw/Mn= 1.05, hydrophile-lipophile balance (HLB)= 18.2) was used as the continuous phase and an EA solution composed of 25 mg mL^{-1} of PLA (Mn= 13,600, Mw/Mn= 1.14 or Mn= 52,000, Mw/Mn= 1.29) and 1.25-15 μL mL^{-1} of PFOB was used as the dispersed phase. For confocal microscopy, a trace amount of porphyrin derivative was added to the dispersed phase before the feeding. The obtained O/W emulsion was transferred to a bath filled with 100 mL of ultra pure water through Teflon tubing (Φ= 0.5 mm, L= 20 cm) whose exit tip was submerged in the water. The EA was then rapidly removed from the droplet to the massive amount of pure water by solvent diffusion with or without gentle stirring, leading to precipitation of PLA microcapsules. The microcapsules were washed with ultra pure water three times by centrifugation (himac CF 15R, Hitachi, Japan) (3,000 rpm, 3 min) to remove the surfactant, followed by freeze-drying overnight, yielding dried PLA microcapsules.

2.3 Interfacial tension measurements

Interfacial tension measurement was carried out by the pendant drop method using an equipment of DSA10 (Kruss, Germany). An EA droplet containing 25 mg mL^{-1} of PLA, and a pure PFOB droplet were formed in an aqueous solution of 1 wt% of w-PEG-*b*-PLA saturated with EA. The interfacial tensions were calculated from the droplet images using the image analysis software. The measurements were carried out at 20°C.

2.4 Sample Characterization

Emulsions and microcapsules were observed using an optical microscope (OLYMPUS BX50, Japan) equipped with a digital camera (OLYMPUS CS 230). Porphyrin derivative labelled microcapsule dispersion was observed by a confocal laser scanning microscope (CLSM) equipped with a 1 mW helium-neon laser (Zeiss LSM-510, Japan). The red fluorescence was observed with a long-pass 560 nm emission filter under 543 nm laser illumination. Morphology of the microcapsules after freeze-drying was observed by a scanning electron microscope (SEM, S-4700, Hitachi Ltd., Japan) at intensity of 1 kV under various magnifications. A sputter-coater (E-1030 Ion-Sputter, Hitachi Ltd. Japan) was used to coat the samples with Pd-Pt to prevent the samples from charge up. Before the observation, the freeze-dried samples were stored in a desiccator. We evaluated the microcapsule size and the size distribution on the microscopic images by using image analysis software (Winroof, Mitanishoji Co., Ltd., Japan). In the analysis, the size distribution was expressed by coefficient of variation (CV) that is defined as the ratio of the standard deviation to the mean diameter. We used 200 microcapsules in each calculation.

3. RESULTS AND DISCUSSION

Continuous preparation of monodisperse microcapsules with a liquid oil core and a biocompatible polymeric shell of PLA via emulsion-solvent diffusion has been performed in this study. The process follows our previous technique to prepare monodisperse compact PLA microparticles using a combined method of microfluidic emulsification and subsequent solvent diffusion (30). This method does not require toxic materials and time-consuming solidification process. The dispersed phase is a mixture of PLA, EA, and PFOB. EA is a good solvent for PLA with high solubility to water, whereas PFOB that is used for a model-encapsulated reagent acts as a non-solvent for PLA. The continuous phase is an aqueous solution saturated with EA containing biocompatible w-PEG-*b*-PLA as a surfactant. The diblock copolymer enhances the stability of EA/water emulsion.

In detail, the organic phase and the aqueous phase are first separately introduced into the Y-shaped microfluidic device by using syringe pumps to produce monodisperse polymer droplets. Then, the polymer droplets travel to the downstream of the channel and are finally poured into a solidification bath filled with enough amount of ultra pure water via Teflon tubing. Due to higher solubility of EA in water, once the polymer droplets touch with pure water, the rapid diffusion of EA to the outer aqueous phase begins. The solvent diffusion leads to a rapid reduction of the droplet sizes and steep increase in volume fraction of PLA and PFOB within the droplets. As a result of the concentration increase, the droplets readily reach the bimodal boundary, which induces internal phase separation and results in formation of PFOB phase as tiny droplets and concentrated PLA/EA phase within each droplet. At this stage, we can see two interfaces; one is between PFOB phase and concentrated PLA/EA phase within the droplets and the other is between the dispersed organic phase and the continuous aqueous phase as we recognised emulsion. As the diffusion of EA proceeds, tiny PFOB droplets within each polymer droplet tend

to coalesce to minimize the interfacial area and eventually form a single big core within the emulsion droplets.

