Dry fabrication of poly(*dl*-lactide-co-glycolide) microspheres incorporating a medium molecular drug by a ball mill method

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SUMMARY   Poly(*dl*-lactide-co-glycolide) acid (PLGA) microspheres is a useful carrier for controlled drug release. However, the organic solvents used in their conventional manufacturing process may affect the chemical structure of a macromolecular drug. Thus, we investigated the applicability of a dry fabrication method for PLGA microspheres. Cyanocobalamin (MW = 1,355) (VB_{12}) was used as a model drug, and it formed agglomerates under mild conditions with powdered PLGA in a generic ball milling system. Light and scanning electron microscopy showed the formation of PLGA microspheres and few agglomerates. The obtained microspheres had the particle size injectable as suspensions, namely smaller than 150 μm specified for subcutaneous and intramuscular injections by the Japanese Pharmacopoeia. The observed and theoretical drug contents were consistent. PLGA microspheres fabricated using a combination of small (ϕ3 mm) and large (ϕ10 mm) balls showed low initial burst of cyanocobalamin release *in vitro*. The *in vitro* drug release profile was equivalent with that of the microspheres fabricated by a conventional oil-in-water emulsion solvent evaporation method, while the drug release profile was influenced by the brand of the PLGA used. To prevent drug loss during fabrication, the dry fabrication method using a ball mill should be applied to prepare PLGA microspheres containing a medium macromolecular drug.

Keywords   biodegradable polymer, microspheres, solvent-free, long-acting injectable formulation, mechanical alloying

1. Introduction

Biodegradable polymeric microspheres are injectable formulations that promote long-acting drug efficacy in clinical practice. Although oral formulations are widely used for patient care, these formulations still hold some risks, such as risks of noncompliance, asphyxiation due to deterioration of swallowing ability, cognitive decline, polypharmacy, and finite supervised administration. Given these risks, there is a growing importance of long-acting injectable formulations administered under supervision.

Conventional methods for fabricating biodegradable polymeric microspheres include the emulsion-based solvent evaporation method (*1*) and the spray drying method (*2*). However, most biodegradable polymers used in long-acting injectable formulations, such as polylactide (PLA) and poly(*dl*-lactic-co-glycolic) acid (PLGA), are insoluble in an aqueous solvent. Thus, in the preparation of microspheres using the conventional methods, organic solvents, such as methylene chloride, are essential to dissolve the polymers. However, the use of organic solvents in the fabrication of microspheres leads to problems that can increase product cost, such as installation of a removal facility dedicated for volatile organic compounds, risk control for exposure of the environment and workers to organic solvents, and residual solvents in the formulation. Following the recent launch of biomedicines as therapeutic agents for refractory or rare diseases, a paradigm shift of therapeutic agents from chemical compounds to biomedicines is expected in drug development. However, the use of organic solvents in the manufacturing process of biomedicines can inactivate them, thus limiting the development of long-acting injectable formulations of biomedicines.

In the fabrication of biodegradable polymeric microspheres, not only the use of organic solvents but also the complicated manufacturing process leads to high production costs. The manufacturing processes of microspheres are, so far, more complicated than those of the conventional injectable formulation. The complicated processes are a disadvantage in terms of the aseptic guarantee because these processes are performed under aseptic conditions. In addition, containment technology is also needed if the

active pharmaceutical ingredients encapsulated in microspheres are highly potent.

We have investigated and proposed a new microsphere fabrication method without the use of halogenated solvents (*3*). Although the method results in low toxicity in the manufacturing process, the risks mentioned above remain. To completely solve these problems, a new fabrication method without the use of any solvents is needed. Recently, we reported a dry coating method (*4*). The method is based on the agglomeration that occurs in pulverization using a ball mill. Dry grinding with a ball mill is a popular technique used in the pharmaceutical industry; moreover, low critical particle sizes can be achieved through pulverization. In general, it is difficult to reduce particle size below 10 μm by dry milling. Core particles of approximately 10 μm are not pulverized in the dry milling process; however, the exothermic effect is maintained. Exothermic energy can melt the coating materials, leading to the formation of agglomerates of core particles and coating materials. The remarkable characteristics of this method is that coating materials larger than core particles can be applied. The dry coating technology used in the present study is related to mechanofusion, which is mainly used for modifying the surface of particles. For the conventional mechanofusion and dry coating procedures, smaller particles were used as guest particles and larger particles as hosts (*5-8*). In this case, guest particles corresponded to the coating materials. Nanoparticles are often used as guest particles to obtain particles of micrometer size, which refers to nanocomposites (*9,10*). However, there are some technical challenges in the fabrication of small guest particles, such as nanoparticles. Our reported technique has an advantage of no technical difficulties in obtaining coating materials.

