Development of PEG–PLA/PLGA microparticles for pulmonary drug delivery prepared by a novel emulsification technique assisted with amphiphilic block copolymers

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ABSTRACT

We developed a novel “spray dry-based” method for preparing surface-modified particle via “block copolymer-assisted” emulsification/evaporation for pulmonary drug delivery. The method included three steps: (1) o/w emulsion containing both hydrophobic polymers and amphiphilic block copolymers was obtained by emulsification of water and a polymer-containing organic solvent, (2) the o/w emulsion was misted with a nebulizer, and (3) the emulsion mists were dried by a heater. In this way, the hydrophobic polymers and the hydrophobic part of the amphiphilic block copolymers gradually tangled during the evaporation of organic solvents from the o/w emulsion. Consequently, the hydrophilic polymer chain was introduced on the particle surface. The particle surface can be easily modified although there are no reactive groups in the hydrophobic polymer molecules. We successfully obtained dry PEG–PLA/PLGA microparticles by controlling the weight ratio of the block copolymer and the hydrophobic polymer. The introduction of PEG to the particle surface involves an increase in the Zeta potential of the particles. Interestingly, the “dimpled” microparticles having a diameter of approximately 2 μm were obtained. The “dimpled” microparticles can serve as drug carriers for pulmonary drug delivery, because the particles have a large surface area. We expect that this novel surface-modification technique will enable efficient fabrication of particles in drug delivery systems.

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1. Introduction

There are many methods for the administration of drugs. The routes of administration include intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administrations. For example, the oral is the simplest route of drug administration. However, the oral administration has a risk of drug decomposition by digestive organs. The transdermal is also a simple route of drug administration, even though an adsorption efficiency of drugs is generally low in this method. The injection method results in high adsorption efficiency, an early effect of injected drugs’ pharmacological actions, and no risk of drug decomposition by digestive organs. However, because the method can create pain at injection sites, it is difficult to administer injection-based drugs to patients with belonephobia. On the other hand, in recent years, there has been a growing interest in the pulmonary route of drug administration, because pulmonary administration is simple, shows early effects of drugs’ pharmacological actions, and has no risk of drug decomposition [1–3]. Pulmonary administration is a method that works through the lungs. Among the several types of drug carriers including liposomes [4,5], micelles [6–9], and gels [10–12], polymeric “particles” are suitable for pulmonary drug administration [13]. The drug-incorporated particle is aspirated into the lung and decomposed by transcytosis, and finally, the drug is absorbed into the body. The drug–absorption efficiency with this method is expected to be high because the lungs have relatively large surface areas, thin alveolar epithelial cells, and significant blood capillaries.

Some noteworthy properties are necessary for a drug carrier that can be used for pulmonary administration. First, it is important to use biocompatible or biodegradable materials such as polyethylene glycol (PEG) and poly(lactide-co-glycolide) (PLGA). Second, it is important to control the diameter and density of particles [14–17]. Large particles (over 10 μm) are trapped by part of the pharynx or part of the bronchi. In contrast, small particles (below 1 μm) are emitted out with breath. The appropriate diameter of the particles for pulmonary drug delivery is reported to be 1–5 μm [17]. Further, as long as solvent evaporation-based methods are used to prepare particles (where the resulting particles are obtained in suspended manners), the resulting particles have a high density. Exceptionally, in the case when the porous particles can be prepared, particles having a low density are obtained even though
solvent evaporation-based methods are used [18]. In contrast, particles having a low density are generally obtained with spray dry-based preparation methods. Third, it is important to modify the surface of the particles. The particles having functional molecules (in particular, functional polymers) on the surface can bind to the tissue surface and, consequently, tend to be absorbed into tissue. In general, chemical conjugation of polymers to particles is expensive and time-consuming because conjugation chemistry needs to be optimized for each polymer-particle combination. In addition, commercially available biocompatible and biodegradable polymers, such as PLGA and polylactide (PLA) have been known to be materials of which surface modification of the resulting particles is difficult, because PLGA and PLA have almost no reactive groups in their molecules. From these points of views, there is a need for microparticle preparation methods that rests on suitable carriers for pulmonary drug delivery.

In our previous paper [19], we reported a novel technique for preparing nanoparticles modified with hydrophilic polymers on the surface of the particles via “block copolymer-assisted” emulsification/evaporation process. Because the method is based on solvent evaporation, the resulting surface-modified particles are obtained in an aqueous solution in suspended manners. For the aforementioned reason, it is obvious that a spray dry-based method is suitable for the preparation of the microparticles for pulmonary drug delivery. However, the spray dry-based techniques of the surface modification of particles have not been reported.

