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Layered Paving of Vesicular Nanoparticles Formed with Cerasome as a Bioinspired Organic−Inorganic Hybrid

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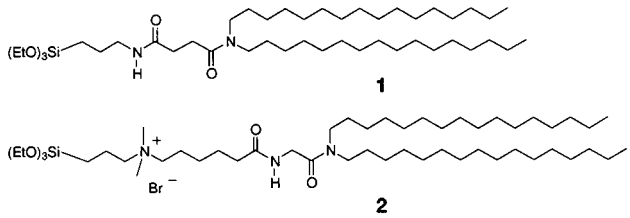
Lipid bilayer vesicles with an inner aqueous compartment have been extensively employed as biomenbrane models in the field of supramolecular chemistry. Over the years it has become apparent that the bilayer vesicles are effectively utilized as nanocapsules for the drug delivery or gene transfection systems,[1] artificial cell membranes,[2] and artificial enzymes.[3] From the point of view of recently growing nanotechnology, however, the bilayer vesicle with a nanometer size seems to have another potential as a fascinating nanomaterial candidate for designing functional supramolecular devices.[4] For such a purpose it would be important to develop a new methodology to form hierarchically integrated vesicular assemblies, since the multicellular bodies in the biological system can create highly organized architectures and exhibit many more functions as compared to the unicellular ones.

While the lipid bilayer vesicles have a wide variety of advantages in the control of the aggregate morphology, particle size, lipid composition, and various physical properties based on molecular design of the lipid components, the morphological stability seems to be generally insufficient to create hierarchical nanoarchitectures. For example, we have previously developed synthetic peptide lipids bearing an amino acid residue interposed between a polar head moiety and a hydrophobic double-chain segment through the peptide bond.[5] Formation of the hydrogen-belt domain in the peptide lipid membrane stabilized the small unilamellar vesicles without morphological change for over several months. Even for such stable

Chart 1



bilayer vesicles, however, the aggregate morphology could not be maintained in the presence of polyelectrolyte with opposite charges to afford membrane fusion,[6] and the behavior is now common knowledge in lipid membrane chemistry.[7] In this communication, we report the first successful three-dimensional integration of lipid vesicular nanoparticles on a substrate by employing the layer-by-layer assembling method. As the lipid bilayer vesicles for the integration we chose a couple of cationic and anionic Cerasomes recently developed by us.[8,9] A Cerasome is a novel bioinspired organic−inorganic hybrid composed of a liposomal membrane with a ceramic surface. The Cerasomes were prepared from organoalkoxysilane proamphiphiles (**1** and **2**) under sol−gel reaction conditions in a manner similar to that reported previously.[8] Formation of the bilayer vesicular structures of the Cerasomes was confirmed by negative-staining transmission electron microscopy (TEM). The observed diameter from TEM observation was 70−300 and 20−100 nm for the Cerasomes prepared from **1** and **2**, respectively. These Cerasomes had characteristic properties as lipid bilayer vesicles, e.g., phase transition behavior from gel to liquid-crystalline state. Phase transition temperatures, T_m, for the Cerasome were measured by differential scanning calorimetry (DSC) with a Seiko-Instruments DSC-6100 calorimeter. The values of T_m for multilamellar vesicles of the Cerasomes derived from **1** and **2** were 10.5 and 25.7 °C, respectively. These Cerasomes

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(9) Preparation and characterization of compound **1** has been reported in ref 8a. Compound **2** was synthesized as follows: condensation of N^{α}-(*tert*-butoxycarbonyl)glycine with dihexadecylamine, deprotection of the *tert*-butoxycarbonyl group, condensation of 6-bromohexanoyl chloride, substitution of the bromide by the dimethylamino group, and quaternization of the amino moiety with 3-bromopropyltriethoxysilane. Yield, 39.4%. Anal. Calcd for $C_{51}H_{106}BrN_3O_5Si\cdot 0.5H_2O$: C, 63.91; H, 11.25; N, 4.38. Found: C, 63.98; H, 11.29; N, 4.40. HRMS (FAB$^+$, *m/z*) calcd for $C_{51}H_{106}N_3O_5Si$ ([M − Br]$^+$), 868.7901; found, 868.7606.

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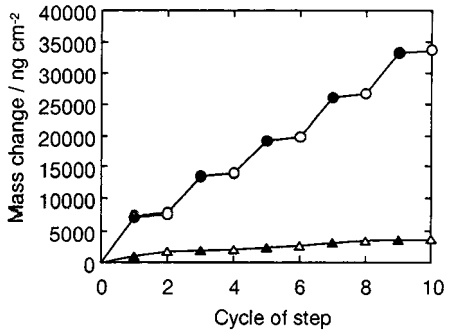

Figure 1. Adsorbed mass change of QCM upon layer-by-layer assembling of the Cerasomes and traditional synthetic liposomes: solid circle, the anionic Cerasome; open circle, the cationic Cerasome; solid triangle, the anionic liposome; open triangle, the cationic liposome.

perform as polyanionic and polycationic nanoparticles in the neutral pH region, since the isoelectric points determined by pH dependence of the ζ-potential were 4.3 and 12.0.