The objective of this study is to evaluate the feasibility of our dry coating technique in the fabrication of PLGA microspheres. We compared the microspheres fabricated by the dry technique with those prepared by the conventional oil-in-water (o/w) solvent evaporation technique in terms of encapsulation efficiency and drug release profiles. The results of this study will encourage the use of an alternative, facile method for fabricating long-acting injectable formulations, such as PLGA microspheres.

2. Materials and Methods

2.1. Materials

PLGA with a lactic acid: glycolic acid ratio of 50:50 and inherent viscosity midpoint of 0.2 dl/g was purchased from Sigma-Aldrich Co., LCC. (St. Louise, US; Resomer® RG502 and Resomer® RG502H) and FUJIFILM Wako Chemicals Corporation (Osaka, Japan; PLGA5020), and was kindly gifted by Corbion (Amsterdam, Netherlands; PURASORB®PDLG5002). Cyanocobalamin (VB_{12}) was purchased from Enzo Life Science, Inc. (New York, US). Polyvinyl alcohol (PVA, POVAL® 220C) was obtained from KURARAY Co., Ltd. (Tokyo, Japan). All other chemicals used were of reagent grade. There are no ethical aspects to declare in this study.

2.2. Fabrication of microspheres

A. Dry fabrication method

Briefly, 150 mg mixture of pulverized VB_{12} particles (9.07 ± 0.47μm) and PLGA (21.86 ± 0.45μm) was placed in an agate ball mill pot (φ40 mm; h 40 mm) with balls (agate φ10 mm and zirconia φ3 mm). The ball mill was then rotated at 22-23°C using a planetary mill (Pulverisette 6; FRITSCH GmbH, Idar-Oberstein, Germany). One cycle consisted of rotation at a speed of 250 rpm for 30 min followed by resting for 5 min. The fabrication condition of each sample is shown in Table 1.

B. Conventional o/w emulsion solvent evaporation method

For microsphere fabrication using the conventional o/w emulsion solvent evaporation method, 30 mg pulverized VB_{12} particles (ca. 10 μm) were dispersed, and 120 mg PLGA were dissolved in 0.75 mL methylene chloride to prepare the oil phase. The oil phase was emulsified into 0.5% PVA aqueous solution 5 mL using a homogenizer (ULTRA-TURRAX® T18; IKA®-Werke GmbH & Co., KG, Staufen, Germany) for 5 min at 22-23°C. The resultant emulsion was added to 100 mL water all at once as needed and stirred at room temperature to remove the solvent for 3 h. This constituted the solvent evaporation process. The hardened particles were sieved through 149-μm sieves to remove any aggregates and collected by 20-μm sieves to be lyophilized.

2.3. Microscopic observations

The obtained microspheres were observed by a scanning electron microscopy (SEM) (JSM-5500LV; JOEL Ltd., Tokyo, Japan). Samples for SEM observation were prepared through deposition on a gold-palladium plate at 15 mA for 3 min (Quick Auto Coater JFC-1500; JOEL Ltd.).

2.4. Determination of particle size

The sizes of the fabricated microspheres were determined using a laser diffraction particle size analyzer (SALD-2200; Shimadzu Co., Ltd., Kyoto, Japan). Briefly, A few mg the microspheres were dispersed in 0.05% tween 80 solution and the suspension were applied to the analyzer.