The equilibrium structure of the microcapsules can be predicted by using spreading coefficient theory established by Torza and Mason, which is based on the interfacial energy of O/W interface (33). According to the theory, if droplets of two immiscible liquids (phase 1 and phase 3) are brought into contact in the third mutually immiscible liquid (phase 2), the final equilibrium morphology can be predicted by calculating spreading coefficient values using each interfacial tension (γ_{12}, γ_{23}, and γ_{31}). The spreading coefficients S_i for each phase are defined as

$$S_i = \gamma_{jk} - (\gamma_{ij} + \gamma_{ik})$$
$$S_j = \gamma_{ik} - (\gamma_{jk} + \gamma_{ij})$$
$$S_k = \gamma_{ij} - (\gamma_{ik} + \gamma_{jk})$$

In our system, two immiscible drops correspond to a pure liquid PFOB (phase 1) and EA dissolving 25 mg mL^{-1} of PLA (phase 3), and the third immiscible phase is an aqueous solution of 1 wt% w-PEG-*b*-PLA saturated with EA (phase 2) as shown in Figure 2A. We measured two initial interfacial tension values: (1) between phase 1 and phase 2 and (2) between phase 2 and phase 3. It should be noted that we could not measure the interfacial tension between pure liquid PFOB (phase 1) and EA solution dissolving PLA (phase 3) since these solutions were completely miscible at the initial experimental condition, and there was therefore no interface, which is considered that the interfacial tension would be considerably lower value than that of other two interfaces. The measurement results were γ_{12}= 8.51 mN m^{-1} and γ_{23}= 2.53 mN m^{-1} (Table 1). If we assume that the interfacial tension value between phase 1 and phase 3, corresponding to γ_{12}, is close to 0 mN m^{-1}, the spread coefficient values of our system will be $S_1 < 0$, $S_2 < 0$ and of $S_3 > 0$,

indicating that the equilibrium configuration of the microcapsules is core-shell morphology (Figure 2B).

With the aid of microfluidics, we obtained monodisperse O/W emulsion without any satellite droplets as shown in Figure 3a. It indicates that the droplets are stabilized by w-PEG-*b*-PLA at the interface of the emulsion droplets. We then carried out the precipitation of the polymer in water bath with stirring at 120 rpm. The resultant microcapsules after solvent diffusion are shown in Figure 3b. Highly monodisperse microcapsules (d= 35.0 μm, CV= 4.0%) with core-shell structure were observed, indicating that internal phase separation occurred in the course of the solvent diffusion. Understanding the detailed internal microcapsule structure, we prepared the microcapsules with a trace amount of porphyrin derivative as a fluorescent marker. This fluorescent marker colours hydrophobic PLA red but does not colour PFOB and it also does not affect the stability of the emulsion. CLSM image has proved that each microcapsule possesses relatively uniform shell thickness and a large cavity located at the radial centre of the microcapsules (Figure 3c). It is also important to be noted that the shell of microcapsules has small pores although the configuration roughly corresponds to the theoretical prediction. The small cavities would be derived from small droplets of PFOB phase before coalescence. The diffusion of EA to the outer aqueous phase is a much faster process than the evaporation of dichloromethane reported by other groups. Due to higher solubility of EA in water, the microcapsules are obtained at a non-equilibrium configuration. The precipitation starts from the surface of EA droplets, therefore, some of phase separated small PFOB droplets before coalescence where it is located near the surface would be entrapped in the polymer matrix forming the shell of the microcapsules during the precipitation process. SEM observation after freeze-drying clearly shows that most of the microcapsules keep a spherical shape with smooth

surface (Figure 3d). In addition, we found that the microcapsules had hollow internal structure due to evaporation of the PFOB core during freeze-drying and many dimples at the core-shell interface (Figure 3d, inset). The dimples would be another clue that rapid precipitation of polymer provides non-equilibrium structure of microcapsules. These results show that our simple process is capable of continuous production of well-defined monodisperse PLA microcapsules with hydrophobic oil core and hollow microcapsules.

