

## Chemical Modification of Skeletonized Vesicles via Hydrolysis

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Holes can be drilled in the membrane of a tumor cell attacked by an activated macrophage. This process results in the leakage of the cytoplasmic interior and the death of the cell. In order to simulate the biological process, phase-separated large vesicles were used.<sup>1,2</sup> Polymerization of bilayer membrane composed of polymerizable and non-polymerizable lipids induced phase separation by the direct binding of polymerizable lipids through covalent bonds.<sup>3</sup> Consequently, the vesicles were composed of a polymerized lipid matrix surrounding the labile domains of non-polymerizable lipids. The labile domains were removed from the vesicles (skeletonization) by dissolving the non-polymerizable lipids with surfactant or organic solvent, or by cleaving them with chemical or enzymatic reaction.

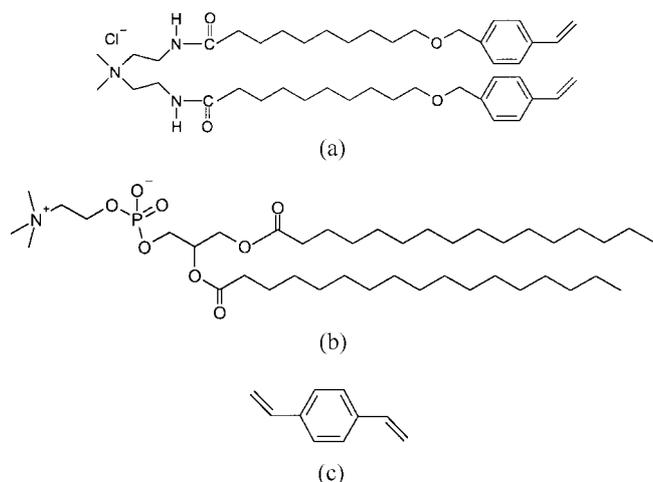
If the skeletonized vesicles are chemically modified to have reasonable affinity with organic solvent, application of the colloidal particles will be more extended. Because of large specific surface area and surface diversity, the new materials may be utilized as stationary phase in chromatography, catalyst, etc. As far as we know, however, any work related to this concept has not been published yet in the literature. In this study, we employed, *N,N*-bis[10-(4-vinylbenzyloxy)decanoylaminoethyl]-*N,N*-dimethylammonium chloride (BDAC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocho-

line (DPPC), and divinyl benzene (DVB) as polymerizable surfactant, non-polymerizable lipid, and cross-linking agent, respectively, as shown in Figure 1.

BDAC was synthesized in this laboratory and details on the synthesis will be reported elsewhere. The surfactant did not form vesicles itself probably due to the bulky styryl group at the end of the both tails.<sup>4,5</sup> A chloroform solution of BDAC, DPPC, and 2,2'-azobisisobutyronitrile (AIBN) in a molar ratio of 3 : 2 : 0.6 was evaporated slowly to form a thin film which was then dried in high vacuum and hydrated with an appropriate amount (5.0 mg lipid/mL) of phosphate buffered solution (pH 7.5). The lipid suspension was extruded through two stacked Nuclepore polycarbonate filters (3 times with 0.4  $\mu\text{m}$  filter and 3 times with 0.1  $\mu\text{m}$  filter) at 50 °C using a stainless steel extruder (Lipex Bio membranes) to prepare unilamellar vesicles. According to dynamic light scattering measurement, the average diameter of the extruded vesicles was  $110 \pm 10$  nm. DVB was injected into the vesicle dispersion with a syringe (BDAC/DVB = 1 : 1, mol/mol), and the resulting mixture was stirred for 2 days at room temperature to ensure a complete incorporation of DVB into the lipid bilayers.<sup>4</sup> The suspension was polymerized under nitrogen bubbling at 60 °C for 24 h. The extent of polymerization was monitored by measuring the UV absorbance of BDAC at 254 nm. The polymerization was allowed to continue until the absorbance remained unchanged. According to the FT-IR spectrum, characteristic peaks of the vinyl groups of BDAC and DVB disappeared completely, indicating that the comonomers have reacted almost completely.

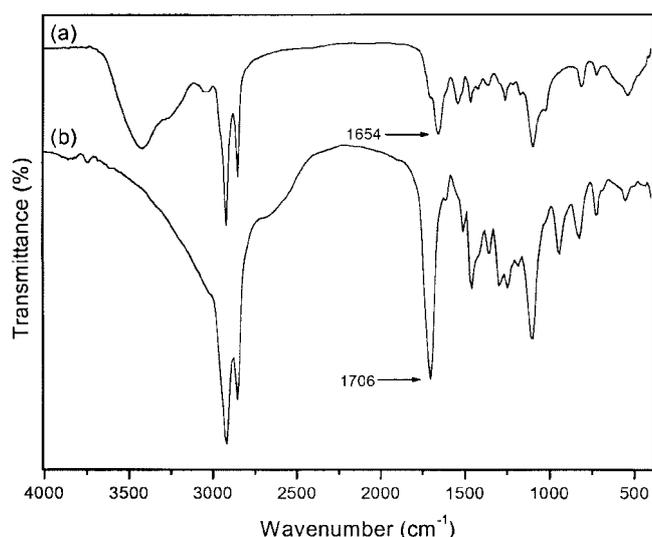
The polymerized vesicle suspension was incubated in the presence of Triton X-100 for 1 h. The molar ratio of Triton X-100 to lipids (BDAC + DPPC) was 10, which should be more than enough to dissolve BDAC in the vesicles because unpolymerized vesicles of BDAC and DPPC were readily dissolved by the surfactant when the molar ratio of the surfactant to the lipids was only 2. After the incubation, the suspension was chromatographed on Bio-Gel A column to remove DPPC-containing micelles. The FT-IR spectrum of the resulting suspension indicated the complete removal of DPPC since the characteristic peaks of ester groups in DPPC were not observed after the surfactant treatment.