Table 1. Fabrication condition of VB$_{12}$ microspheres

Sample No.	Fabrication Method	VB$_{12}$/PLGA (VB$_{12}$ loading)	PLGA	Ball[2]	Cycle[3]	Total process time
#1	Dry fabrication method	30 mg/120 mg (20%)	Resomer®RG502	4 agate balls	2	1h
#2	Dry fabrication method	30 mg/120 mg (20%)	Resomer®RG502	4 agate balls	6	3h
#3	Dry fabrication method	30 mg/120 mg (20%)	Resomer®RG502	4 agate balls	12	6h
#4	Dry fabrication method	30 mg/120 mg (20%)	Resomer®RG502	4 agate balls	24	12h
#5	Dry fabrication method	15 mg/135 mg (10%)	Resomer®RG502	4 agate and 4 zirconia balls	24	12h
#6	Dry fabrication method	15 mg/135 mg (10%)	Resomer®RG502	4 agate and 10 zirconia balls	24	12h
#7	Dry fabrication method	15 mg/135 mg (10%)	Resomer®RG502	4 agate and 15 zirconia balls	24	12h
#8	Dry fabrication method	15 mg/135 mg (10%)	Resomer®RG502	4 agate balls	24	12h
#9	Dry fabrication method	30 mg/120 mg (20%)	Resomer®RG502	4 agate and 15 zirconia balls	24	12h
#10	Dry fabrication method	45 mg/105 mg (30%)	Resomer®RG502	4 agate and 15 zirconia balls	24	12h
#11	Conventional method[1]	30 mg/120 mg (20%)	Resomer®RG502	-	-	-
#12	Dry fabrication method	30 mg/135 mg (20%)	PURASORB®PDLG5002	4 agate and 15 zirconia balls	24	12h
#13	Dry fabrication method	30 mg/135 mg (20%)	Resomer®RG502H	4 agate and 15 zirconia balls	24	12h
#14	Dry fabrication method	30 mg/135 mg (20%)	PLGA5020	4 agate and 15 zirconia balls	24	12h

[1] o/w emulsion solvent evaporation method, [2] agate ball: ϕ 10 mm, zirconia ball: ϕ 3 mm, [3] milling for 30 min followed by resting for 5 min.

2.5. Determination of VB$_{12}$ content in microspheres

Microspheres loaded with VB$_{12}$ (10 mg) were weighed in a test tube and dissolved in methylene chloride (2 mL), to which 9.6 mM phosphate buffered saline (pH 7.4; 5 mL) was added. VB$_{12}$ was extracted after overnight shaking. After centrifugation for 5 min at 2,000 rpm, the upper aqueous layer was collected, and the concentration of VB$_{12}$ in the aqueous layer was determined by the absorbance at 360 nm using a hybrid multimode microplate reader (Synergy H4; BioTek Instruments, Winooski, LV, USA) based on absorptiometry. VB$_{12}$ loading and encapsulation efficiency were calculated using the following equations:

$$VB_{12}\ loading\ (\%) = \frac{Weighed\ VB_{12}\,(mg)}{Weighed\ VB_{12}\,(mg) + Weighed\ PLGA\,(mg)} \times 100$$

$$Encapsulation\ efficiency\ (\%) = \frac{\%\ actual\ VB_{12}\ content\ in\ microspheres}{\%\ VB_{12}\ loading\ in\ microspheres} \times 100$$

2.6. Dissolution test

Dissolution test was performed by the rotating bottle method. Briefly, microspheres loaded with VB$_{12}$ (10 mg) were weighed in a test tube, to which 9.6 mM phosphate buffered saline (pH 7.4; 10 mL) at 37°C was added as a dissolution medium. The microspheres were then dispersed, rotated at 25 rpm at 37°C in an air-conditioned incubator (BioShaker V-BR-36; TITEC, Koshigaya, Japan), and collected at predetermined times. After centrifugation for 1 min at 2,500 rpm, all the medium was collected from the tube as a sample for dissolution assay and replaced with a fresh medium (10 mL). The dissolution test was performed in triplicate. The VB$_{12}$ that remained after the dissolution test was extracted and determined using the method described above.

2.7. Related substance analysis by HPLC

The aqueous layer obtained in the 'Determination of VB$_{12}$ content in microspheres' section was further analyzed by high-pressure liquid chromatography (HPLC) (Prominence; SALD-2200; Shimadzu Co., Ltd.) under the conditions of purity test in the Japanese Pharmacopoeia 17 edition. Namely, VB$_{12}$ and the related substances in the aqueous layer were assayed under the following conditions: detector: an ultraviolet absorption photometer (wavelength: 361 nm); column: a stainless steel column of 4.6-mm inside diameter and 25-cm length, packed with octylsilanised silica gel for liquid chromatography (5-μm particle diameter) (InertSustainC8, GLsciences, Tokyo, Japan); column temperature: a constant temperature of approximately 30°C; mobile phase: A (70 mM Na$_2$HPO$_4$ adjusted to pH 3.5 with phosphoric acid) and B (methanol) at an A/B ratio of 53/147; and flow rate: 1.05 mL/min.