We achieved a robust control over the microcapsules size by varying the flow rates on emulsification. The flow rate of the continuous phase (Q_c) was varied between 1,200 μL h^{-1} and 6,000 μL h^{-1} while keeping the dispersed phase flow rate (Q_d) constant at 60 μL h^{-1}. As shown in Figure 4a-c, the diameter of the resultant microcapsules decreased with increasing the Q_c, which was controlled from 20.8 to 34.6 μm, in which the CV values of the core and the microcapsules size remained below 7%. Moreover, it was found that the shell thickness to radius (T/R) ratio was approximately constant at 0.28 regardless of the microcapsule size (Table 2). These results show that our system can produce monodisperse PLA microcapsules with tunable size without any effect on the T/R ratio of the core-shell structure.

We have also demonstrated that the ratio of core and shell of the microcapsules can be modulated by changing the concentration of PFOB from 1.25 to 15 μL mL^{-1}, whilst keeping the other compositions fixed. As shown in Figure 4d-f, independent of the PFOB concentration, monodisperse microcapsules with core-shell structure were successfully obtained. The images show that the T/R ratio decreases with increasing the PFOB concentration and it can be controlled from 0.28 to 0.61 (Table 3). In addition each shell thickness showed good agreement with the theoretical calculation results (Table S1).

Alternative to lower molecular weight PLA (Mn= 13,600, Mw/Mn= 1.14), in the case with PLA having relatively higher molecular weight (Mn= 52,000, Mw/Mn= 1.29) used as a shell forming material, we found that structural complexity of the microcapsules occurred as a result of solvent diffusion. As shown in Figure 5a, monodisperse microcapsules whose surfaces were covered with a lot of small droplets were obtained, which looks like Pickering emulsion (34). The CLSM image revealed that the microparticles also had core-shell structure (Figure 5b). In the image, the microcapsules showed dark rough surface, indicating the existence of the PFOB phase forming small droplets. Moreover, after freeze-drying, we obtained monodisperse hollow PLA microcapsules with dimpled surface as shown in Figure 5c. The size of dimples was apparently polydisperse and the dimples did not penetrate the shell of the microcapsules. The distinct surface morphology would be caused by the increase in the viscosity of the PLA solution. Scheme 1 shows the proposed formation mechanism of these microcapsules. In the case with the preparation condition that monodisperse PLA microcapsules with smooth surface were obtained, the viscosity of the dispersed phase was low. That is why, tiny PFOB droplets that stem from the emulsion droplet during internal phase separation can be easily movable in the polymer droplet and smoothly migrate towards the centre due to the higher interfacial tension between the PFOB phase and the continuous phase while small PFOB droplets coalesce each other before polymer precipitation (Scheme 1A). On the other hand, in the case with higher molecular weight PLA, the viscosity of the solution is relatively high due to increase in the degree of polymer entanglement in the solution. By increasing the viscosity, the small PFOB droplets formed by internal phase separation become difficult to move and to migrate towards the centre. In addition, it is much easier for the emulsion composition to reach the bimodal boundary in the droplets as a result of solvent diffusion, which leads to rapid solidification of the polymer droplets from the surface.

Since the volume shrinkage of the polymer droplets is much faster and PFOB is immiscible with water and a non-solvent for PLA, a partial phase separated small PFOB droplets before coalescence would be left on the surface of the microcapsules as a spherical form and the other PFOB droplets that are escaped from the precipitation front would migrate to the centre of the polymer droplet with coalescence prior to the complete solidification. Consequently, monodisperse core-shell microcapsules covered with a number of small PFOB droplets are formed in the collection bath (Scheme 1B).