From these points of views, in the present study, we developed a novel “spray dry-based” method for preparing surface-modified particle via “block copolymer-assisted” emulsification/evaporation for pulmonary drug delivery [Fig. 1]. First, o/w emulsion containing both hydrophilic polymers and amphiphilic block copolymers was obtained by emulsification of water and a polymer-containing organic solvent. Second, the o/w emulsion was misted with a nebulizer. Third, the emulsion mists were dried with a heater. In this way, the hydrophilic polymers and the hydrophobic part of the amphiphilic block copolymers gradually tangled during the evaporation of organic solvents from o/w emulsion. Consequently, the hydrophilic polymer chains were introduced on the particle’s surface. Particle surfaces can be easily modified although there are no reactive groups in the hydrophilic polymer molecules. In this study, we have clarified the factors affecting both the stability of o/w emulsion and the surfaces of the resulting particles.

2. Experimental

2.1. Materials

Ethylene oxide (Sumitomo Seika Chemicals Co., Ltd., Osaka, Japan) was purified by distilling it with CaH₂. Dl-lactide (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was recrystallized twice from ethyl acetate. 2-Methoxyethanol was distilled with sodium under reduced pressure. Potassium naphthalene was obtained by mixing potassium and naphthalene in anhydrous tetrahydrofuran (THF) for 18 h. PLGA (monomer ratio of lactide/glicolide: 3, molecular weight: 10,000) was purchased from Wako Pure Chemical Industries (Osaka, Japan). All the other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of methoxy-terminated poly(ethylene glycol)-polylactide (PEG–PLA) block copolymers

A PEG–PLA block polymer was synthesized by ring-opening polymerization of both ethylene oxide and dl-lactide in THF according to the previously reported method [20–22] with slight modifications (Fig. 2). 2-Methoxyethanol (1 mmol) and potassium naphthalene (1 mmol) were mixed in THF for 1 h. The purified ethylene oxide (80–130 mmol) was added to the obtained potassium 2-methoxyethoxide solution (total volume: 50 ml). After stirring for 48 h, the THF solution of purified dl-lactide (40 mmol) was added to the solution. After the reaction, the resulting block copolymer was precipitated into cold 2-propanol, stored in a freezer for 12 h, centrifuged at 10,500 rpm, and lyophilized in benzene. The average molecular weight of the obtained block copolymer was determined by the use of gel permeation chromatography (GPC) (column: TSKgel G3000HHR, TOSOH, Japan, eluent: DMF in the...
presence of 10 mM LiBr, flow: 1 ml/min, column temperature: 40 °C) and 1H NMR (AL-300, 300 MHz, JEOL Ltd., Tokyo).

2.3. Effect of organic solvents on the stability of o/w emulsion

We obtained o/w emulsion with ultrasonic irradiation after mixing an aqueous solution (13.4 ml) and organic solvents (0.56 ml). The organic solvents used were dichloromethane, toluene, and their mixture. The density of the mixed organic solvents was adjusted to be 1.00 g/cm³. After the organic solvent was added to the aqueous solution (the volume fraction of the organic solvent was adjusted to be 2.0–40.0% (v/v)), the mixture was sonicated with ultrasonic irradiation (UH-50, SMT Co., Ltd., Japan, sonication time: 10 min, frequency: 20 kHz, output: 50 W). The obtained o/w emulsion was placed under static observation.

2.4. Preparation of surface-modified PLGA particles using a novel spray dry-based emulsification/evaporation process

The preparation of surface-modified PLGA particles involved a novel spray dry-based block copolymer-assisted emulsification/evaporation method. PLGA (0.07–0.09 g) and the synthesized block copolymers (0.01–0.03 g) were dissolved in a dichloromethane–toluene mixed solvent (2.0 ml). After the organic solvent (0.56 ml) was added to the aqueous solution (13.4 ml), the mixture was sonicated with ultrasonic irradiation (UH-50, SMT Co., Ltd., Japan, sonication time: 10 min, frequency: 20 kHz, output: 50 W). The obtained emulsion was then transferred to a nebulizer. The prepared o/w emulsion was sprayed with a nebulizer and dried by heating (carrier gas: N₂, spray flow rate: 1.01/min, inlet temperature: 120 °C, outlet temperature: 49 °C). Finally, the particles were collected on a glass filter. The surface morphology of the surface-modified PLGA particles was observed by means of a scanning electron microscope (SEM, VE-9800, KEYENCE Co., Ltd.). The specimens for SEM observation were prepared by mounting the sample on an aluminum plate and coating a thin platinum film (approximately 10 nm in thickness) on a sample under a reduced pressure with MSP-15 ion water (Vacuum Device Inc., Ibaraki, Japan). The volume–averaged diameter and the zeta potential of the particles were determined by the use of dynamic light scattering (DLS) with a Zetasizer Nano ZS (Malvern Instruments, England).

