Characterization of pH-Responsible Polymer Nano-Film Synthesized on Self-Assembled Phospholipid Layer Fabricated by Plasma-Assisted Method

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We fabricated the pH-responsible polymer nano-film possessing carbamate groups, which were easily decomposed in acidic condition, in the linker moieties. In pH 7.4 phosphate buffer saline the number average diameter of pH-responsible polymer nano-film was about 350 nm and its size distribution had almost unchanged for 3 days. In pH 5.0 acetate buffer solution the scattering intensity of polymer nano-film in DLS measurement gradually decreased and it was difficult to measure the particle diameter of polymer nano-film after 15 h due to the lower scattering intensity. It was suggested that most of the polymer nano-film might be decomposed until 15 h.

Keywords: Polymer nano-film, pH-responsible, Plasma irradiation, Self-assembled phospholipid layer, Cyclodextrin

1. Introduction

The nano-film (or nano-sheet) is a new type of material that possesses a two-dimensional polymeric structure with nano-meter thickness. Many of nano-film needs the supporting material [1-5]. Recently, free standing nano-films fabricated from molecular, atomic, and ionic components have been extensively investigated for systems with analytical and biomedical applications, such as separation matrices and drug delivery carriers [6-11].

We have developed the method to introduce a durable surface wettability on several hydrophobic polymers, such as poly(ethylene naphthalate), polyethylene, and nylon-12 by plasma-assisted method [12-16]. We have also demonstrated that a self-assembled phospholipid (phosphatidyl choline (PC)) layer (LDPE-PC-SA) could be fabricated on a hydrophobic polymer with a durable surface wettability and possessed fluidity being similar to cellular membrane [17].

Recently, we have reported that the polymer nano-film possessing hydrophilic and hydrophobic surface can be synthesized on the surface of LDPE-PC-SA containing stearic acid (StA) (Fig. 1) and that the morphology of polymer nano-film in organic solvents (methanol and chloroform) and water was estimated by 1H-NMR spectra measurement and atomic force microscope (AFM) measurement [18,19]. It was clarified that that polymer nano-film kept a spread form in methanol and a particle-like shape in chloroform and water.

In the previous paper, we demonstrated that the polymer nano-film could be applied to the drug carrier [20]. Although about 15% of drug incorporated in polymer nano-film was released within 24 h, it was suggested that complete drug release might be difficult for the drug to be trapped.
in the hydrophobic core. In this communication, we fabricated the pH-responsive polymer nano-film in which the linker moiety might be degradable in acidic condition (pH 5). The size of pH-responsive polymer nano-film was estimated by dynamic light scattering (DLS) measurement. We also studied the progressive changes in scattering intensity to reveal the degradation of the polymer nano-film at pH 5.

2. Experimental

2.1. Materials

Hexamethylene diisocyanate and 10% Pd/C were purchased from Tokyo Chemical Industry Co., Ltd. and Kojima Chemicals Co., Ltd., respectively. Per-6-amino-β-cyclodextrin (Per-6-ABCD) and benzyl 3-hydroxypropionate were synthesized according to the literatures [21,22].

2.2. Synthesis of di[2-(benzoyloxy carbonyl)ethyl] hexamethylene-1,6-dicarbamate (I)

Benzyl 3-hydroxypropionate (0.9 g, 5.0 mmol) was dissolved in dry CH₂CN (15 mL). To the solution hexamethylene diisocyanate (0.45 g, 2.7 mmol) was added dropwise. This solution was refluxed for 20 h, and then evaporated. The residue was recrystallized from CHCl₃ – hexane to yield 0.8 g (56%) of I. ¹H-NMR (CDCl₃) δ 1.31 – 1.48 (8 H, methylene of alkyl group), 2.68 (4 H, t, J = 5 Hz, -CH₂-COO-), 3.15 (4 H, t, J = 6.5 Hz, -NH-), 3.15 (4 H, s, benzyl), 7.35 (12 H, m, phenyl).

2.3. Synthesis of di(2-carboxyethyl) hexamethylene-1,6-dicarbamate (Linker I)

Di[2-(benzoyloxy carbonyl)ethyl]hexamethylene-1,6-dicarbamate (0.2 g, 0.38 mmol) was dispersed in the mixture of methanol (10 mL) and ethyl acetate (1 mL) and 10% Pd/C (20 mg) was added. The mixture was stirred under hydrogen gas for 16 h. The reaction mixture was filtered and evaporated. The residue was recrystallized from methanol – CHCl₃ to yield 40 mg (30%) of Linker I. ¹H-NMR (CD₂OD) δ 1.02 – 1.37 (8 H, m, methylene of alkyl group), 2.50 (4 H, t, J = 6.5 Hz, -CH₂-COOH), 2.97 (4 H, m, -CH₂-NH-), 4.15 (4 H, t, J = 6.5 Hz, -CH₂-OCOHNH-), 4.83 (2 H, br, -COOH).

