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RESEARCH ARTICLE

Development of poly(lactic-co-glycolic acid) microparticles with pH-sensitive drug release behaviors

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Abstract We developed a novel pH-sensitive microparticle using poly(lactide-co-glycolide) (PLGA) and 3-diethylaminopropylated poly(L-lysine) [poly(Lys-DEAP)] (pK_b of DEAP ~6.5). Here, the design of the microparticle takes advantage of the unique pH-sensitive feature of poly(Lys-DEAP) as either a non-ionic characteristic of DEAP moieties at pH 7.4 or an ionic characteristic of DEAP moieties at acidic pH. In particular, the ionized DEAP in PLGA microparticles modulated acidic pH-activated microparticle-destruction and allowed accelerated release of the encapsulated drug content when the pH of the solution was decreased to from 7.4 to 6.8 or 6.0. We believe that this microparticle system is expected to improve the treatment efficacy for lung diseases with chronic respiratory acidosis.

Keywords PLGA microparticle · 3-diethylaminopropylated poly(L-lysine) · Tiotropium

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Introduction

Chronic lung diseases usually cause abnormalities in alveolar gas exchange, resulting in an acid–base disturbance in regional alveolar epithelium (Barnes 2004; Halbert et al. 2006; Pauwels and Rabe 2004; Bruno and Valenti 2012). For example, chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) characterized by airway obstruction exhibit acute or chronic respiratory acidosis (e.g., $6.0 < pH < 7.35$), due to an acute or chronic rise in the alveolar carbon dioxide tension (Barnes 2004; Lopez and Murray 1998). This acidosis reflects severity of chronic lung diseases and indicates progressive deterioration in alveolar hypoventilation (Bruno and Valenti 2012; Budweiser et al. 2008). Recently, due to high treatment cost for chronic lung diseases, there has been increasing interest in various cellular and molecular mechanisms of chronic lung diseases and follow-up therapeutic drug designs (Bivas-Benita et al. 2005; Koumis and Samuel 2005). However, these approaches have achieved limited success, owing to the very long drug development cycles.

A direct targeting method for this acidosis using conventional therapeutic drugs may be implemented to achieve improved therapeutic effect, without the consideration of new drug discovery. For example, hybridizing acidic pH-responsive polymer component into the well-known drug-encapsulating poly(lactide-co-glycolide) (PLGA) microparticles (Mundargi et al. 2008; Park et al. 2014) may offer a unique opportunity for developing a functional drug-carrier for the treatment of chronic lung disease. This drug carrier design, operating therapeutic action at a micro-sized scale if specific pH signal is given, is a challenging task.

In this study, we sought to develop pH-sensitive PLGA microparticles (hereafter termed 'PHMS') via a water-in-oil-in-water ($W_1/O/W_2$) multi-emulsion method (Lee et al.

2007). In the emulsion step, tiotropium (a therapeutic model drug for treating COPD) (Disse et al. 1999; Koumis and Samuel 2005) dispersed in aqueous micro-droplets (5 mM sodium tetraborate) was coated with a mixture of PLGA and 3-diethylaminopropylated poly(L-lysine) [poly (Lys-DEAP)] dissolved in dichloromethane (DCM). It has been reported that non-ionic (hydrophobic) DEAP moieties of poly(Lys-DEAP) at normal pH (pH 7.4) changes to ionic (hydrophilic) DEAP moieties at acidic pH (Lee et al. 2014; Oh et al. 2009; Park et al. 2011). Such characteristics of DEAP moieties are expected to provide acidic pH-induced microparticle-destruction property to PLGA microparticles and accelerated drug release at acidic pH, as shown in Fig. 1.