In order to confirm the formation mechanism of the microcapsules with dimpled surface, we carried out to monitor the time course of the microcapsule formation during solvent diffusion. However, due to several limitations in volatility of EA, quick diffusion of EA into the aqueous phase, and rapid change in the density of the droplets within a few seconds, it was difficult to observe the microcapsule formation process under typical experimental conditions. Therefore, we did the model experiment using the emulsion (prepared by homogenizer) on a slide glass covered with a cover slip on a microscope stage. In the experiment, we induced solvent diffusion of EA by adding pure water into the system from the gap between the slide glass and the cover slip. Because of step-by-step addition of pure water from one side of the sample, the diffusion time scale was longer than that of our typical experimental process and the diffusion was spread heterogeneously in the sample, which gave the resultant microcapsules with irregular shape. However, we confirmed that in the middle stage, the surface roughness was formed as a result of pinning a portion of tiny PFOB droplets at the surface, which indicated that the proposed mechanism would be plausible (Figure S1).

The diameter of the microcapsules with dimpled surface can be tuned by changing the Q_c on the microfluidic emulsification. We obtained monodisperse dimpled PLA microcapsules with

11.7 to 40.6 μm in the diameter (Figure 6A, C, and D). It was also found that the dimple size decreased with declining the microcapsule size (Figure 6B and E). It should be noteworthy that the dimple size prepared with gentle stirring is smaller than that prepared without stirring although there is no difference in the microcapsule size. This result indicates that the stirring during solvent diffusion facilitates the polymer precipitation and suppresses the fusion of small PFOB droplets until some of them are stabilized on the surface.

4. CONCLUSIONS

Monodisperse PLA microcapsules encapsulating a liquid PFOB core were successfully produced by simply diluting monodisperse O/W emulsion with pure water under stirring. Rapid extraction of EA from the droplets into outer aqueous phase led to internal phase separation between concentrated PLA/EA phase and PFOB phase and rapid solidification of the droplets, resulting in core-shell microcapsules with non-equilibrium structures. The core-shell ratios and the diameter of the microcapsules could be modulated by varying the compositions of the dispersed phase and flow rates upon the emulsification. Our process has enabled to prepare monodisperse PLA microcapsules having a hydrophobic oil core and hollow structure, with either smooth or dimpled surfaces. We believe that such PLA microcapsules with controllable structures have great potential for carriers of functional molecules used in biomedical fields such as cosmetics, pharmaceutics and contrast imaging.

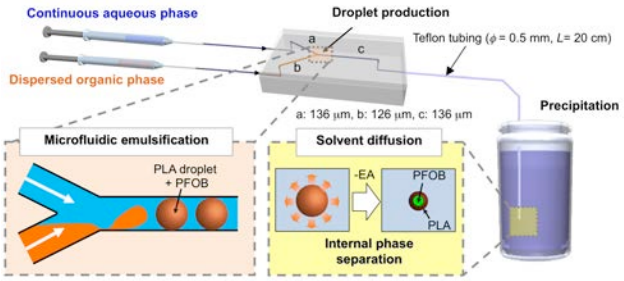


Figure 1 Schematic illustration of the process to continuously produce monodisperse PLA microcapsules encapsulating liquid PFOB in the core through microfluidic emulsification and solvent diffusion, coupled by internal phase separation.

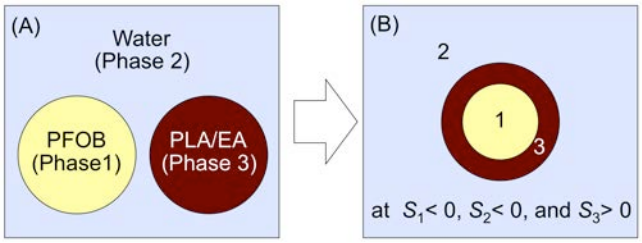


Figure 2 (A) Designation of each phase to calculate spreading coefficients. (B) Expected equilibrium core-shell configuration obtained at $S_1 < 0$, $S_2 < 0$, and $S_3 > 0$.