2.4. Preparation of pH-responsive polymer nano-film

According to the literatures, the self-assembled phospholipid layer incorporating stearic acid (StA) (LDPE-StA-PC-SA) was fabricated, and then Per-6-amino-β-cyclodextrin (Per-6-ABCD) was immobilized on the LDPE-StA-PC-SA film [17, 18]. To 100 mL of water was added 1 mL of 0.047 μmol/mL 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) water solution and 50 μL of 29 μmol/mL Linker I in DMSO. This solution was kept at 30 °C for 30 min. The LDPE-StA-PC-SA film immobilizing Per-6-ABCD (1 × 3 cm, 40 films) was soaked into this solution at 30 °C for 16 h. These films were washed with water. The films were immersed into 60 mL of ethanol and kept at 30 °C for 3 h. This ethanol solution was concentrated to about 7 mL in vacuo. The concentrated solution was transferred into a pre-swollen semi-permeable membrane (Spectra/Por® 1 Dialysis Membrane Standard RC Tubing MWCO: 6 - 8 kD, Spectrum Laboratories, Inc.). The both sides of the tube were sealed with dialysis tubing closures (Dialysis Tubing Closures Standard Closure Type, 80 mm). The solution was dialyzed against 300 mL of methanol for 15 h. And then the dialysis membrane was immersed into 300 mL of pH 7.4 phosphate buffer saline (PBS) to obtain the polymer nano-film solution.

Fig. 1. Schematic illustration of the fabrication of polymer nano-film possessing hydrophilic and hydrophobic side with the self-assembled phospholipid layer containing stearic acid.
2.5. Dynamic light scattering measurement

Dynamic light-scattering was measured using a DLS-5500G Photal dynamic light-scattering spectrophotometer (Otsuka Electronics) equipped with a He/Ne laser. A scattering angle of 90° was used to evaluate the size of polymer nano-film. The hydrodynamic diameter and the polydispersity factor, represented as $\mu^2/\Gamma^2$, were calculated using the Stokes-Einstein equation and the cumulant method. The number-average particle diameter was determined by histogram method with Marquardt calculation.

3. Results and Discussion

3.1. Fabrication of pH-responsible polymer nano-film

To fabricate a pH-responsible polymer nano-film we designed pH-degradable linker moiety. Figure 2 shows the structure of pH-degradable linker, di(2-carboxyethyl) hexamethylene-1,6-dicarbamate (Linker I). It is well-known that the carbamate group (-NH-COO-) could easily be decomposed in acidic condition, so that it is expected that Linker I might be broken down in the acidic organelle such as endosomes and lysosomes. Figure 3A shows the size distribution of the pH-responsible polymer nano-film at pH 7.4. The size distribution was slightly broad and the number average particle diameter was about 350 nm. We separately confirmed that the size distribution at pH 7.4 had almost unchanged for 3 days.

3.2. Degradation of pH-responsible polymer nano-film

The degradation of pH-responsible polymer nano-film was performed in pH 5.0 acetate buffer solution. Figure 3B shows the size distribution of polymer nano-film after 1 h at pH 5.0. The number average particle diameter in Fig. 3B (pH 5.0) was larger than that in Fig. 3A (pH 7.4). The similar result was obtained in a previous paper [20]. Free amino groups in Per-6-ABCD moiety would be protonated in acidic condition, so that the particle size of polymer nano-film at pH 5.0 might be larger than that at pH 7.4 by the electric repulsion among Per-6-ABCD moieties. After 2h the number average particle diameter decreased, but after then this value was almost unchanged (Fig. 3C and 3D).

On the other hand, the scattering intensity, which related to the amount of nanoparticles having the diameter of more than 10 nm in our experimental setup, decreased with increasing the time. Figure 4 shows the progressive changes in relative scattering intensity of polymer nano-film at pH 5.0. The scattering intensity at pH 7.4 was used as the baseline. The particle diameter at 4 h could be measured by changing the operational condition, but it was difficult to estimate the particle diameter after 15 h due to the lower scattering intensity. It was suggested that most of the polymer nano-film might be decomposed until 15 h.

![Fig. 2. Structure of the linker moiety, di(2-carboxyethyl)hexamethylene-1,6-dicarbamate (Linker I).](image-url)

![Fig. 3. Size distribution of pH-responsible polymer nano-film (A) at pH 7.4, (B) at pH 5 after 1 h, (C) 2 h and (D) 4 h.](image-url)
Fig. 4. Progressive changes in relative scattering intensity in DLS measurement. ○; pH 7.4, ●; pH 5.0. The scattering intensity at pH 7.4 was used as the baseline.

4. Conclusion

The conclusions drawn from the present study can be summarized as follows.

We fabricated the pH-responsible polymer nano-film possessing carbamate groups in the linker moieties. Although the number average particle diameter at pH 7.4 was about 350 nm, that at pH 5.0 after 1h was about 405 nm. It was considered that the larger particle size at pH 5 might be ascribed to the electric repulsion among the protonated amino groups in Per-6-ABCD. The progressive changes in scattering intensity at pH 5.0 indicated that the polymer nano-film could gradually be decomposed at pH 5.0.

We are now actively elaborating the application to a drug carrier possessing more effective drug release property using polymer nano-film.

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