Preparation of Bionanocapsules by the Layer-by-Layer Deposition of Polypeptides onto a Liposome

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ABSTRACT: Hollow nanoparticles (bionanocapsules) were prepared using liposomes as a template for the layer-by-layer deposition of biopolymers. We carried out the alternative deposition of poly-L-lysine (P_{Lys}) and poly-L-aspartic acid (P_{Asp}) onto a negatively charged liposome, which was prepared from dilauroyl phosphatidic acid (DLPA) and dimyristoylphosphatidylcholine (DMPC). Efficient separation of nanocapsules from unbound polymers was achieved by ultrafiltration. ζ-Potentials of nanocapsules changed between positive and negative charges at each deposition. FE-TEM revealed that the liposome was covered with a thin polymeric layer. A fluorescent probe, 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS), was encapsulated into nanocapsules. The release of HPTS was suppressed by the polymer deposition, and the release rate was tunable by coverage of the first P_{Lys} layer and the ratio of DLPA.

Introduction

Hollow particles, which have a structure composed of a cavity and a thin wall, have attracted much attention because they possess unique functions, which are different from solid particles, such as low density, a large gap in refractive index, and thermal insulation. Their applications are diverse, such as composites for weight savings and pigments for a whitening effect in the paper and paint industries, inks for paper display in the publishing industry, agents for hiding blotches and producing opaqueness in the cosmetic industries, and capsules for drug delivery in pharmaceuticals. A variety of fabrication techniques have been developed to generate hollow particles, e.g., interfacial polycondensation and emulsion polymerization, which are based on interfacial and colloidal sciences such as phase separation and micelle formation.[1] For instance, Okubo and co-workers have reported the preparation of micrometer-sized, monodisperse, and cross-linked particles having a single hollow by a method based on the self-assembly of phase separated polymers.[2]

Nanosized hollow particles (nanocapsules) have become a subject of special interest in many applications including vehicles for drug release and delivery and capsules for further high resolution of inks and displays. However, it is difficult to prepare a nanosized hollow capsule with a narrow size distribution. Recently, the one-step synthesis by miniemulsion polymerization has been developed to obtain hollow nanoparticles.[3] It has also been reported that association of diblock copolypeptides could allow the formation of spherical vesicles.[4] This is based on a self-assembly similar to the construction of biocapsules such as a viral capsid. Such proteinous and lipid capsules found in biological areas are expected to be a powerful candidate for drug and gene delivery.[5] A liposome is a typical nanocapsule composed of a lipid bilayer and has been widely utilized for drug delivery and cosmetics. On the other hand, encapsulation is one of the promising methods to generate the hollow structure. A sophisticated approach to fabricate ultrathin polyelectrolyte multilayer films, which is based on the alternate deposition of polyanions and polycations, has received a lot of attention recently.[6] Such assembly of the electrolytes (layer-by-layer deposition) is believed to be driven by electrostatic attraction and complex formation between polyanions and polycations. Colloid particles,[7] protein aggregates, biological cells,[8,9] and drug crystals have been utilized as micrometer-sized or nanometer-sized templates to assemble multilayer films. However, the core materials must be calcinated or chemically etched by the use of toxic agents.[10] This process is complicated and rupture of the capsule is sometimes induced, and it is not so easy to encapsulate substances into the capsule.[11,12] Recently, Abbott, Caruso, and co-workers have reported a new, nontoxic route. They used oil droplets as templates, which can be simply dissolved using ethanol.[13] Here, we utilized a liposome as a template that is originally a spherical vesicle for the layer-by-layer deposition (Figure 1).

There are some advantages to preparing a nanocapsule by the use of a liposomal template. Nanocapsules with different sizes would be readily obtained by tuning the size of liposome with a membrane extrusion process. We can incorporate a variety of substances into liposomes at the step of liposome preparation. Introduction of the polymer chain on the liposome surface has often been performed to improve the dispersion stability and stiffness of the lipid layer.[14] Sometimes, adsorption of the polymer chain makes the lipid membrane leaky or makes the liposomes aggregated. These suggest that we must take affinities of polymer chains for membranes and their conformational changes upon adsorption into consideration in the interaction of the polymer with the lipid membrane.[15−18]

Here, we produced a biomaterial-derived nanocapsule by the layer-by-layer deposition of polypeptides on the liposome. The first polymeric layer was generated by adsorption of cationic poly-L-lysine (P_{Lys}) onto the negatively charged liposomes, which were prepared with a mixture of dilauroyl phosphatidic acid (DLPA), which has a negative charge, and dimyristoylphosphatidylcholine (DMPC) at different ratios. The second polymer was produced by depositing poly-L-aspartic acid (P_{Asp}) on the P_{Lys}-adsorbed liposome. To make the layer thicker, the sequential deposition of oppositely charged polyelectrolytes was repeated onto the surface. We measured surface potentials and observed morphologies at each deposition, and then we investigated the incorporation of fluorescent probes and the release to evaluate the potential as a nanocapsule.