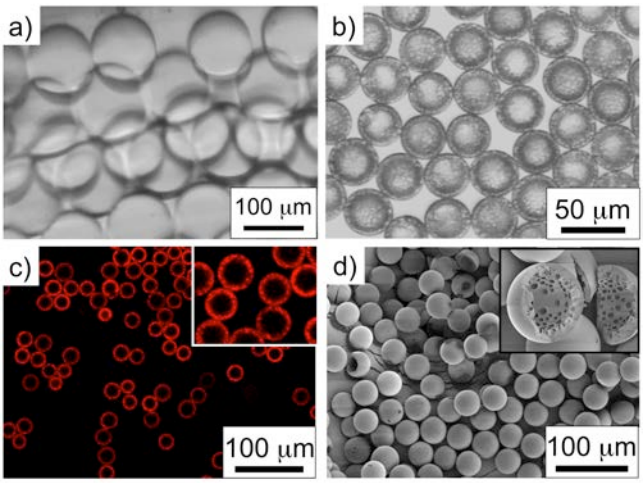


Figure 3 Optical micrographs of (a) monodisperse oil-in-water emulsion droplets in Teflon tubing and (b) monodisperse PLA microcapsules containing PFOB prepared with stirring at 120 rpm during solvent diffusion (Q_d = 60 μL h^{-1}, Q_c = 1,200 μL h^{-1}). (c) Confocal micrograph of porphyrin derivative labelled monodisperse PLA microcapsules. The inset is the magnified image (Q_d = 60 μL h^{-1}, Q_c = 6,000 μL h^{-1}). (d) SEM image of the microcapsules after freeze-drying. The inset image shows the magnified cross sectional view of the microcapsule (Q_d = 60 μL h^{-1}, Q_c = 1,200 μL h^{-1}).

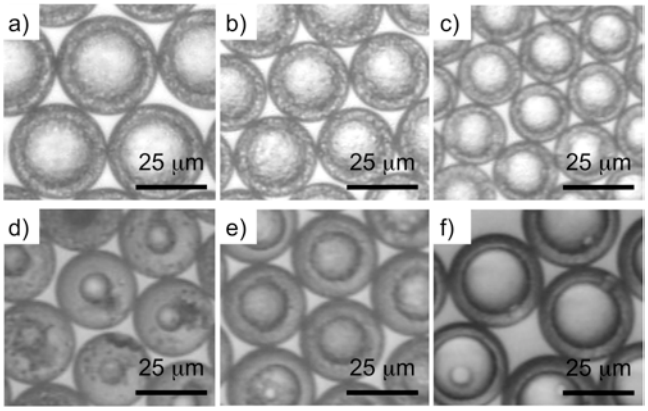


Figure 4 (a-c) Optical micrographs of monodisperse PLA microcapsules prepared by changing Q_c at a fixed Q_d (60 μL h^{-1}). The Q_c was (a) 1,200 μL h^{-1}, (b) 3,000 μL h^{-1}, and (c) 6,000 μL h^{-1}. The C_{PFOB} was constant at 15 μL mL^{-1}. (d-f) Optical micrographs of monodisperse PLA microcapsules prepared by varying the concentration of PFOB in the dispersed phase while keeping each flow rate constant (C_{PFOB}= (d) 1.25 μL mL^{-1}, (e) 5 μL mL^{-1}, and (f) 15 μL mL^{-1}, at Q_d= 60 μL h^{-1} and Q_c= 1,200 μL h^{-1}).