2.8. Fourier transform infrared ray spectrometry (FT-IR)

Microspheres were analyzed by an attenuated total reflection method by FT-IR (FT/IR6600; JASCO International Co., Ltd., Tokyo, Japan). The samples were placed on a φ2.5-mm diamond prism and scanned at a range of 500-4,000 cm^{-1}.

2.9. Statistical analysis

Experiments were replicated at least thrice. The results are presented as means ± standard error of the means (s.e.m.).

3. Results

3.1. Fabrication conditions

First, we investigated the effect of processing time at
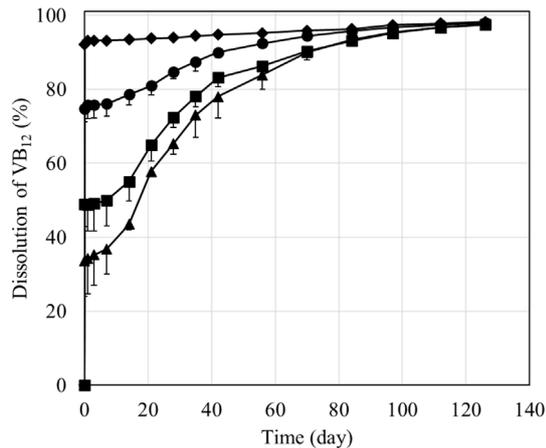
Figure 1. Effect of processing time on *in vitro* VB$_{12}$ release from the microspheres fabricated by the dry fabrication method. The processing time was ♦: 1 h (sample No.: #1); ●: 3 h (sample No.: #2); ■: 6 h (sample No.: #3); ▲: 12 h (sample No.: #4). The microspheres were fabricated under the following conditions: media, four balls (ϕ10 mm); PLGA, Resomer®RG502; loaded drug, 20%VB$_{12}$. The data represent mean ± S.E. (*n* = 3 batches).

Figure 2. Effect of the addition of small balls (ϕ3 mm) on the properties of microspheres. (a) *In vitro* VB$_{12}$ release; ●: 4 large and 4 small balls (sample No.: #5); ■: 4 large and 10 small balls (sample No.: #6); ▲: 4 large and 15 small balls (sample No.: #7); ○: 4 large balls alone (sample No.: #8). The data represents mean ± S.E. (*n* = 3 batches). **(b)** Optical micrograph of microspheres fabricated using *i*) 4 large balls (ϕ10 mm) alone and *ii*) 4 large (ϕ10 mm) and 10 small (ϕ3 mm) balls. The sample were dispersed in soybean oil. The bar indicates 50 µm. Resomer®RG502 was used as PLGA and 10% VB$_{12}$ was the loaded drug. The processing time was 12 h.

20% drug loading on drug release profiles. The results are shown in Figure 1. The fabrication was performed using 20% VB$_{12}$ as a loaded drug and 4 large balls (ϕ = 10 mm) as media. The result showed that processing time reduced the initial burst (release rate at 1 h). For the microspheres processed for 12 h, the initial burst was 34.2 ± 9.5%. Ball milling of PLGA without VB$_{12}$ did not provide microparticles after over 30 min of processing time, resulting in a large clump.

The effect of small (ϕ = 3 mm) and large (ϕ = 10 mm) balls on the amount of VB$_{12}$ released from microspheres were evaluated. Figure 2a shows VB$_{12}$ release from microspheres loaded with 10% VB$_{12}$ prepared by the dry fabrication method. Although large balls alone provided large initial burst, the combination of large and small balls reduced the initial burst. Microspheres prepared using large balls alone showed poor content uniformity, according to microscopic observation. On the contrary, microspheres fabricated using large and small balls showed good uniformity (Figure 2b). The effect of drug loading on drug release profiles is shown in Figure 3. Drug loading affected the initial burst, thus contributing to the drug release profile. Drug loading at 10%, 20%, and 30% resulted in initial bursts of 25.2 ± 1.9%, 15.6 ± 4.2%, and 58.2 ± 3.0%, respectively. FT-IR analysis shows that the any peaks of VB$_{12}$ and PLGA did not shift in the microspheres (Figure 4). The peaks of the VB$_{12}$-related substances did not increase, and the unknown peaks were not generated during the dry fabrication procedure (Table 2).