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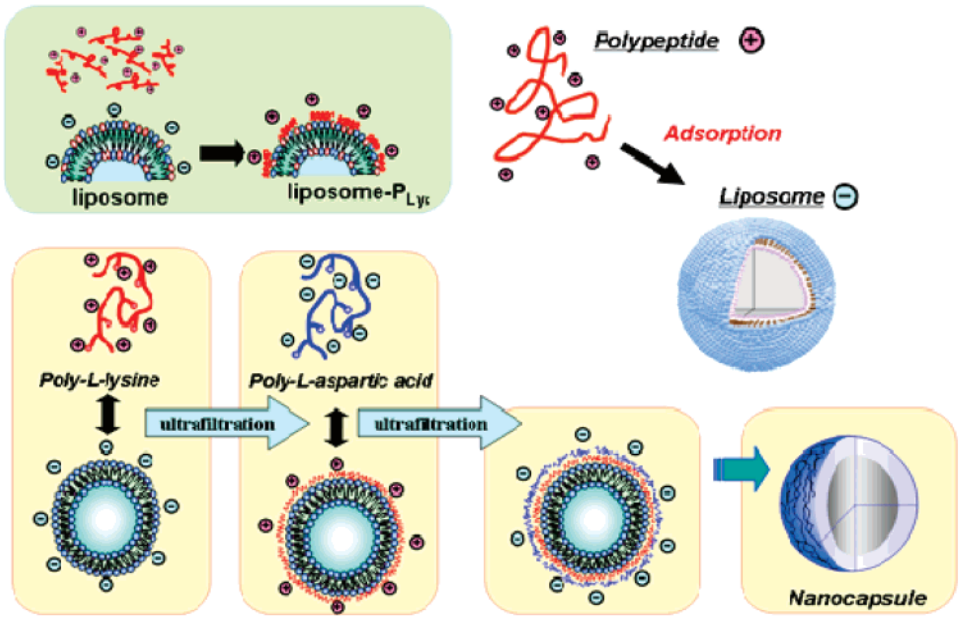
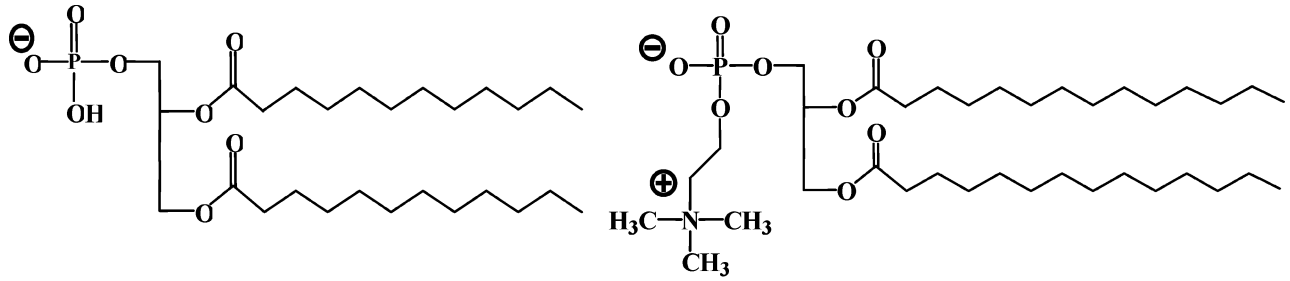


Figure 1. Preparation of nanocapsules by the layer-by-layer deposition of polypeptides onto a liposome template.

Scheme 1. Chemical Structures of Lipids Used for a Liposomal Template



Dilauroyl phosphatidic acid (DLPA) **Dimyristoyl phosphatidylcholine (DMPC)**

Experimental Section

Materials. Dilauroyl phosphatidic acid (DLPA) and dimyristoylphosphatidylcholine (DMPC) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and the Nippon Oil and Fats Co. (Tokyo, Japan), respectively (Scheme 1). Poly-L-lysine (P_{Lys}, MW 30000−70000) was purchased from Wako Pure Chemical. Poly-L-aspartic acid (P_{Asp}, MW 15000−50000) and 1-hydroxypyrene-3,6-trisulfonic acid (HPTS) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Ammonium molybdate was purchased from TAAB Laboratories Equipment Ltd. (Berkshire, England). The 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) protein quantitation kit was purchased from Molecular Probes (Eugene, OR). The other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the chemicals were used as received. The water used in all experiments was prepared in a water purification system (WT-100, Yamato Scientific Inc., Tokyo, Japan) and had a resistivity higher than 18.2 M ohm cm.

Preparation of Liposomes. The negatively charged liposomes were prepared as follows. DMPC and DLPA were dissolved at different molar ratios in methanol. Methanol was removed by rotary evaporation to yield a thin lipid membrane over the inner surface of a round-bottom flask. The membrane was dispersed in 1 mL of HEPES buffer (10 mM HEPES, pH 7.4) using a bath-type sonicator at 40 °C. After three cycles of freezing and thawing, the liposome suspension was extruded 20 times through a membrane with pores of 100 nm at 40 °C using a LipoFast Basic (Avestin Inc., Ontario, Canada). The phospholipid concentrations were measured by an assay using Phospholipids A-Test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan). To encapsulate HPTS into the liposomes, 1 mL of 50 mM HPTS aqueous solution was added to the lipid membrane instead of HEPES buffer and then liposomes were prepared in the same manner. The suspension of HPTS-encapsulated liposome was purified by gel permeation chromatography on a Sephadex G-25 (Amersham Pharmacia Biotech, Oakville, Ontario, Canada) column equilibrated with HEPES (pH 7.4). To determine the HPTS concentration in the liposome, 0.9 mL of the liposome suspension (phospholipids concentration: 1.1 mM) was lysed with 0.1 mL of 10% Triton X-100 and the emission intensity at 512 nm was measured.