Materials and methods

Materials

N^{ε}-benzyloxycarbonyl-L-lysine, triphosgene, anhydrous 1,4-dioxane, *n*-hexane, anhydrous dimethylformamide (DMF), hexylamine, diethyl ether, trifluoroacetic acid (TFA), 33 % HBr in acetic acid, ethanol, sodium tetraborate ($Na_2B_4O_7$), dimethylsulfoxide (DMSO), 3-diethylaminopropyl (DEAP) isothiocyanate, poly(vinyl alcohol) (PVA; M_W 12–23 kDa), NaCl, and dichloromethane (DCM) were purchased from Sigma-Aldrich (USA). PLGA (RG503H; lactide/glycolide = 50/50, M_W = 24 kDa) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Tiotropium was provided by Dongkook Pharm. Corporation (Republic of Korea).

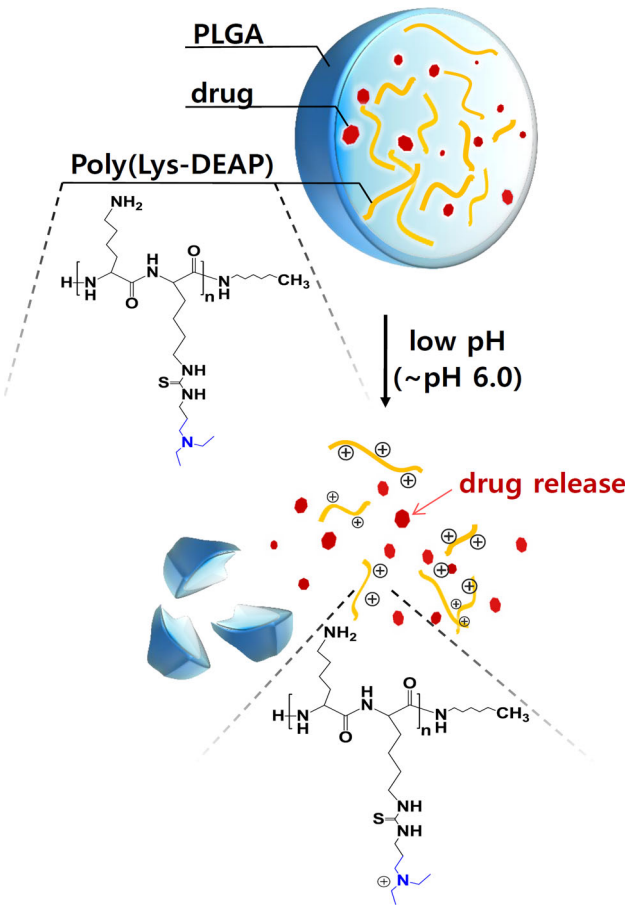

Fig. 1 Schematic concept of the proposed pH-sensitive PLGA microparticles

Polymer synthesis

Poly(N^{ε}-benzyloxycarbonyl-L-lysine) [poly(Lys-cbz)] was prepared as described by Oh et al. (2009) and Lee et al. (2014). Briefly, N^{ε}-benzyloxycarbonyl-L-lysine (1 mmol) reacted with triphosgene (1 mmol) in anhydrous 1,4-dioxane (20 mL) at 50 °C for 2 h was precipitated after mixing with excess *n*-hexane, yielding *N*-carboxy-(N^{ε}-benzyloxycarbonyl)-L-lysine anhydride. Next, *N*-carboxy-(N^{ε}-benzyloxycarbonyl)-L-lysine anhydride (0.03 mmol) was ring-opening polymerized in anhydrous DMF (30 mL) containing hexylamine (initiator, 0.001 mmol) at room temperature for 3 days. The obtained solution was mixed with excess diethyl ether, resulting in producing the precipitated poly(Lys-cbz). The cbz groups of poly(Lys-cbz) were removed using TFA (5 mL)/33 % HBr in acetic acid (5 mL) at room temperature for 30 min. The solution was added into excess ethanol/diethyl ether (50:50 vol.%), filtered, and lyophilized (Oh et al. 2009; Lee et al. 2014). Finally, the free pendant amine groups of poly(Lys) (0.06 mmol) were coupled with DEAP isothiocyanate (5 mmol) in DMSO (30 mL) containing TEA (1 mL) at room temperature for 3 days. After dialyzing the resulting solution against fresh distilled water using a dialysis membrane tube (Spectra/Por, MWCO 3.5 kDa, Spectrum Lab. Inc., Rancho Dominguez, USA) and performing the freeze drying procedure, poly(Lys-DEAP) was lyophilized (Oh et al. 2009; Lee et al. 2014). The chemical structure and number average molecular weight (M_n) of polymers were analyzed using a Bruker 300 MHz NMR Spectrometer (Bruker, Germany).