3.2. Comparison of properties of microspheres fabricated by the dry fabrication and those prepared by the conventional method

Figure 3. Effect of VB$_{12}$ loading on *in vitro* VB$_{12}$ release from the microspheres fabricated by the dry fabrication method. Drug loading was ●: 10% (sample No.: #7); ■: 20% (sample No.: #9); ▲: 30% (sample No.: #10). The microspheres were fabricated under the following conditions: media, 4 large (ϕ10 mm) and 15 small balls (ϕ3 mm); PLGA, Resomer®RG502. The processing time was 12 h. The data represent mean ± S.E. (*n* = 3 batches).

We investigated the comparison of morphological feature, particle size, encapsulation efficiency and drug release profiles of microspheres fabricated by the dry fabrication method and the conventional o/w emulsion solvent evaporation. For the morphological feature, the

conventional method provided the spherical feature. The dry fabrication method provided the aggregates gathered by several particles (Figure 5a). For the particle size distribution, the microspheres fabricated by both methods had the injectable particle size, not larger than 150 μm that is specified for suspensions for injection by the Japanese Pharmacopoeia (Figure 5b, Table 3). For

encapsulation efficiency, the dry fabrication method did not loss the drug during the fabrication process, however, encapsulation efficiency by the conventional method was $36.4 \pm 1.8\%$ (Table 3). The drug release was equivalent between the microspheres fabricated by both methods (Figure 5c).

3.3. Compatibility of the dry fabrication method with PLGA from various manufacturers

We evaluated the compatibility of the dry fabrication method with PLGA from various manufacturers under the following conditions: lactic acid: glycolic acid ratio, 50:50; inherent viscosity midpoint, 0.2 dl/g. The drug release profiles are shown in Figure 6. The order of the initial burst rate was as follows: PLGA5020 (Wako) > Resomer®RG502H (Sigma-Aldrich) > Resomer®RG502 (Sigma-Aldrich) > PURASORB®PDLG5002 (Corbion). The duration of drug release was in the order of the microspheres prepared by using Resomer®RG502 (Sigma-Aldrich) > Resomer®RG502H (Sigma-Aldrich) > PLGA5020 (Wako) > PURASORB®PDLG5002 (Corbion). With Resomer®RG502 (Sigma-Aldrich), the drug release lasted for 126 days.

4. Discussion

In general, in the process of ball milling, exothermic heat is generated by the collision between balls and samples, thus inducing the local melting of samples. The occurrence of melting in the process of amorphization by ball milling has been reported (*11*). Local melting at points of impact can induce binding between particles. Although the PLGA used in this study did not have a melting point owing to its amorphous state, binding between particles was considered to occur approximately at a softening point of 50-60°C. In contrast, VB_{12} is known to have a high melting point (> 300°C). VB_{12} particles with higher melting points are considered to be attached and bound by softened PLGA particles. PLGA particles with VB_{12} attached were pulverized and aggregated during repeated milling. Therefore, VB_{12} particles at a smaller proportion should be encapsulated
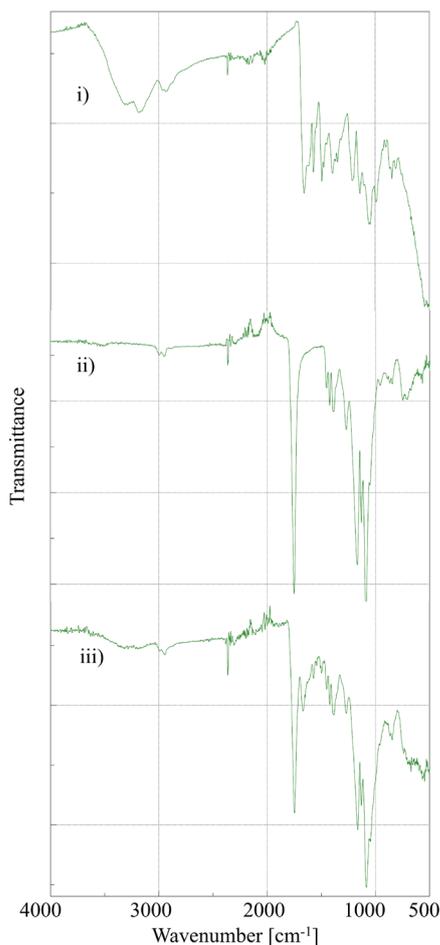
Figure 4. FT-IR analysis. *i*) VB_{12} powder, *ii*) PLGA powder, *iii*) microspheres fabricated by the dry fabrication method (sample No.: #4). The microspheres were fabricated under the following conditions: media, 4 large (ϕ10 mm) and 15 small (ϕ3 mm) balls; PLGA, Resomer®RG502; loaded drug, 20% VB_{12}; processing time 12 h.