Preparation of Nanocapsules by the Layer-by-Layer Deposition. The first polyelectrolyte layer was deposited onto the negatively charged liposome from the P_{Lys} aqueous solution containing 10 mM HEPES. A 0.5 mL aliquot of the P_{Lys} solution was added to 0.5 mL of the liposome suspension so as to be 0.5 mM at the final lipid concentration. The adsorption at different concentrations of P_{Lys} was carried out for 30 min at 25 °C and pH 7.4 with stirring at 700 rpm. Then, excess polyelectrolytes were removed by 7 times repeated ultrafiltration of the suspension using the Amicon filtration membrane (100kD MW cutoff, Millipore, Bedford, MA) for 2 min at 3000 rpm and 25 °C. The concentration of polypeptides in the supernatant solution was estimated by the CBQCA method in Hanks' balanced salt solution (HBSS) buffered solution. This method is based on the fact that CBQCA generates a highly fluorescent derivative upon conjugation with amines in the presence of cyanide or thiols.[19] First, 100 μL aliquots of samples were pipetted into microplate wells. Then, 35 μL of HBSS was added to each well followed by the addition of 5 μL of a 20 mM KCN solution and 10 μL of a 4 mM CBQCA solution. After

incubation at room temperature with shaking for 60 min, the fluorescence was determined using a fluorescence plate reader with a 460 nm excitation and a 545 nm emission. The obtained capsule was referred to as liposome−P_{Lys}. To fabricate the second layer, the aqueous solution containing the oppositely charged P_{Asp} was added at a final concentration of 500 ppm to the suspension of liposome−P_{Lys}, which was prepared by depositing P_{Lys} at 500 ppm on a liposome of DLPA/DMPC = 0.5/0.5. Adsorption, purification, and suspension were carried out in the same manner. The obtained capsule was referred to as liposome−P_{Lys}−P_{Asp}. To make the polymeric layer thicker, the sequential deposition was repeated with the same procedure under the same conditions outlined for the first layer.

Characterization of Liposomes and Nanocapsules. The liposomes and nanocapsules were suspended in 10 mM HEPES. The size of the liposomes was evaluated by dynamic light scattering using a photon correlator (PAR-3, Otsuka Electronics, Osaka, Japan). Electrophoretic mobilities of the bare and polymer-coated liposomes suspended in HEPES (pH 7.4) were measured using a ZEECOM ζ potential analyzer (Microtec. Co., Tokyo, Japan). The mobility u was converted into a ζ-potential using the Smoluchowski relation $\zeta = u\eta/\epsilon$, where η and ϵ are the viscosity and permittivity of the solution, respectively. To detect a lipid phase change of liposomes, high-sensitive differential scanning calorimetry (DSC) measurements were performed with a NanoDSC2 6100 (Calorimetry Sciences Corp. Utah). To detect the secondary structural changes upon the adsorption of P_{Lys}, measurements by circular dichroic (CD) spectroscopy for liposomes and polymer-coated liposomes, which were suspended in 10 mM phosphate buffered solution (PB), were carried out with a JASCO J-600 spectropolarimeter at 25 °C. Scanning was repeated 30 times at a speed of 20 nm min^{-1}. The stability of the capsules was evaluated by adding 10% Triton X-100 to the suspension and measuring the intensity at 512 nm. The morphology of samples was observed with a field emission transmission electron microscope (FE-TEM). Samples for FE-TEM were prepared by deposition of aqueous suspension of the nanocapsules upon a carbon-coated copper grid. The mixture was allowed to air-dry for 1 min. After the extra solution was blotted off, the sample was stained with 1 wt % ammonium molybdate.

Release from Nanocapsules. HPTS was encapsulated into the liposome composed of 50 mol % DLPA (DLPA/DMPC = 0.5/0.5). Release of the loaded HPTS from nanocapsules was studied using a fluorescent assay. The liposomes were lysed by adding 0.10 mL of 10% Triton X-100 and the intensity (I_0) of HPTS in the lysate was measured. The liposomes or nanocapsules suspended in 0.45 mL of 10 mM HEPES (pH 7.4) were poured into a dialysis unit (M_w cutoff 10kD, Slide-A-Lyzer MINI, Pierce Biotechnology, Inc., Rockford, IL), and dialyzed against 45 mL HEPES. After a defined time interval, the fluorescence intensity (I) at 512 nm of HPTS released into HEPES was measured at an excitation wavelength of 413 nm using a spectrofluorometer (F2000 Fluorescence spectrophotometer, Hitachi, Ltd., Tokyo, Japan). Release of HPTS was calculated from I and I_0 using expression 1.

$$\text{release (\%)} = I/I_0 \times 100 \quad (1)$$

Results and Discussion

The liposomes prepared by extrusion through a membrane with pores of 100 nm were around 100 nm in diameter and possessed a highly negative charge at pH 7.4 due to a phosphate group of DLPA. As shown in Table 1, the negative charge increased with an increase in the ratio of DLPA to DMPC. This indicates that obtained liposomes can display the negative region onto which a positively charged polymer chain would adsorb via the strong electrostatic attraction. In addition to the electrostatic interaction, the membrane fluidity plays an important role in the assembly of polymer chains onto the lipid bilayer.[20] At a fluid−solid-phase transition, the lipid bilayers are switched from the liquid crystalline (LC) to the gel phase.

Table 1. Surface Charges and Transition Temperatures of Liposomes[a]

DMPA:DMPC (molar ratio)	EPM, μm s^{-1} cm V^{-1}	ζ-potential, mV	transition temp (T_c), °C
0:1	0.00	0.00	24.5
0.1:0.9	−4.38	−56.1	no datum
0.5:0.5	−7.28	−93.3	29.2
1:0	−10.7	−137	30.6

[a] Hollow nanoparticles were prepared using a liposome as a template for the layer-by-layer deposition.