Microparticle preparation

PLGA microparticles were fabricated using the conventional $W_1/O/W_2$ multi-emulsion method (Lee et al. 2007; Park et al. 2014). Tiotropium (20 mg) dispersed in 10 mM sodium tetraborate (1 mL; W_1 phase) was vigorous emulsified with PLGA (RG503H; 200 mg) and poly(Lys-DEAP) (0–50 mg) dissolved in DCM (3 mL; O phase) (Table 1). The resulting solution was injected into a 1 wt% PVA and 0.9 wt% NaCl aqueous solution (800 mL; W_2

Table 1 Compositions used for preparing microparticle formulations

Sample	W_1 phase	O phase (in DCM 3 mL)		W_2 phase
		PLGA	Poly(Lys-DEAP)	
Tio-MS	Drug 20 mg	200 mg	0 mg	PVA (1 wt%)/NaCl (0.9 wt%)
			10 mg	
Tio-PHMS1			50 mg	
Tio-PHMS2				

phase) (Table 1). The $W_1/O/W_2$ emulsification was performed for 5 min, using a homo-mixer (Primix Corp, Japan) at 4,000 rpm, and was hardened by mild stirring for 2 h at room temperature. The particles were collected by centrifugation at 2,000 rpm for 2 min and were washed three times with 0.9 wt% NaCl aqueous solution (Park et al. 2014). The final particles were then freeze-dried for 2 days. The yield of microparticle fabrication was 80–85 wt%.

Drug or polymer loading efficiency

The microparticles (10 mg) were dissolved in immiscible solvents such as 1 mM sodium tetraborate (2 mL) and DCM (4 mL). Tiotropium dissolved in 1 mM sodium tetraborate or polymer [poly(Lys-DEAP)] in DCM was analyzed using a HPLC (Agilent 1100 series, USA) on a CAPCELL PAK C_{18} column (250 × 4.6 mm, 5 μm, Shiseido Co. Ltd., Japan) at ambient temperature. The samples in a mobile phase (60 vol.% 10 mM ammonium acetate buffer/40 vol.% acetonitrile, a flow-rate of 0.8 ml/min) were analyzed at 235 nm (for tiotropium) or 660 nm [for poly(Lys-DEAP)] (Park et al. 2014; Wang et al. 2007). Drug (or polymer) loading efficiency (%) was calculated by a weight percentage ratio of actual drug (or polymer) dose loaded into PLGA microparticles to initial feeding drug (or polymer) dose. Drug (or polymer) loading content (%) was calculated by a weight percentage ratio of encapsulated drug (or polymer) dose to the total amount of PLGA microparticles harvested (Park et al. 2014; Yoo et al. 2011).

Morphology and particle size of formulations

The morphology and particle size of microparticles were confirmed with a Field Emission Scanning Electron Microscopy (FE-SEM, Hitachi S-4800, USA). Before sample analysis, the dried formulations were vacuum coated with carbon (Yoo et al. 2011).