3. Results and discussion

3.1. Synthesis and characterization of PEG–PLA block copolymer

The obtained PEG–PLA block copolymers were characterized by 1H NMR and gel permeation chromatography (GPC). The 1H NMR spectra of the PEG–PLA block copolymer shown in Fig. 3 indicates that the block copolymers were synthesized successfully (the peak around 1.8 ppm corresponds presumably to some impurities). Table 1 summarizes the results of the synthesis of the PEG–PLA block copolymers. The number–average molecular weights of the PEG and PLA units were determined on the basis of GPC and 1H NMR, respectively. The number–average molecular weights of the obtained block copolymers were 7040 (PEG unit: 3640, PLA unit: 3400, and Mₘ/Mₙ: 1.15) and 6520 (PEG unit: 2500, PLA unit: 4020, and Mₘ/Mₙ: 1.21).

3.2. Evaluating the effect of organic solvents on the stability of o/w emulsion

In our novel method, surface-modified particles were prepared by a spray-drying of the o/w emulsion containing both the particle-forming hydrophobic polymer and the block copolymer (Fig. 1). An emulsion is a system consisting of two immiscible liquid phases, one of which (the dispersed phase) is dispersed throughout the other (the continuous phase) in fine droplets. If the emulsion is not highly stable, the emulsion aggregates before particle formation, and consequently, there was no preparation of surface-modified particles with a narrow size distribution. Therefore, it is necessary to evaluate the stability of o/w emulsion for the successful avoidance of the phase separation during particle formation.

We evaluated the effect that the organic phase’s volume fraction would have on the stability of the o/w emulsion prepared from water, toluene, and dichloromethane. Toluene and dichloromethane were used for preparing a series of mixed organic solvents, because they have low boiling points and because evaporation removes them from the emulsion relatively easily. The density of the mixed organic solvent was adjusted to be 1.00 g/cm³, because our previous studies showed that an organic solvent with a density of 1.00 g/cm³ yielded a highly stable emulsion [15]. Fig. 4 shows our findings regarding the effects that the organic phase’s volume fraction had on the stability of the o/w emulsion. The volume fractions of the organic phase were 2.0, 4.0, 10.0, 20.0, and 40.0% (v/v) from left to right in each image in the figure. The lower the organic phase’s volume fraction in the o/w emulsion became, the more difficult it was to separate the emulsion into organic and aqueous phases. In contrast, when the volume fraction of the organic phase was high, the gradient of the presence of the emulsion was observed (the phenomenon was confirmed by visual observation and DLS measurement). The emulsion with the highest stability was obtained when the volume fraction of the organic phase was adjusted to be below 4.0% (v/v). From the view point of
the productivity of particles, we concluded that the most suitable volume fraction of the organic phase was 4.0% (v/v).

3.3. Effect of the presence of amphiphilic block copolymer on the diameter of o/w emulsion

It is well known that the particles whose diameter is 1–5 μm are suitable for pulmonary drug administration [17]. In this study, an emulsification was used for the preparation of microparticles. Therefore, it is necessary to control the droplet size of o/w emulsion for the successful preparation of the particle with a desired diameter.

We evaluated the effect of the weight ratio of amphiphilic block copolymer and PLGA on the diameter of the o/w emulsion droplets. The diameter of the o/w emulsion droplets containing amphiphilic block copolymers and PLGA was determined by DLS. Fig. 5 shows the effect of amphiphilic block copolymers on the diameter of the o/w emulsion droplets. The results show that the diameter of the o/w emulsion droplets decreased, reached a saturated value, and gradually increased, as the weight ratio of the amphiphilic block copolymers and PLGA increased. The surface of the o/w emulsion droplets was gradually covered with amphiphilic block copolymers as the weight ratio of amphiphilic block copolymers and PLGA increased. As a result, the diameter of o/w emulsion droplets decreased as the ratio increased. The surface charge of the oil droplets decreased as the amphiphilic block copolymers covering the surface increased. We assumed that the increment of the droplets’ diameter relative to the o/w emulsion derived from the aggregation of the droplets where the surface charge decreased. It seemed that the diameter of the o/w emulsion was independent of whatever kind of amphiphilic block copolymer was involved. We confirmed that the optimal o/w emulsion occurred when the weight ratio of amphiphilic block copolymer and PLGA was varied from 3/7 to 1/9.