When the molar ratios of DLPA and DMPC were 0/1.0 and 1.0/0, the transition temperatures of liposomes were 24.5 and 30.6 °C, respectively. The associated DSC curves exhibited a sharp peak, whereas for a molar ratio of 0.5/0.5 a broad peak could be observed and the transition temperature was 29.2 °C. These results are shown in Table 1.

To generate the first polyelectrolyte layer, adsorption of P_{Lys} onto the negatively charged liposomes was carried out at pH 7.4 and 25 °C. The adsorption time was set at 30 min unless otherwise stated because the amount of adsorbed P_{Lys} leveled off at 20 min incubation. In the layer-by-layer electrostatic assembly onto flat substrates, alternate dipping of substrates into the polymer solutions enables us to generate the polyelectrolyte multilayer.[21] As for particulate templates, separation and recovery of the polymer-adsorbed particles from the polymer solution have usually been achieved by centrifugation.[22] We first attempted to separate liposome−P_{Lys} by ultracentrifugation. However, a pellet of liposomal aggregates was obtained and it was hard to disperse the pellet to nanosized capsules in the medium. Thus, we tried to separate the P_{Lys} solution by ultrafiltration through a porous membrane. After the ultrafiltration was repeated seven times to completely remove unbound polymer chains, we could obtain the dispersed capsules without coagulation, and their size was similar to that of the templates. Figure 2 shows adsorption isotherms for P_{Lys} onto liposomes composed of different DLPA ratios. P_{Lys} chains were scarcely adsorbed on a liposome composed of DMPC alone (DLPA:DMPC = 0:1.0), whereas the amount of P_{Lys} adsorbed on the liposomes containing DLPA sharply increased with P_{Lys} concentration.

It was also found in Figure 2 that the saturated amounts of adsorbed P_{Lys} were dependent on the ratio of DLPA. This suggests that adsorption sites were provided by the moieties of DLPA. When adsorption was carried out at the lipid molar ratio of 0.5/0.5, the profile exhibited Langmuir's adsorption isotherm and the amount leveled off at approximately 140 ng cm^{-2}. It was often found that liposomes gathered to form aggregates at low surface coverage. This is probably because aggregation was caused by neutralization of the charges and bridging of polymer chains among liposomes.[23] As can be seen in Figure 2, the liposome composed of DLPA alone (DLPA:DMPC = 1.0:0) showed the two-step adsorption isotherm. The liposome composed of DLPA alone possesses a large adsorption region and high membrane fluidity compared with other liposomes (Table 1). Since adsorbed polymer chains could spread out from the coil state to the extended state on the lipid surface when P_{Lys} concentration was relatively low, the subsequent adsorption might be inhibited by surface occupation by polymer chains spreading over the liposome surface. When the P_{Lys} concentration was increased, such spreading could be suppressed by simultaneous adsorption of a number of polymer chains and the polymer chain might be adsorbed onto the surface so as to form the tethered structure. This leads to a significant increase in the adsorption amount. These suggest that adsorption behavior

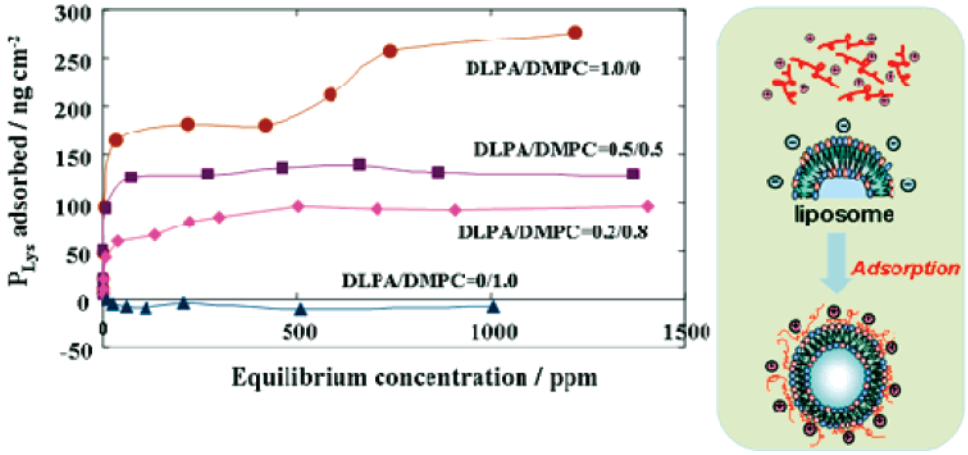


Figure 2. Adsorption isotherms for poly-L-lysine (P_{Lys}) onto the liposome (DLPA/DMPC = 0.5/0.5) at pH 7.4 and 25 °C.

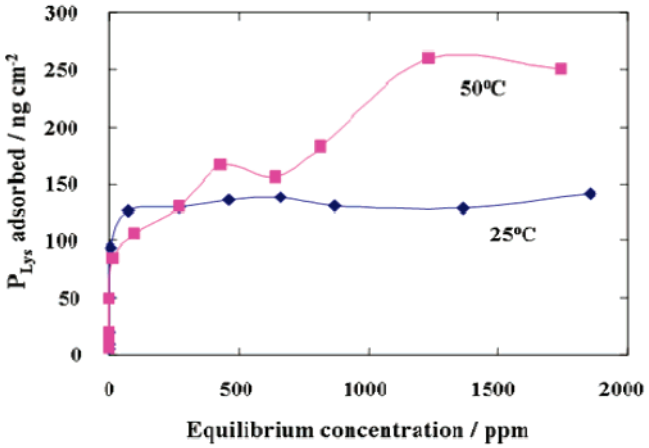


Figure 3. Effect of temperature on adsorption of poly-L-lysine (P_{Lys}) onto the liposome. Deposition was carried out onto the liposome (DLPA/DMPC = 0.5/0.5) at pH 7.4 and at 25 and 50 °C.