Table 2. Evaluation of VB$_{12}$ related substances increase by the dry microsphere fabrication process

	Cyanocobalamin (%)		Related Substances (%)												
Retention time (min)	6.78	(Total)	3.14	3.49	3.70	4.03	4.89	5.33	5.49	5.79	6.22	8.07	9.42	10.07	11.19
VB_{12} PBS solution	97.37	2.63	0.06	0.04	0.12	0.01	0.05	0.17	0.26	0.22	0.09	0.06	0.98	0.26	0.30
Extracted solution from VB_{12} PBS solution	97.71	2.29	0.01	0.03	0.07	0.03	0.01	0.10	0.40	0.20	0.09	0.08	0.79	0.24	0.25
Extracted solution from VB_{12} loaded microspheres[*]	97.36	2.64	0.04	0.03	0.11	0.01	0.01	0.05	0.47	0.22	0.10	0.06	0.96	0.28	0.30

[*]The microspheres were fabricated under the following conditions: media, 4 large (ϕ10 mm) and 15 small balls (ϕ3 mm); process time 12h ; PLGA, Resomer®RG502; drug loading 10%.

by PLGA at a larger proportion in the microspheres. With one cycle consisting of milling for 30 min and resting for 5 min, the total milling time required to encapsulate ca.85% VB_{12} particles may be 12 h for Resomer® RG502, judging from the initial burst of 15% in the 20% VB_{12}-loaded Resomer® RG502 microspheres. From our preliminary study, approximately 15% initial burst were considered to be the lower limit, which indicated that

there was no difference in the initial burst between VB_{12}-loaded microspheres fabricated through 12 and 18 h of total milling (data not shown). This initial burst rate was also equivalent to that obtained with the conventional fabrication method, indicating that the initial burst of VB_{12} from Resomer® RG502 microspheres obtained through 12 h of milling was due to the properties of the polymer and the size of the microspheres, not due to the fabrication method. The subsequent release after the initial burst was also equivalent to that obtained with the conventional method.

In general, the risk for drug degradation by exothermic heat due to collision is known as a precaution point in a milling process. This may become a major disadvantage of the dry fabrication method using a ball mill. In this study, however, VB_{12} degradation and interaction between VB_{12} and PLGA were not detected by HPLC and FT-IR analyses, respectively.