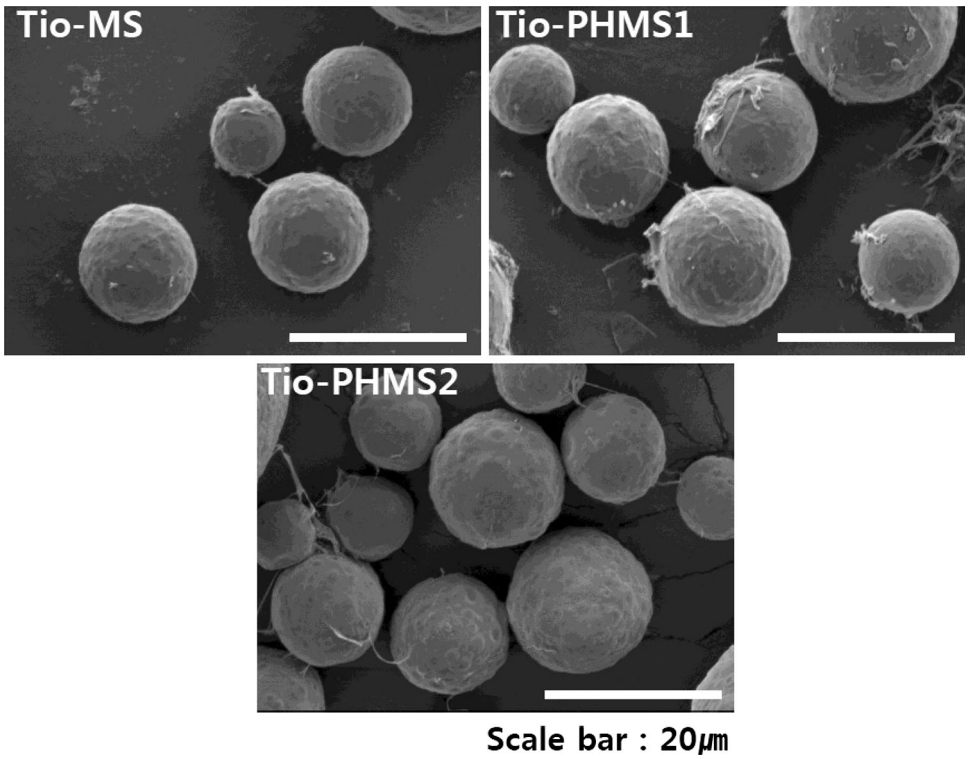

Fig. 2 FE-SEM images of Tio-MS, Tio-PHMS1, and Tio-PHMS2 microparticles prepared from the compositions listed in Table 1

Table 2 Characterization of microparticles

Sample	Drug loading efficiency (wt%)	Drug loading content (wt%)	Poly(Lys-DEAP) loading efficiency (wt%)	Poly(Lys-DEAP) loading content (wt%)
Tio-MS	67.0	8.4	–	–
Tio-PHMS1	47.1	5.2	59.4	3.3
Tio-PHMS2	51.4	6.1	53.0	15.8

Drug or polymer release

The microparticles (10 mg) were added into a dialysis membrane tube (Spectra/Por, MWCO 8–10 kDa, Spectrum Lab. Inc., Rancho Dominguez, USA), and were then immersed in a vial containing fresh PBS pH 7.4–6.0 (20 mL, ionic strength: 0.15; 0.01 % sodium azide) at 37 °C. The outer phase of the dialysis bag was withdrawn and replaced with fresh PBS (pH 7.4–6.0) solution at predetermined time intervals. The tiotropium or polymer [poly(Lys-DEAP)] concentration in each solution was monitored using a HPLC on a CAPCELL PAK C_{18} at ambient temperature (Park et al. 2014; Yoo et al. 2011). The samples in a mobile phase (60 vol.% 10 mM ammonium acetate buffer/40 vol.% acetonitrile, a flow-rate of 0.8 ml/min) were analyzed at 235 nm (for tiotropium) or 660 nm [for poly(Lys-DEAP)] (Park et al. 2014). In addition, the morphology changes of microparticles incubated in a vial containing fresh PBS pH 7.4–6.0 at 37 °C for 48 h were confirmed with a FE-SEM.