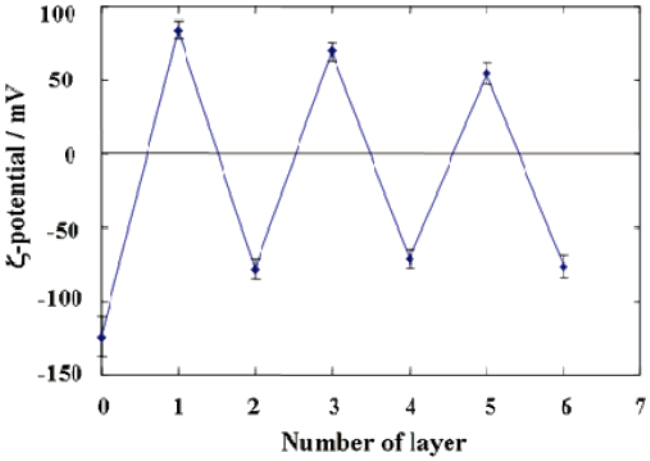


Figure 5. Changes in ζ-potential of nanocapsules at each deposition. Deposition was carried out onto the liposome (DLPA/DMPC = 0.5/0.5) at pH 7.4 and 25 °C.

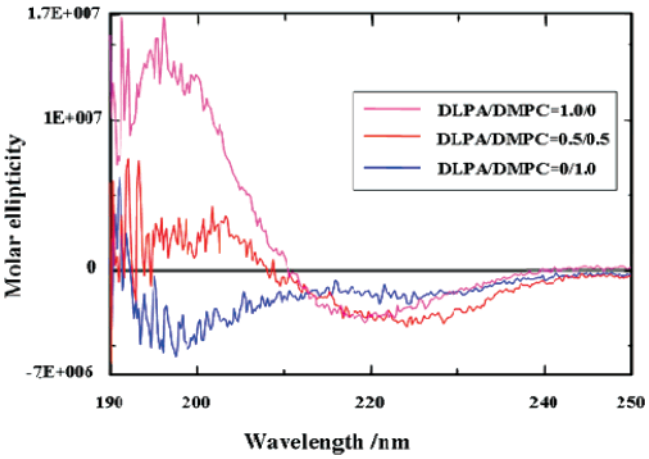


Figure 4. CD spectra of poly-L-lysine (P_{Lys}) layers adsorbed on the liposome with different DLPA ratios. Deposition was carried out at the initial P_{Lys} concentration of 50 ppm at pH 7.4 and 25 °C.

is governed by surface reorganization, which could be brought about by both a conformational change of adsorbed polymer chains and a lateral movement of the lipid molecules. The latter is closely linked to the fluidity of the lipid bilayer, which is influenced by temperature. Therefore, we carried out the adsorption experiment at 50 °C (the lipid molar ratio of 0.5/0.5) to clarify the effect of the fluidity on adsorption. It can be seen from Figure 3 that the adsorption amount significantly increased and leveled off at approximately 250 ng cm^{-2}. It is comparable to that of the liposome composed of DLPA alone at 25 °C. Probably, adsorption sites gathered through the lateral movement of the lipid bilayer to newly generate the large adsorption regions because the fluidity increased with increasing temperature from 25 to 50 °C. As we expected, this indicates that not only the electrostatic interaction but also the membrane fluidity plays an important role in the assembly of polymer chains onto the lipid bilayer. Polyelectrolytes tend to precipitate at high temperatures. P_{Lys} used in this study exhibits a temperature-dependent conformational change from α-helix to β-sheet at high pH.[24,25] To understand temperature effects on adsorption, we must take into account temperature-induced changes in the conformation of polymer chains. Some researchers reported that the polymer−solvent interaction, which is variable with temperature, would determine the thickness of deposited films.[26−28]

The features of nanocapsules such as mechanical structures and physicochemical properties are provided by the polymer layer generated by deposition. In particular, the first layer of P_{Lys} is a key factor to obtain the firm template for subsequent deposition. The P_{Lys} chain in solution exists as a charged random-coil at pH 7. In the pH 9−11 range, the chain with a high molecular weight undergoes a pH-induced conformational transition from a random coil to an α-helix, which is stabilized by intramolecular hydrogen bonding along its backbone. The adsorption experiment was performed at pH 7.4 and 25 °C and the secondary structure of the first layer of P_{Lys} was investigated by circular dichroic (CD) spectroscopy (Figure 4). When P_{Lys}