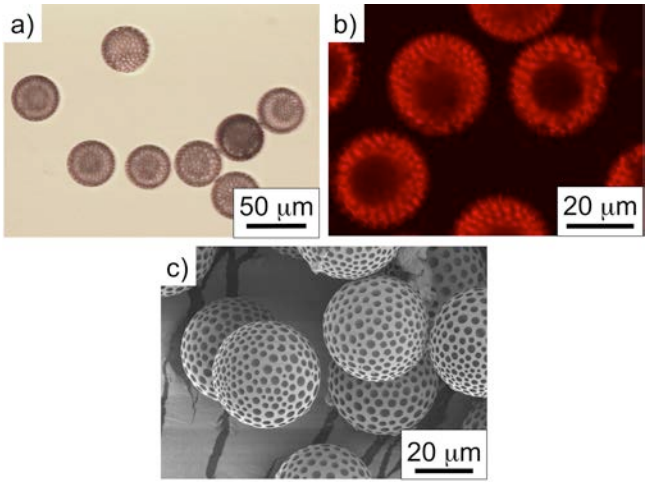
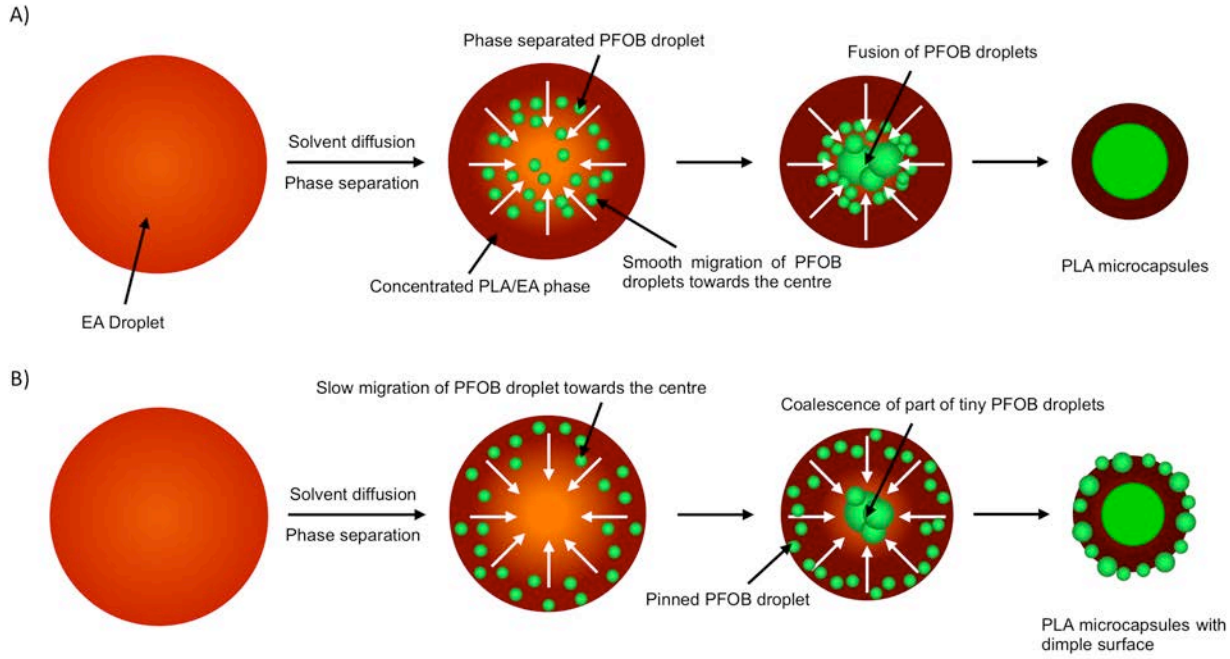


Figure 5 (a) Optical and (b) Confocal micrograph of porphyrin derivative labelled monodisperse PLA microcapsules covered with small PFOB droplets after solvent diffusion. (c) SEM image of

the microcapsules after freeze-drying. The microcapsules were produced by using PLA with higher molecular weight (Mn= 52,000, Mw/Mn= 1.29).



Scheme 1 Schematic illustration of proposed mechanism of the formation of PLA microcapsules with (A) smooth surface and those with (B) dimpled surface.