The skeletonized vesicles were then stirred in a 12% aqueous HCl solution at 50 °C for 48 h, and centrifuged. The supernatant was decanted off and the precipitate was resuspended with water, followed by the centrifugation. This



**Figure 1.** Chemical structures of (a) BDAC, (b) DPPC, and (c) DVB.

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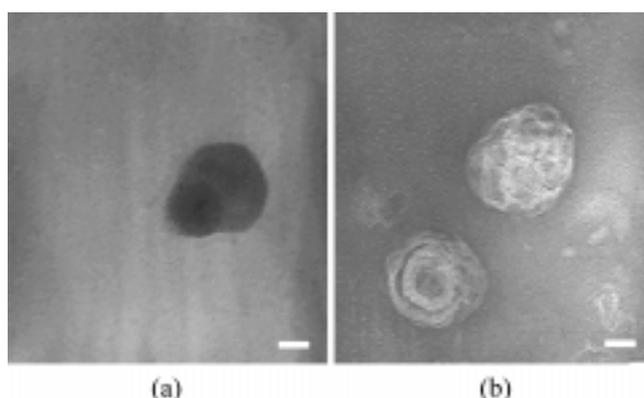


**Figure 2.** FT-IR spectra of (a) before and (c) after acidic hydrolysis.

procedure was repeated three times. According to the FT-IR spectra, the absorption peaks of amide groups at about 3400 and 1654  $\text{cm}^{-1}$  in Figure 2a disappeared, and instead the peaks of carboxyl groups at about 3500-2400 and 1706  $\text{cm}^{-1}$  in Figure 2b emerged clearly. This result indicates that the hydrophilic head groups were removed from the skeletonized vesicle surface.

The modified vesicles were visualized by transmission electron microscopy (TEM, JEOL-JEM 1200EX microscope) after staining with uranyl acetate solution. Most of the hydrolyzed vesicles retained their original spherical shapes, but a so-called parachute-like morphology was also occasionally observed, as shown in Figure 3a. Jung and coworkers observed the parachute-like structures from the polymerization of styrene in dioctadecyldimethylammonium bromide vesicles.<sup>6,7</sup> They claimed that the structures resulted from complete phase separation between the styrene polymer and the vesicle-bilayer matrix. A similar argument can be applied here in explaining the morphology of the skeletonized vesicles. Namely, polymerization of DVB proceeds in the bilayer latex-like fashion almost independently from BDAC. Preferential sorption of DVB to the DVB polymer occurs due to the higher solubility of DVB in its growing polymer than in the polymerizing BDAC bilayer, resulting in development of a new environment where the DVB polymers are growing until DVB is depleted. If the new environment formed in the non-polymerizable (DPPC) domains, the DVB polymer would be possibly released in the form of freely floating latex beads during the skeletonization.

We actually skeletonized the vesicles composed of DPPC and BDAC after polymerization. They were not parachute-like, but approximately spherical shaped, as shown in Figure 3b. The vesicle surface appears to be undulated. The surface morphology may form during the sample preparation, and/or may be also due to the cross-linked polymer within the bilayer because the polymer forces the aggregates to adopt



**Figure 3.** TEM micrographs: (a) after chemical modification of skeletonized vesicles prepared from BDAC, DPPC and DVB, (b) before chemical modification of skeletonized vesicles prepared from BDAC and DPPC only. The scale bar corresponds to 20 nm.

geometries which are ideal with respect to the polymer conformation rather than the minimization of the surface between water and the surfactant.<sup>4</sup> However, the acidic hydrolysis disrupted the vesicles, and most of the new aggregates could not be identified. This result suggests that DVB was copolymerized with BDAC in the bilayers composed of BDAC, DPPC, and DVB, and the cross-linked hybrid vesicles could retain their original shapes even after the hydrolysis.

In conclusion, vesicles composed of BDAC, DPPC, and DVB were polymerized and skeletonized. Finally, the hydrophilic head groups of BDAC components in the polymerized membrane were successfully removed by acidic hydrolysis to obtain porous vesicles with surface covered by carboxyl groups. The surface morphology of the resulting vesicles will be reported in the near future.

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