The dry fabrication method showed considerable differences from the conventional method in terms of encapsulation efficiency and microsphere morphology.
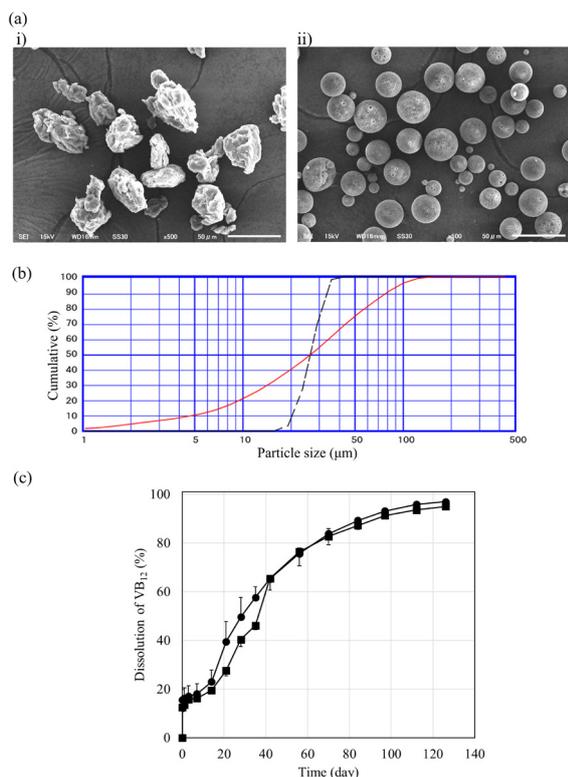
Figure 5. Comparison of microsphere properties between the dry fabrication method and the conventional o/w emulsion solvent evaporation method. (a) Scanning electron micrograph of microspheres fabricated by the *i)* dry fabrication method (sample No.: #9) and *ii)* conventional method (sample No.: #11). **(b)** Size distribution of microspheres fabricated by the dry fabrication method (sample No.: #9); solid line and conventional method (sample No.: #11); dashed line. **(c)** *In vitro* VB_{12} release from the microspheres fabricated by the dry fabrication method (sample No.: #9) (●) and conventional method (sample No.: #11) (■). The data represent mean ± S.E. (*n* = 3 batches). The microspheres were fabricated under the following conditions: media, 4 large (ϕ10 mm) and 15 small (ϕ3 mm) balls; PLGA, Resomer®RG502; loaded drug, 20%VB_{12}; processing time 12 h.
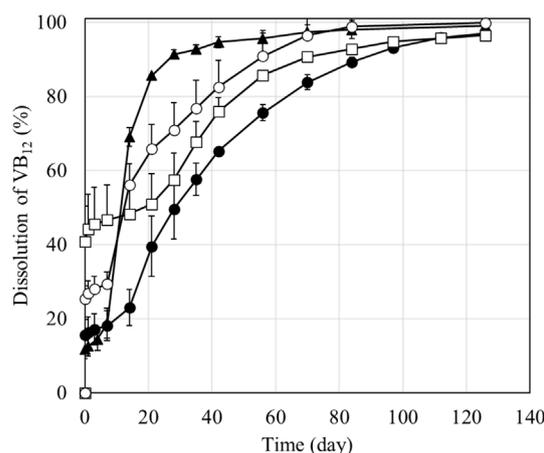
Figure 6. Compatibility of the dry fabrication method with PLGA from various manufacturers. *In vitro* VB_{12} release from the microspheres fabricated using Resomer®RG502 (sample No.: #9) (●), PURASORB®PDLG5002 (sample No.: #12) (▲), Resomer®RG502H (sample No.: #13) (○), and PLGA5020 (sample No.: #14) (□). The microspheres were fabricated under the following conditions: media, 4 large (ϕ10 mm) and 15 small (ϕ3 mm) balls; PLGA, Resomer®RG502; loaded drug, 20%VB_{12}; processing time 12 h. The data represent mean ± S.E. (*n* = 3 batches).

Table 3. Comparison of particle size and encapsulation efficiency of the microspheres fabricated by the dry fabrication and conventional methods

Sample No.	VB_{12} loading (%)	Fabrication Method	Particle size (μm)				Encapsulation efficiency (%)
			Mean	D_{25}	D_{50}	D_{75}	
#7	10	Dry fabrication method	40.9 ± 3.6	22.7 ± 2.9	43.5 ± 2.4	88.6 ± 5.8	95.8 ± 2.6
#9	20	Dry fabrication method	27.4 ± 6.1	14.4 ± 5.2	28.5 ± 8.0	66.4 ± 9.6	104.1 ± 5.0
#10	30	Dry fabrication method	32.0 ± 6.0	16.3 ± 5.0	41.1 ± 8.8	83.3 ± 13.5	95.4 ± 2.1
#11	20	Conventional method[*)]	26.2 ± 0.1	22.9 ± 0.1	26.3 ± 0.1	30.1 ± 0.1	35.3 ± 1.6

[*)]o/w emulsion solvent evaporation method. Data represents Mean ± S.E. (*n* = 3 batches).

Regarding encapsulation efficiency, the dry fabrication method showed an advantage over the conventional method. In the conventional o/w emulsion solvent evaporation method, leakage of a water-soluble drug into the external water phase during the fabrication process is known to be a major disadvantage. In contrast, there was no risk of drug leakage in the dry fabrication method owing to the solvent-free nature of the method. However, there were some precaution points on encapsulation with the dry fabrication method. One is the affinity balance between PLGA particles, drug particles, and materials used in the mill. Drug and PLGA particles can be attached on the inner surface of the mill. If there is a difference in the degree of attachment to the inner surface between drug and PLGA particles, excess amount of the component attached to the inner surface can be unused for microencapsulation, thus affecting the encapsulation efficiency of the drug into the microspheres. In this study, we used balls and a pot from agate for ball milling. In the microspheres fabricated by the dry fabrication method in this study, the observed VB_{12} content in microspheres was in agreement with the theoretical VB_{12} content; hence, the material appeared to be suitable for the dry fabrication of VB_{12}-PLGA microspheres. If there was a difference between the observed and theoretical drug concentrations, the materials should be further validated for suitability for the aimed microspheres.

