Application to Nano Drug Carrier Using Polymer Nano-Film Synthesized on Self-Assembled Phospholipid Layer Fabricated by Plasma-Assisted Method

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We prepared the polymer nano-film containing drug (5-Fluorouracil, 5-FU) in phosphate buffer saline (PBS, pH 7.4). The size of polymer nano-film containing 5-FU was estimated by dynamic light scattering measurement. The particle size of polymer nano-film at pH 5 was bigger than that at pH 7.4. It was considered that this phenomenon might be ascribed to the electric repulsion among Per-6-ABCD moieties in polymer nano-film at pH 5. The drug release from the polymer nano-film containing 5-FU was also studied at pH 5. It was considered that the increase of particle diameter at pH 5 could induce the release of 5-FU from the polymer nano-film.

Keywords: Polymer nano-film, Nano drug carrier, 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].

A durable surface wettability was introduced on several hydrophobic polymers, such as polyethylene, polyethylene, and nylon-12 by plasma-assisted method [12-16]. Furthermore, a self-assembled phospholipid (phosphatidyl choline (PC)) layer was fabricated on a hydrophobic polymer with a durable surface wettability [17]. It was also shown that the self-assembled phospholipid layer (LDPE-PC-SA) possessed fluidity being similar to cellular membrane.

We have reported the polymer nano-film possessing hydrophilic and hydrophobic surface with LDPE-PC-SA containing stearic acid (StA) [18]. Figure 1 shows the schematic illustration of fabrication of polymer nano-film. Per-6-amino-β-cyclodextrin (Per-6-ABCD) was immobilized onto the LDPE-PC-SA containing StA, and then crosslinked with 4,4’-[1,2-ethanediyl-di-(oxy-ethylamino)]-bis(4-oxo-2-butenoic acid) (EBBA) to fabricate the polymer nano-film. In the previous paper, 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 [19]. It was clarified that that polymer nano-film kept a spread form in

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methanol and a particle-like shape in chloroform and water.

In this communication, we prepared the polymer nano-film containing drug (5-Fluorouracil, 5-FU) in phosphate buffer saline (PBS, pH 7.4). The size of polymer nano-film containing 5-FU was estimated by dynamic light scattering (DLS) measurement. We also studied the drug release profile from the polymer nano-film containing 5-FU at pH 5.

2. Experimental

2.1. Materials

Per-6-amino-β-cyclodextrin (Per-6-ABCD) and 4,4’-[1,2-ethanediyl-di(oxyethylamino)]bis-(4-oxo-2-butenoic acid) (EBBA) were synthesized according to the literatures [18,20].

2.2. Preparation of polymer nano-film containing 5-FU

The self-assembled phospholipid layer incorporating stearic acid (StA) (LDPE-StA-PC-SA) was fabricated according to the literature [17]. The LDPE-StA-PC-SA film immobilizing Per-6-ABCD was prepared according to the literature [18]. To 5 mL of 0.016 nmol/mL EBBA solution was added 4.8 mg of EDC-HCl. This solution was kept at 30 ºC for 2h. The LDPE-StA-PC-SA film immobilizing Per-6-ABCD was soaked into this solution at 5 ºC for 24 h. This film was washed with water. The film was immersed into 5 mL of ethanol and kept at 40 ºC for 12 h. This ethanol solution was transferred into a pre-swollen semi-permeable membrane tube (Spectra/Por® 2 Dialysis Membrane Standard RC Tubing MWCO: 12-14,000, Spectrum Laboratories, Inc.; molecular weight cutoff, 12,000 – 14,000 g/mol). The both sides of the tube were sealed with dialysis tubing closures (Dialysis Tubing Closures Standard Closure Type, 35 mm). The solution was dialyzed against 200 mL of methanol for 24 h. To a solution in the dialysis membrane was added 10 mg of 5-FU. After closing the both sides, the dialysis membrane was immersed into 300 mL of PBS to obtain the polymer nano-film containing 5-FU.