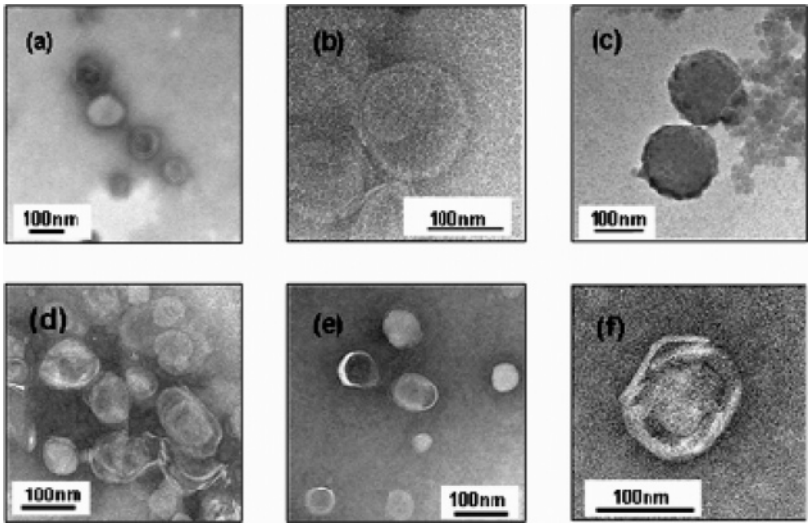


Figure 6. FE-TEM images of a parent liposome (a), liposome−P_{Lys} (b), liposome−P_{Lys}−P_{Asp} (c), liposome−P_{Lys}−P_{Asp}−P_{Lys} (d), liposome−P_{Lys}−P_{Asp}−P_{Lys}−P_{Asp} (e), and liposome−P_{Lys}−P_{Asp}−P_{Lys}−P_{Asp}−P_{Lys} (f). Deposition was carried out onto the liposome (DLPA/DMPC = 0.5/0.5) at pH 7.4 and 25 °C.

was mixed with the DMPC liposome, the spectral datum on the CD analysis revealed that the conformation of P_{Lys} chains remained in a random-coil. This coincides with the fact that P_{Lys} chains were scarcely adsorbed on the DMPC liposome in Figure 2. P_{Lys} adsorbed on the liposomes containing DLPA exhibited typical spectra of β-sheet formation with a minimum ellipticity at 218 nm and a maximum at 195 nm (Figure 4). It was also found that the tendency of β-sheet formation became stronger with the ratio of DLPA. It have been reported that P_{Lys} undergoes a conformational change from random coil to β-structure upon adsorption.[16] Our results suggest that the positive charge of the adsorbed segments of the P_{Lys} chain was weakened upon adsorption onto the negatively charged liposomal surface and then a part of the chain could adopt the extended conformation sufficiently to form a β-sheet structure.

The second layer was deposited on the liposome−P_{Lys} surface by the electrostatic complex formation of poly-L-aspartic acid (P_{Asp}) with liposome−P_{Lys}. The adsorption experiment was carried out at pH 7.4 and 25 °C and at a P_{Asp} concentration of 500 ppm. As can be seen in Figure 5, the ζ-potentials of the parent liposome and liposome−P_{Lys} were −73.6 and +70.9 mV, respectively. After depositing P_{Asp} on liposome−P_{Lys}, the potential decreased to −61.5 mV. When the alternative adsorption of P_{Lys} and P_{Asp} on the liposomes was repeated to thicken the capsule layer, the ζ-potentials changed between positive and negative charges at each deposition (Figure 5). This at least indicates the coverage on the surface with the polypeptides at each deposition. In order to unambiguously prove the buildup of multilayer films by the alternative deposition, we need more detailed studies including confocal microscopy and flow cytometry using a fluorescently labeled polymer, and observation of the thickness by SEM and AFM. These studies are currently under way. The stability of the obtained nanocapsules was estimated by measuring transmittance of their suspensions after addition of a strong surfactant, Triton X-100. The transmittance of the parent liposomes changed to almost 100% due to the lysis of the lipid bilayer, whereas that of nanocapsules did not change at all and still remained suspended in the aqueous medium.

This suggests that generated polymeric layers strengthened the wall of nanocapsules. To provide direct evidence for achievement of the layer-by-layer deposition, we observed the samples with negative staining FE-TEM (Figure 6). The

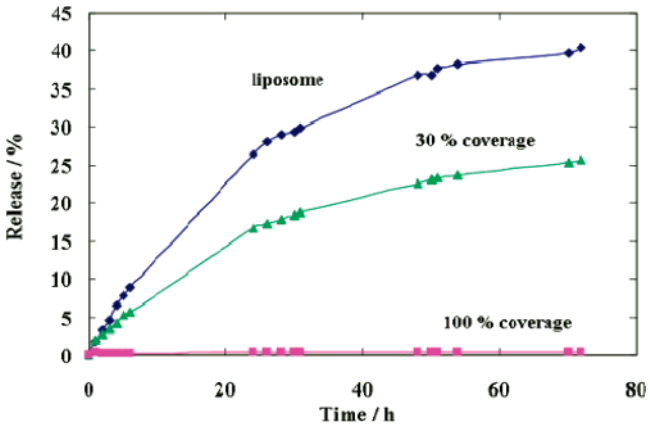


Figure 7. Release of encapsulated HPTS from a parent liposome and liposome−P_{Lys} with 30 and 100% coverage. Deposition was carried out onto the liposome (DLPA/DMPC = 0.5/0.5) at pH 7.4 and 25 °C.

obtained samples maintained the spherical shape and no rupture of the capsule wall was observed although they underwent deformation by freeze-drying. The contour of the shell layer of liposome−P_{Lys}−P_{Asp} could be observed although those of parent liposome and liposome−P_{Lys} were unclear. The thin shell became well-demarcated by further polymer deposition. Upon collapse of liposome−P_{Lys}−P_{Asp}−P_{Lys}−P_{Asp}−P_{Lys}, a dented shell layer like a rubber balloon was observed, as shown in Figure 6. This strongly suggests that a firm shell composed of the polymer multilayer was generated on the liposome surface by the layer-by layer deposition of polypeptides.