Another precaution point is the content uniformity of each particle. Microsphere fabrication by using 4 large balls (ϕ10 mm) resulted in a mixture of high and low particle content. At the end of the fabrication process, most powders stuck on the top inner part of the pot, and the powder in the pot wall next to the sticking matter was mainly PLGA particles. This finding indicated that mixing did not proceed well. Uniformity is greatly related to the mixing process of the loaded particles during the ball milling. The large ball media generate a large space between balls or between balls and the pot wall. It is difficult for the powder stuck in the space to be stirred. To improve mixing, small ball media were added to fill the space. Although it is known that the close-packed structure provides minimum space, such close-packed composition of ball media, such as the five-sized particles, was reported to facilitate particle size reduction. Because the excess reduction of particle size is inconvenient for microencapsulation, we attempted to improve uniformity using volume ratios of small and large balls of up to 1/10. The addition of a small number of small balls improved content uniformity between particles both microscopically and macroscopically. However, further addition of small balls did not result in more improvement. These results indicated that the addition of a small number of small balls improved the mixing of powder during dry fabrication. As content uniformity was improved, variations in release profiles, especially in the initial burst between different

manufacturing batches were also improved. Moreover, the degree of the initial burst was reduced by the addition of small balls. A possible reason is that deviation of VB_{12} encapsulation in some parts of the microspheres can occur when mixing was conducted with large balls alone, and that the deviation can be eliminated through the addition of small balls. Increasing the percentage of drug loading led to an increase in the degree of the initial burst. Hence, the overloading of VB_{12}, including unencapsulated VB_{12} particles, was considered to occur in some part of the microspheres owing to inadequate mixing, leading to increased initial burst. However, the fraction of the overloaded microspheres was decreased by increasing the degree of mixing through the addition of small balls, resulting in a decrease in the initial burst.

Another precaution in the microspheres prepared by the dry fabrication method is the needle-passing at injection. The microspheres prepared by the conventional method are complete spheres. The spherical particles are known to be the shape with the best needle-passing property. The microspheres prepared by the dry fabrication method were not spheres. Although the microspheres showed good needle-passing property in this study, they are at risk of showing a weak needle-passing property. An effort to round the particles prepared by ball milling has been reported in the field of mechanical alloying; polymer particles characterized by an irregular shape were sprayed into the thermal chamber to round the particles (*12*). Although thermal rounding is a promising technique for rounding the particles, considerable attention should be paid to the thermal stability of the encapsulated drug.

The compatibility of the dry fabrication with PLGA from various manufacturers also is an important factor in the practical aspect of manufacture. In this study, we evaluated PLGA obtained from three manufacturers and showing equivalent inherent viscosity. PLGA is classified into two types according to the terminal carboxyl group: alkyl ester capped PLGA and free carboxylic acid PLGA. Microspheres prepared using alkyl ester capped (Resomer®RG502) and free carboxylic acid (Resomer®RG502H) showed equivalent initial bursts, although they also showed some differences in drug release profiles. This indicated that the feasibility of the dry fabrication method was high and independent of the end capping of PLGA. On the contrary, microspheres prepared using PLGA from different manufacturers showed great differences in the initial burst. This indicated that the feasibility of the dry fabrication may be dependent on the brand of the PLGA used.

The present study in the 150 mg scale indicated the usefulness of the dry fabrication method for injectable PLGA microspheres. At the early stage of new drug research, only a small amount of candidate compounds is often available. In such a situation, because a new chemical entity-loaded, long-acting, injectable formulation could not be fabricated by a conventional

method in terms of applicable minimum scale and drug loss, it is difficult to evaluate its pharmacologic effectiveness. Its availability in a small scale and its low drug loss in the manufacturing process are the advantages of the dry fabrication method. However, scale-up ability is an important point to be clarified for commercial manufacturing in the future. Another related theme is the optimization of powder volume rate to the pot volume, which affects the productive efficiency. Thus, further studies are needed to evaluate these points.

In conclusion, the dry fabrication method can be an alternative method for fabricating long-acting injectable formulations, as it showed an advantage of low drug loss during the fabrication process. In this study, only one drug, VB_{12}, was used to evaluate the feasibility of the dry fabrication method. Clarifying the properties of drugs that are suitable for this method is important to advance the studies on the dry fabrication method. We are planning to investigate the drug properties suitable for the dry fabrication method.

Acknowledgements

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