2.3. 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 90º was used to evaluate the size of polymer nano-film containing 5-FU. 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 and weight-average particle diameter were determined by histogram method with Marquardt calculation.

2.4. Drug release from polymer nano-film

The pH 7.4 solution (3 mL) of polymer nano-film containing 5-FU was transferred into a pre-swollen semi-permeable membrane tube (Spectra/Por® 2 Dialysis Membrane Standard RC Tubing MWCO: 12-14,000, Spectrum Laboratories, Inc.). The both sides of the tube were sealed with dialysis tubing closures. The membrane tube containing polymer nano-film was immersed into pH 5.0 acetate buffer solution (200 mL) at room temperature. The outer-layer solution was periodically sampled and the released 5-FU was assayed by measuring the UV absorbance at 265 nm.

![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.](image-url)
3. Results and Discussion

3.1. DLS measurement of polymer nano-film containing 5-FU

The size of polymer nano-film containing 5-FU was estimated by DLS measurement. Figure 2 shows the size distribution of polymer nano-film at pH 7.4 and pH 5. The pH values of 7.4 and 5 would correspond to those in blood and acidic organelle (endosomes, lysosomes), respectively. The particle distribution was slightly broad at each pH. The number average particle diameters at pH 7.4 and pH 5 were 329 nm and 498 nm, respectively. As it was considered that Per-6-ABCD moiety would be protonated in the acidic condition, the particle size of polymer nano-film at pH 5 might be bigger due to the electric repulsion among Per-6-ABCD moieties in polymer nano-film. Figure 3 shows the plots of the scaled average characteristic line width (diffusion coefficient) against angle. Although the value of diffusion coefficient slightly increased with increasing the angle at pH 7.4, these values were almost same against angle at pH 5. This result suggested that the polymer nano-film containing 5-FU was nearly spherical morphology at pH 7.4 and more spherical at pH 5. In the previous paper, it was reported that the polymer nano-film containing no 5-FU might form cylindrical morphology at pH 7 [18]. It was assumed that the incorporated 5-FU could affect on the morphology of polymer nano-film.

3.2. Nature of drug release profile from polymer nano-film containing 5-FU

Drug load (%) was calculated using the following equation:

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\text{Drug load} = \frac{\text{weight of 5-FU in the polymer nano-film}}{\text{weight of drug-loaded polymer nano-film}} \times 100 \quad (1)
\]

The drug load of the present polymer nano-film was about 50%. The incorporated 5-FU was not released at pH 7.4 from polymer nano-film within a detectable extent. Figure 4 shows the progressive changes in drug release from polymer nano-film containing 5-FU at pH 5. It is seen from Fig. 4 that the degree of drug release from polymer nano-film increases until about 1.5 h and then tends to level off. About 15% of 5-FU was released within 24 h. It was supposed that the increase of particle diameter at pH 5 could induce the release of 5-FU from polymer nano-film. However, it was also considered that complete release of 5-FU from the polymer nano-film might be difficult, because 5-FU was trapped in the hydrophobic core of polymer nano-film.

Fig. 2. Size distribution of polymer nano-film containing 5-FU at pH 7.4 and pH 5.

Fig. 3. Plots of the scaled average characteristic line width (diffusion coefficient) against angle of polymer nano-film containing 5-FU.

Fig. 4. Drug release profile from polymer nano-film containing 5-FU at pH 5.
4. Conclusion

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

It was observed that the particle size of polymer nano-film at pH 5 might be bigger than that at pH 7.4 due to the electric repulsion among Per-6-ABCD moieties in polymer nano-film. About 15% of 5-FU was released from the present polymer nano-film within 24 h. It was supposed that the increase of particle diameter at pH 5 could induce the release of 5-FU from the present polymer nano-film.

We are now actively elaborating the control of the size of polymer nano-film and a drug carrier possessing more effective drug release property using polymer nano-film.

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