Three-dimensional integration of the Cerasomes was accomplished by adopting the alternate layer-by-layer assembling technique[10] and monitored by measuring the adsorption mass changes on a 9 MHz quartz crystal microbalance (QCM) from USI System Co., Japan.[11] All experiments were conducted at pH 9.0 and 25.0 °C. A precursor film composed of five polyion layers with a cationic surface was made on the QCM resonator by alternate assembling of poly(diallyldimethylammonium chloride) and potassium poly(vinyl sulfate). The substrate was then immersed in an aqueous solution of the anionic and cationic Cerasome with intermediate water washing and drying under a nitrogen stream. The concentration of **1** and **2** in aqueous solutions of both Cerasomes was fixed to 0.5 mmol dm^{-3}. This process was periodically interrupted to measure the QCM resonance frequency. In Figure 1 the odd- and even-number steps were indicated for the anionic and cationic Cerasome adsorption, respectively. For comparison, the mass change for alternate layer-by-layer assembling of the traditional synthetic liposomes monitored by QCM was also shown in Figure 1. In this case, dihexadecyl phosphate (DHP) and dimethyldioctadecylammonium chloride was employed to prepare anionic and cationic liposome, respectively. The average values of the mass change for the Cerasomes adsorption were much larger that those of traditional liposomes, reflecting formation of the layered assembly of each Cerasome. In addition, these values were larger for the anionic and smaller for the cationic Cerasome layer, reflecting the size of the Cerasome in the aqueous solution.

To clarify the integrated structure of the Cerasomes, a similar alternate layer-by-layer assembly of the anionic and cationic Cerasome was formed on a mica substrate and the surface structure was examined by atomic force microscopy (AFM) with a SPI3800N

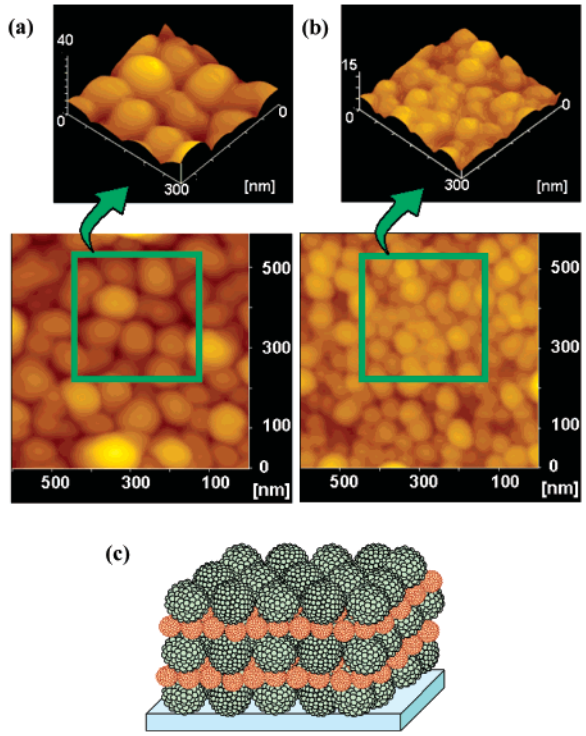

Figure 2. Tapping mode AFM images of the first layer formed with the anionic Cerasomes (a) and the second layer formed with the cationic Cerasomes (b), and schematic drawing of the three-dimensional assembled structure of the anionic and cationic Cerasomes (c).

System from Seiko Instruments Inc., Japan, in tapping mode with a 20 μm scanner. AFM images after the first and second assembling step in Figure 1 were shown in Figure 2, panels a and b, respectively. The Cerasome particles closely packed like a stone pavement were clearly observed in both layers. In addition, the difference in the particle size for each layer indicates the cationic and anionic Cerasomes undoubtedly formed the layer-by-layer assembly as illustrated in Figure 2c. The layered paving of the vesicular nanoparticles was seen in every layer at least up to the 10th adsorption step. It is noteworthy that the layered paving superstructure was never observed by replacement of the Cerasome to other bilayer vesicles formed with phospholipids or synthetic lipids, e.g., DHP. Thus morphological stability of the Cerasome seems to be extremely higher than that of other bilayer vesicles, since the membrane surface of the Cerasome is covered with the siloxane network to prevent collapse and fusion of the vesicle.

In conclusion, a three-dimensional packed vesicular assembly was successfully prepared by using an organic−inorganic hybrid vesicle, the Cerasome. To the best of our knowledge, this is the first example of a hierarchically integrated vesicular assembly on a solid substrate with the layer-by-layer adsorption method. The present nanoparticle assembly keeping a bilayer membrane structure and an inner aqueous compartment in each unit has a potential for constructing artificial multicellular systems as supramolesular nanodevices.

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