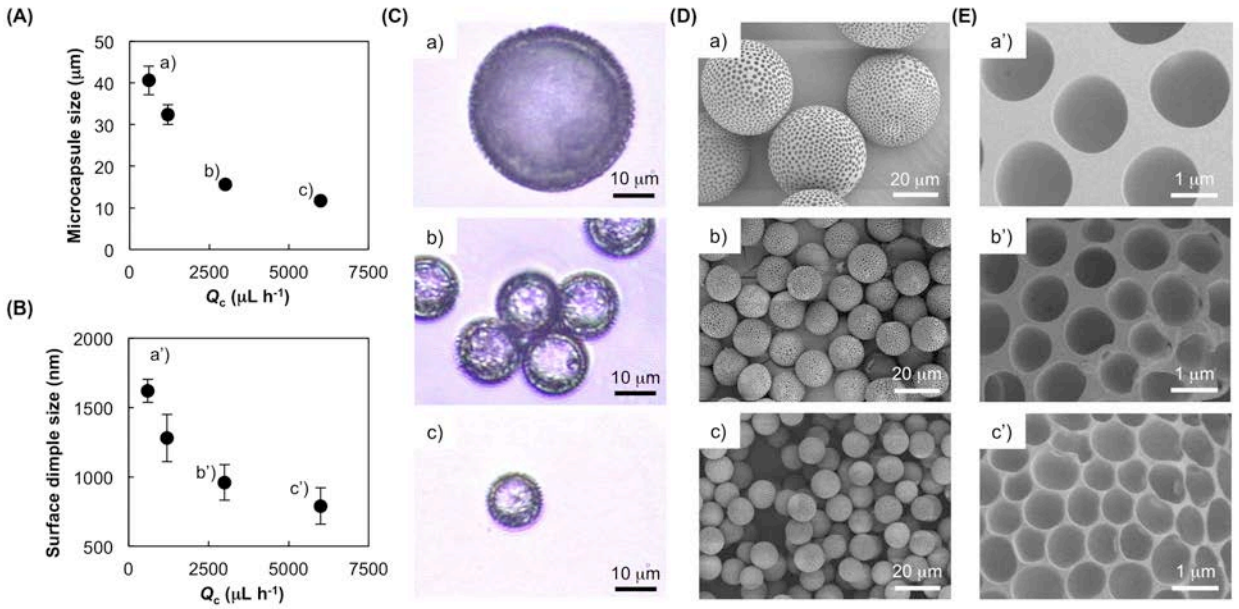


Figure 6 Effect of the Q_c on (A) the diameter and (B) the surface dimple size of the microcapsules. The microcapsules were produced by varying Q_c, while keeping Q_d constant. Q_c= (a, a') 600, (b, b') 3,000, and (c, c') 6,000 μL h^{-1} at Q_d= 60 μL h^{-1}. The C_{PFOB} was 15 μL mL^{-1}. (C) Optical micrographs of the microcapsules before freeze-drying. SEM images of (D) monodisperse PLA microcapsules after freeze-drying and (E) the magnified surface morphology.

Table 1. Interfacial tensions measured by Wilhelmy plate method.

Entry	Interfacial tension (mN m^{-1})
PFOB - Water (γ_{12})	8.51
PLA/EA - Water (γ_{23})	2.53
PFOB - PLA/EA (γ_{13})	NA*

*The interfacial tension was assumed to be almost 0 mN m^{-1} when calculating spreading coefficients.

Table 2. Effect of the Q_c on the microcapsule size, the inner core size, and the shell thickness to radius ratio.

Q_c [μL h^{-1}]	1,200	3,000	6,000
Microcapsule size [μm] (CV)	34.6 (4.0%)	26.8 (6.8%)	20.8 (4.0%)
Inner core size [μm] (CV)	25.0 (4.4%)	19.2 (6.1%)	15.0 (5.2%)
Shell thickness to radius ratio (T/R [-])	0.28	0.28	0.28

Table 3. Effect of the PFOB concentration in the dispersed phase on the microcapsule size, the inner core size and the shell thickness to radius ratio.

C_{PFOB} [μL mL^{-1}]	1.25	5	15
Microcapsule size [μm] (CV)	33.8 (3.1%)	35.5 (3.3%)	38.4 (3.1%)
Inner core size [μm] (CV)	13.1 (9.2%)	20.5 (3.4%)	27.6 (4.6%)
Shell thickness to radius ratio (T/R [-])	0.61	0.42	0.28

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Supporting Information Available

An equation for estimating the theoretical shell thickness of the microcapsules, the calculation results and optical microscopic images illustrating the formation of the microcapsules with dimpled surface. This information is available free of charge via the Internet at http://pubs.acs.org.

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