The liposomes have been utilized as a carrier to encapsulate and deliver many substances, including drugs, proteins, RNA and DNA. Here, a fluorescent probe, 1-hydroxypyrene-3,6,8-trisulfonic acid (HPTS) was encapsulated into the liposome and then the layer-by-layer deposition was carried out on the liposome to investigate the release behavior from the nanocapsule to 10 mM HEPES (pH 7.4).

Figure 7 shows the release-time profile for parent liposomes and nanocapsules. Parent liposomes showed 30−40% release of the encapsulated HPTS for 72 h, whereas we observed no release from liposome−P_{Lys} with 100% coverage, which corresponds to the leveling amount shown in Figure 3. We observed no release from liposome−P_{Lys} to 10 mM MES buffered solution (pH 4.1) and 10 mM phosphate buffered solution (pH 11.4)

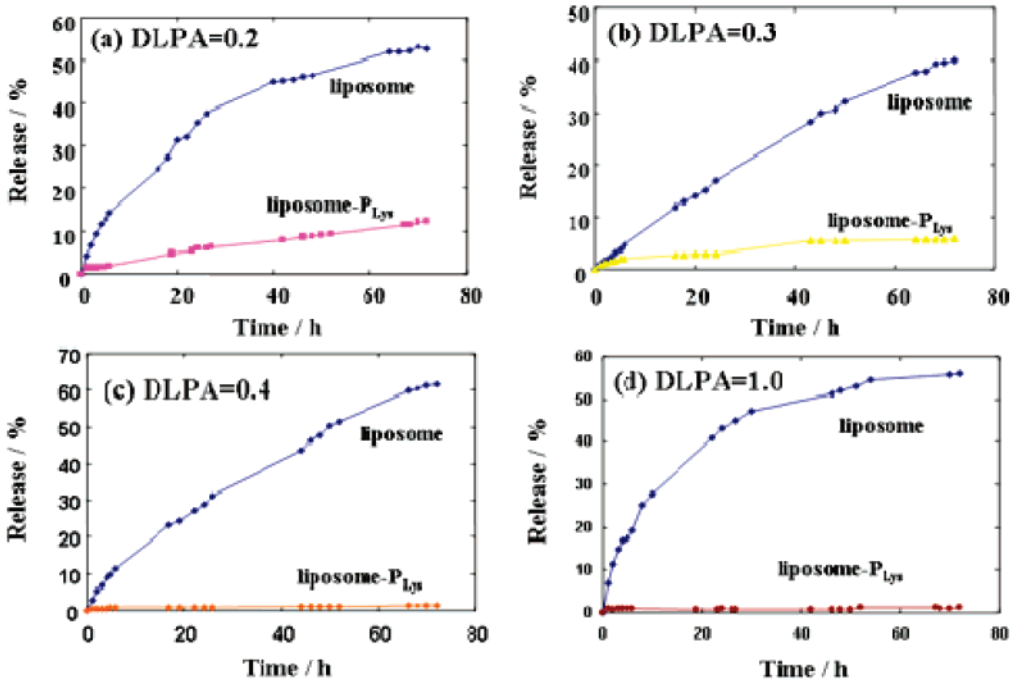


Figure 8. Effect of the DLPA ratio on the release of encapsulated HPTS from a parent liposome and liposome−P_{Lys} with 100% coverage. Deposition was carried out on the liposomes (DLPA/DMPC = 0.2/0.8 (a), 0.3/0.7 (b), 0.4/0.6 (c), and 1.0/0 (d) at pH 7.4 and 25 °C.

(Figure S1). These suggest that the first polymer layer plays an important role to keep the encapsulated HPTS. When the sequential deposition of P_{Lys} and P_{Asp} was carried out, the release was still suppressed as well as that of the first deposition (Figure S2). To tune the membrane permeability, the adsorption amount of the first polymer layer was changed by lowering the P_{Lys} concentration. Liposome−P_{Lys} with 30% coverage, which corresponds to 30% of the leveling-off amount, exhibited a release at a lower rate than a parent liposome (Figure 7). Then, we carried out adsorption of P_{Asp} onto liposome−P_{Lys} with 30% coverage. The release profile for the obtained nanocapsules hardly changed regardless of changing the number of polymer layers. This suggests that, in addition to encapsulation, the membrane permeability of the first layer determines the release behavior, which is tunable by the first adsorption amount of P_{Lys}.

Figure 8 shows the results of the release profiles at 20, 30, and 40 mol % DLPA (DLPA/DMPC = 0.2/0.8, 0.3/0.7, 0.4/0.6, and 1.0/0). We prepared liposome−P_{Lys} with 100% coverage for each liposome with different DLPA ratios. We found that the rate of the release gradually increased with decreasing ratio of DLPA, suggesting that the lipid bilayer also plays a key role in the release. We conclude that the polymer-deposited layers significantly influence the membrane fluidity of the liposomal template and determine the diffusion of substances across the lipid membrane. Further improvements of nanocapsules are still needed to change the permeability through the shell layer by environmental changes such as pH, ionic strengths, and solvents, and to release the encapsulated materials in osmotic pressure- or redox-controlled manners.[29−31]

Conclusion

We have shown that the layer-by-layer deposition of polymers onto the liposome is an easy and useful method to design and synthesize hollow nanoparticles (bionanocapsules) with all biological components. The deposition was governed by electrostatic attraction although the liposome was fluid and extremely brittle as a template. The ζ-potentials changed between positive and negative charges at each deposition. The amount of the deposited polymer was dependent on the concentration of the polymer aqueous solution and the ratio of DLPA in the liposome. The polymer chains deposited on the liposome exhibited typical spectra of β-sheet formation and the tendency was stronger with increasing the ratio of DLPA. The generated polymer layer could allow the storage and release of encapsulated substances. The release rate was tunable by coverage of the first P_{Lys} layer. A variety of nanocapsules will be obtained by selecting polyelectrolytes for the layer-by-layer deposition.