Results and discussion

For the preparation of pH-sensitive polymer, DEAP isothiocyanate was simply coupled with the free pendant amine groups of poly(Lys) {Lys repeating unit: 16.5, as estimated based on the ^{1}H-NMR peaks using the integration ratio of the peaks at δ 0.70 [$-CH_3$, corresponding to the terminal group of poly(Lys)] and δ 4.20 [−CH–, corresponding to the repeating unit of poly(Lys)] (Supplementary Fig. S1)}, resulting in producing poly(Lys-DEAP) (Supplementary Fig. S1). The degree of DEAP substitution [defined as the number of DEAP moieties per repeating unit of poly(Lys)] in poly(Lys-DEAP) estimated based on the ^{1}H-NMR peaks using the integration ratio of the peaks at δ 0.9 ($-CH_3$, corresponding to the DEAP moieties) and δ 4.2 [−CH–, corresponding to the repeating unit of poly(Lys)] was 0.95 (Supplementary Fig. S1).

Next, the PLGA microparticles were prepared by the $W_1/O/W_2$ multi-emulsion method (Lee et al. 2007; Park et al. 2014) using the components described in Table 1.

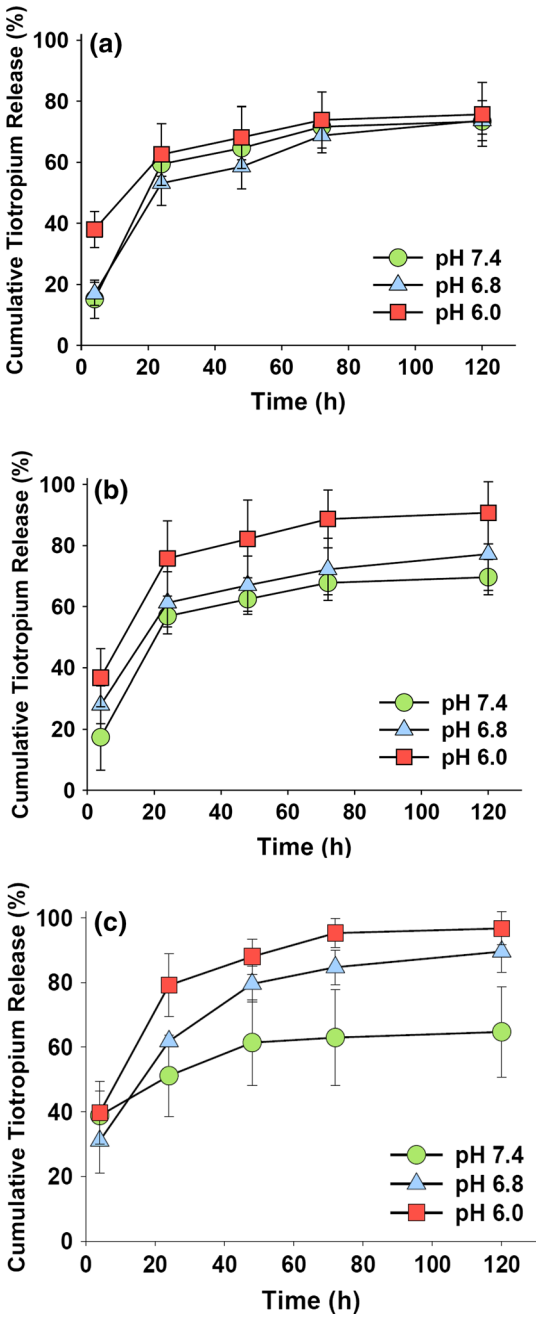

Fig. 3 Cumulative tiotropium release (%) from **a** Tio-MS, **b** Tio-PHMS1, and **c** Tio-PHMS2 microparticles ($n = 3$)