3.4. The surface morphology of the surface-modified PLGA particle

Fig. 6 shows the SEM image corresponding to the surface-modified PLGA particles obtained by a novel spray dry-based emulsification/evaporation method. From these results of SEM images, we found that the dry PEG–PLA/PLGA particles were successfully present when the quantity of the amphiphilic block copolymers was low in the droplets of the o/w emulsion. We confirmed that particles exhibiting “sphere-shape” in SEM images were easily collected from the filter. In such a case, we can judge the particles were wet. In contrast, wet particles were present when the quantity of the copolymers was high. A small amount of PEG chains of the block copolymers was present on the surface of the droplets when the weight ratio of the block copolymers and PLGA was low. Therefore, because the droplets have a thin hydration layer, it is easy to dry the emulsion mists. The results in Fig. 6 reveal that the dry particles were present when the weight ratio of the amphiphilic
block copolymers and PLGA was 1/9 or 2/8. Further, it was found that the zeta potential of the particles increased by the addition of the amphiphilic block copolymer to the o/w emulsion. In general, the anionic surface charge of the PLGA particles was attributable to the free carboxylic acid group of the PLGA molecules present on the particle surfaces (in this study, the zeta potential of PLGA particles was $-29.3 \text{ mV}$). In contrast, it was found that the zeta potential of the surface-modified PLGA particles was $-15.6 \text{ mV}$. The increase in the surface charge indicated the successful incorporation of the PEG chains onto the PLGA surfaces.

**Fig. 7** shows the magnified SEM images of the surface-modified PLGA particles. Interestingly, we successfully obtained the “dimpled” microparticles having a diameter of approximately 2 $\mu$m. Mohamed and van der Walle reported the fabrication of DNA-loaded PLGA microparticles that featured novel surface morphologies (dimples) by using two surfactant series based on hydrophilic–hydrophobic balance and the molecular weight of the hydrophobe [23]. They obtained microspheres with dimpled surfaces having the greatest depth of the dimples when triblock copolymers having hydrophobe blocks with a high molecular weight were used. However, the mechanism of the dimple formation was still unclear in their case. In our case, the same type of “dimpled” microparticles was obtained, as shown in Fig. 7. Although further study of clarifying the formation mechanism of the dimple is necessary, the dimpled microparticles can successfully serve as drug carriers for pulmonary drug delivery, because the particles have a large surface area.

**4. Conclusion**

Pulmonary administration is simple, shows early effects of drugs’ pharmacological actions and carries no risk of drug decomposition. A drug carrier that can successfully function in the pulmonary administration of a drug must possess some notewor thy properties. In particular, surface modification of hydrophobic particles with hydrophilic polymers is an attractive technique for
designing drug carriers for pulmonary drug administration. However, conventional chemical conjugation of polymers to the surface of particles needs to be optimized for each polymer–particle combination. Further, some biodegradable and biocompatible particles have been known to involve difficult surface modification, because polymers as starting materials have almost no reactive groups in their molecules in such a case.

In the present study, we developed a novel spray dry-based method for preparing surface-modified particles via “block copolymer-assisted” emulsification/evaporation for pulmonary drug delivery. We successfully obtained dry microparticles by controlling the weight ratio of the block copolymers and the hydrophobic polymers. The introduction of PEG to the particle surfaces was suggested by an increase in the Zeta potential of the particles. The facile method presented in this manuscript can be a universal tool for modifying the surfaces of microparticles, even though reactive groups might not be present on the surface. In addition, interestingly, we obtained the “dimpled” microparticles having a diameter of approximately 2 μm. Although further study clarifying the formation mechanism of the dimples is necessary, the dimpled microparticles can positively serve as drug carriers for pulmonary drug delivery, because the particles have a large surface area. We expect that this novel surface-modification technique will enable efficient fabrication of particles in drug delivery systems.

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