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Supporting Information Available: Figures showing the release behavior from liposome−PLys under different pH conditions and the influence of the sequential deposition on the release. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Itou, N.; Masukawa, T.; Ozaki, I.; Hattori, M.; Kasai, K. *Colloids Surf. A: Physiochem. Eng. Asp.* **1999**, *153*, 311−316.
(2) Minami, H.; Kobayashi, H.; Okubo, M. *Langmuir* **2005**, *21*, 5655−5658.
(3) Tiarks, F.; Landfester, K.; Antonietti, M. *Langmuir* **2001**, *17*, 908−918.
(4) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244−248.
(5) Yamada, T.; Iwasaki, Y.; Tada, H.; Iwabuki, H.; Chuah, M. KL.; VandenDriessche, T.; Fukuda, H.; Kondo, A.; Ueda, M.; Seno, M.; Tanizawa, K.; Kuroda, S. *Nat. Biotechnol.* **2003**, *21*, 885−890.
(6) Decher, G.; Hong, J. D.; Schmitt, J. *Thin Solid Films* **1992**, *1992*, 831−835.
(7) Caruso, F. *Adv. Mater.* **2001**, *13*, 11−22.
(8) Diaspro, A.; Silvano, D.; Krol, S.; Cavalleri, O.; Gliozzi, A. *Langmuir* **2002**, *18*, 5047−5050.
(9) Richert, L.; Vautier, L. D.; Senger, B.; Stoltz, J. -F.; Schaaf, P.; Voegel, J.-C.; Picart, C. *Biomacromolecules* **2002**, *3*, 1170−1178.
(10) Antipov. A. A.; Shchukin, D.; Fedutik, Y.; Petrov, A. I.; Sukhorukov, G. B.; Mohwald, H. *Colloids Surf. A: Physicochem. Eng. Aspects* **2003**, *224*, 175−183.

(11) Heuberger, R.; Sukhorukov, G.; Voros, J.; Textor, M.; Mohwald, H. *Adv. Functional Mater.* **2005**, *15*, 357−366.
(12) Wang, Y.; Yu, A.; Caruso, F. *Angew. Chem., Int. Ed.*, **2005**, *44*, 2888−2892.
(13) Tjipto, E.; Cadwell, K. D.; Quinn, J. F.; Johnson, A. P. R.; Abbott, N. L.; Caruso, F. *Nano Lett.* **2006**, *6*, 2243−2248.
(14) Maeda, T.; Fujimoto, K. *Colloids Surf. B: Biointerfaces* **2006**, *49*, 15−21.
(15) Hammes, G. G.; Shullery, S. E. *Biochemistry* **1970**, *9*, 2555−2563.
(16) Fukushima, K.; Muraoka, Y.; Inoue, T.; Shimozawa, R. *Biophys. Chem.* **1988**, *30*, 237−244.
(17) Fukushima, K.; Sakamoto, T.; Tsuji, J.; Kondo, K.; Shimozawa, R. *Biochim. Biophys. Acta* **1994**, *1191*, 133−140.
(18) Ge, L.; Mohwald, H.; Li, J. *Colloids Surf. A: Physicochem. Eng. Aspects* **2003**, *221*, 49−53.
(19) Yoo, W. W.; Haugland, R. P.; Ryan, D. K.; Haugland, R. P. *Anal. Biochem.* **1997**, *244*, 277−282.
(20) Xie, A. F.; Granick, S. *Nat. Mater.* **2002**, *1*, 129−133.
(21) Decher, G. *Science* **1997**, *277*, 1232−1237.
(22) Caruso, F. *Chem.—Eur. J.* **2001**, *3*, 413−419.
(23) Bronich, T. K.; Solomatin, S. V.; Yaroslavov, A. A.; Eisenberge, A.; Kabanov, V. A.; Kananov, A. V. *Langmuir* **2000**, *16*, 4877−4881.
(24) Rosenheck, K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 1775−1785.
(25) Sarkar, P. K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 981−989.
(26) Buscher, K.; Graf, K.; Ahrens, H.; Helm, C. A. *Langmuir* **2002**, *18*, 3585−3591.
(27) Tan, H. L.; McMurdo, M. J.; Pan, G.; Patten, P. G. V. *Langmuir* **2003**, *19*, 9311−9314.
(28) Quinn, J. F.; Caruso, F. *Langmuir* **2004**, *20*, 20−22.
(29) Shi, X.; Caruso, F. *Langmuir* **2001**, *17*, 2036−2042.
(30) Antipov, A. A.; Sukhorukov, G. B.; Donath, E.; Mohwald, H. *J. Phys. Chem. B* **2001**, *105*, 2281−2284.
(31) Ma, Y.; Dong, W. -F.; Hempenius, M. A.; Mohwald, H.; Vancso, G. J. *Nat. Mater.* **2006**, *5*, 724−729.

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