The W_1 phase containing tiotropium (drug) was emulsified with the O phase containing PLGA (RG503H) and pH-sensitive polymer, [poly(Lys-DEAP)] (pK_b of DEAP ~6.5, Supplementary Fig. S2). The resulting W_1/O emulsion was vigorously mixed with the W_2 phase containing 1 wt% PVA and 0.9 wt% NaCl aqueous solution. The shape of PLGA microparticles obtained after the DCM evaporation was regular and spherical as visualized in the FE-SEM photograph (Fig. 2). The average particle size of PLGA microparticles ranged from 10 to 15 μm. Table 2

shows the tiotropium or polymer loading results of the harvested PLGA microparticles. The tiotropium loading efficiency of PLGA microparticles was 47.1–67.0 wt%. The polymer [poly(Lys-DEAP)] loading efficiency of PLGA microparticles was 53.0–59.4 wt%. It was found that the incorporation of poly(Lys-DEAP) in PLGA microparticles decreased the tiotropium loading efficiency of PLGA microparticles when compared to that of Tio-MS [without poly(Lys-DEAP)]. In addition, high feeding dose of poly(Lys-DEAP) allowed a high poly(Lys-DEAP) loading content in PLGA mciroparticles (Tio-PHMS2).

The cumulative drug release profiles as a function of pH support the hypothesis that PHMS are stimulated by acidic pH (Fig. 1). Here, we monitored the drug release profiles at pH 7.4 (normal pH of body), 6.8, or 6.0 (acidic pH in chronic lung diseases). As expected, the amounts of tiotropium released from Tio-MS [without poly(Lys-DEAP)] at pH 7.4, 6.8, or 6.0 were not markedly different: a maximum 70 wt% of tiotropium was released from Tio-MS (120 h incubation) at pH 7.4, 6.8, or 6.0, due to the passive diffusion of tiotropium from Tio-MS (Fig. 3a). However, the release rate of tiotropium from Tio-PHMS1 at pH 6.0 was fater than that obtained at pH 7.4 or 6.8 (Fig. 3b). The data reveal that 88 or 65 wt% of encapsulated tiotropium was released from Tio-PHMS1 (72 h of incubation) at pH 6.0 or 7.4, respectively. In particular, Tio-PHMS2 allowed a highly increased tiotropium release at pH 6.0 (Fig. 3c); 95 wt% of encapsulated tiotropium was released from Tio-PHMS2 (72 h of incubation) at pH 6.0. These results indicate that the increased incorporation of poly(Lys-DEAP) in PLGA microparticles (Tio-PHMS2) considerably affected the pH that triggers tiotropium release. Figure 4 shows that poly(Lys-DEAP) release patterns from Tio-PHMS1 were also pH-dependent. 70–80 wt% of encapsulated poly(Lys-DEAP) was rapidly released from Tio-PHMS1 (48 h incubation) at pH 6.0 or 6.8. While, 46 wt% of encapsulated poly(Lys-DEAP) was released from Tio-PHMS1 (48 h incubation) at pH 7.4. Accelerated poly(Lys-DEAP) release at pH 6.0 or 6.8 is attributed to the ionization of poly(Lys-DEAP) at acidic pH (Lee et al. 2014; Oh et al. 2009; Park et al. 2011). Additionally, Tio-PHMS2 showed similar poly(Lys-DEAP) release patterns to that observed with Tio-PHMS1 (data not shown).

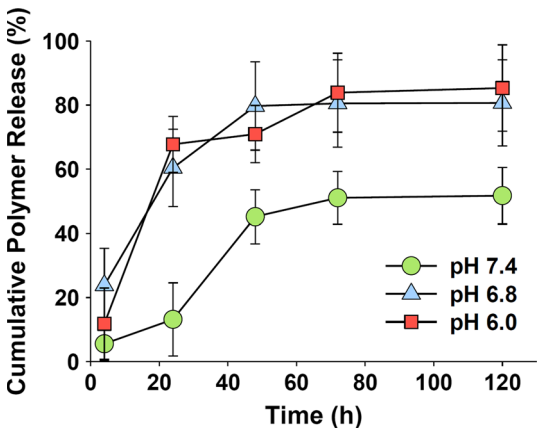

Fig. 4 Cumulative polymer [poly(Lys-DEAP)] release (%) from Tio-PHMS1 microparticles ($n = 3$)

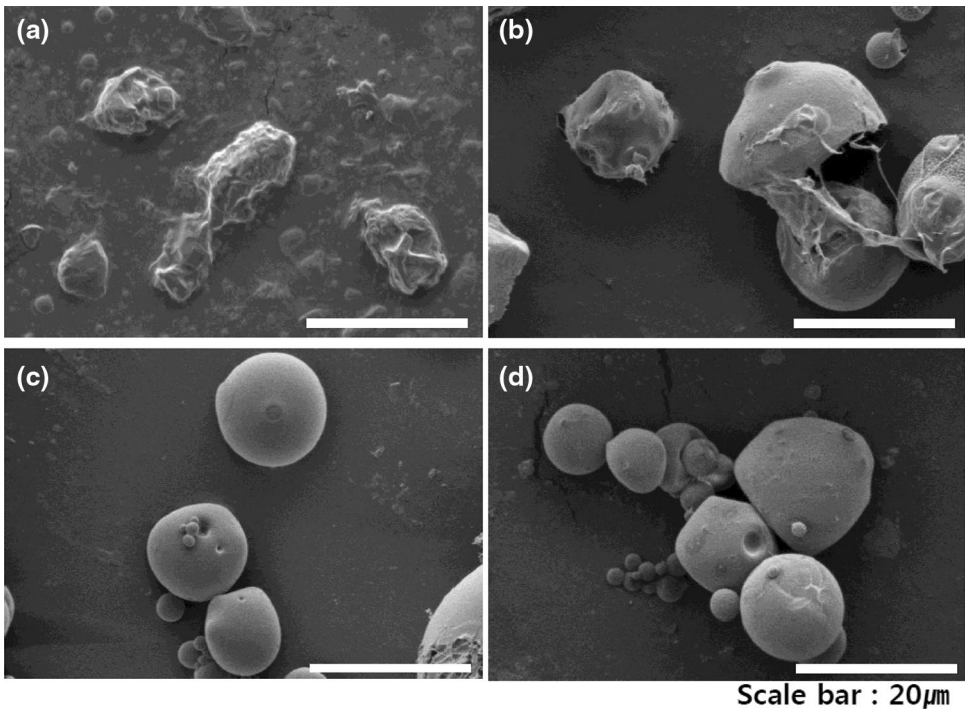

Fig. 5 FE-SEM images of Tio-PHMS2 microparticles incubated at **a** pH 6.0, **b** 6.8, or **c** 7.4 for 48 h. **d** FE-SEM images of Tio-MS microparticles incubated at pH 6.0 for 48 h

Figure 5 shows the morphological changes of PLGA microparticles incubated at different pH values. As expected, the increased incorporation of poly(Lys-DEAP) in PLGA microparticles (Tio-PHMS2) considerably enhanced the microparticle-destruction at pH 6.0 (Fig. 5a) or 6.8 (Fig. 5b), which is comparable with the FE-SEM images of Tio-PHMS2 at pH 7.4 (Fig. 5c) or Tio-MS (Fig. 5d) at pH 6.0. Overall, these results explain why tiotropium release rate of Tio-PHMS2 at pH 6.0 or 6.8 increased (Fig. 3c).

Conclusion

A pH-sensitive PLGA microparticle using poly(Lys-DEAP) was designed and fabricated in an effort to develop new drug-carrier for the treatment of chronic lung diseases. We demonstrated that PHMS system exhibit the acidic pH-stimulated release of the encapsulated drug as a result of the microparticle-destruction at acidic pH due to the ionization of DEAP. Based on these results, the PLGA microparticular system is expected to provide a new route for the development of a lung-implant microparticle model (that is able to increase the drug release rate under a serious disease condition and delay the drug release rate under a mild disease condition) for the treatment of chronic lung diseases. Of course, to confirm and realize these potential, further in vitro/in vivo investigations including therapeutic activity and toxicity studies will be required.

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