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Electroformation of Giant Vesicles on a Non-Electroconductive Substrate

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A cell-sized lipid bilayer membrane vesicle or a giant vesicle (GV) has been used as a model in studies of the basic properties of the membrane and the functions of biomolecules.[1] It is also expected to be the structural foundation in the future construction of an artificial cell.[2]

For preparation of GVs, the electroformation first developed by Angelova and Dimitrov[3] has presently become an indispensable method. In the conventional electroformation, a lipid deposit is made on the surfaces of one or both electrodes of either indium tin oxide (ITO) coated glass plates or Pt wires positioned parallel to each other.[3,4] Application of AC voltage between the two electrodes during the swelling of the lipid in water forms GVs attached on the electrode.

The electroformation allows faster and better-controlled formation of GVs compared with the spontaneous process.[4a] To our knowledge, the lipid was deposited almost always *on the electrode*. However, the effect of electro-osmotic flow of water, which has been widely accepted as the main driving force of the electroformation under AC voltage,[5] or a possible indirect effect of the electric field on the lipid layer should not be confined to the proximity of the electrode surface. In the present study, we demonstrated successful formation of GVs from a lipid deposit on a substrate placed apart from the electrodes under AC voltage.

In the first experiment, a vertically stood Pt wire (diameter 0.50 mm) was touched with a drop of a methanolic solution (2.0 μL, 10.0 mg/mL) of phosphatidylcholine purified from egg yolk (eggPC, Avanti Polar Lipids, Alabaster, AL) near one end, and the fall of the drop along the wire spread the solution over the surface to form a thin film of the lipid. The wire was placed between the two electrodes (also of Pt wires) in a trough (Figure 1). The cell was then filled with ultrapure water, and AC voltage (sinusoidal, 5 V peak-to-peak, 2 Hz) was applied between the electrodes from a function generator. Note that no direct electrical connection was made to the Pt wire that had the lipid deposit on it. The lipid deposit was observed with an inverted optical microscope (Olympus IX-50, Tokyo, Japan) equipped with a digital image processing system.

After the initial swinging of the swelled lipid layers on both sides of the Pt wire, GVs appeared on the layer (Figure 2). The GV formation proceeded as with a conventional setup, and the GVs thus formed showed no significant difference from those formed on the Pt wire electrodes. The diameter of most of the GVs was 10−80 μm, and some were as large as 100 μm. Without the AC voltage, unregulated swelling of the lipid deposit resulted in the formation of only myelin-like figures and other irregular lipid membrane structures, and no GV was observed in 120 min. The GV formation was not spontaneous but was certainly under the influence of the electric voltage, although the lipid was not in direct contact with the electrode.

The substrate may be even non-electroconductive. In the second experiment, eggPC was deposited on the outer surface of a thin borosilicate glass tube (the inner and the outer diameters were 0.20 and 0.30 mm, respectively) and placed between the two Pt electrodes. The experiment was carried out in a similar manner to the first. The inner bore of the tube was filled with water during the experiment to avoid optical diffraction at the air−glass interface.

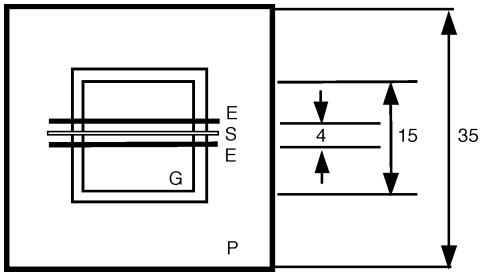

Figure 1. Top view of the electroformation cell with Pt wire electrodes (E). After filling the cell with water, AC was applied between the electrodes, and swelled lipid layers on the substrate (S) were observed through a glass window (G) at the bottom of the trough (P). The sizes are in millimeters.

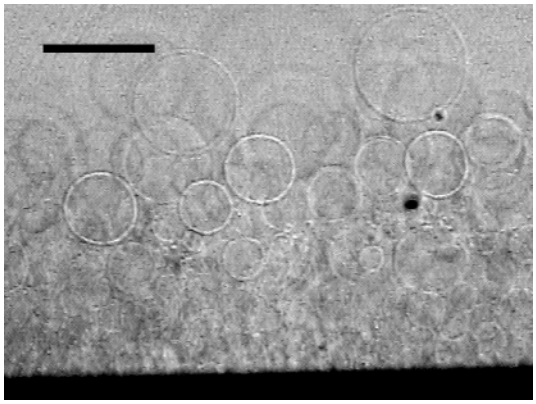

Figure 2. Electroformation on Pt wire substrate. Giant vesicles were formed from an eggPC deposit at 90 min after the application of AC voltage (5 V, 2 Hz) on the surface of the side of the wire. The shadow of the nontransparent Pt wire is seen at the bottom. The bar indicates 50 μm.

The bottom surface of the tube was observable, and the most significant formation of GVs was seen there (Figure 3). On the sides, GVs were formed but only sparsely. Compared with the Pt wire, the optimal GV formation occurred with a thinner swelled lipid layer on the glass surface. No regulated GV formation was seen without the AC voltage.

In the past, examination of a lipid deposit on a thin glass cover slip placed near the electrodes concluded no effect of the AC field on the formation of GVs.[4a] Although the detail awaits further clarification, the apparent contradiction between the previous report and the present result is probably due to the difference in the two experimental setups, most likely the location and/or the form of the lipid deposits. In fact, we also experienced difficulty in some attempts of the electroformation with glass substrates. At the side of the glass tube, the lipid layer movement appeared to be "shielded"

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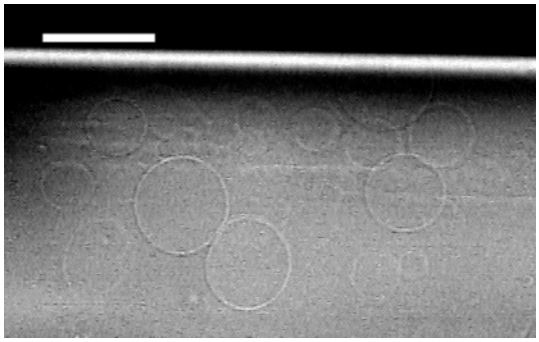

Figure 3. Electroformation on a glass tube substrate. Giant vesicles were formed from an eggPC deposit at 120 min after the application of AC voltage (5 V, 2 Hz) on the outer surface of the bottom of the tube. The shadow of the tube wall is seen at the top. The bar indicates 50 μm.

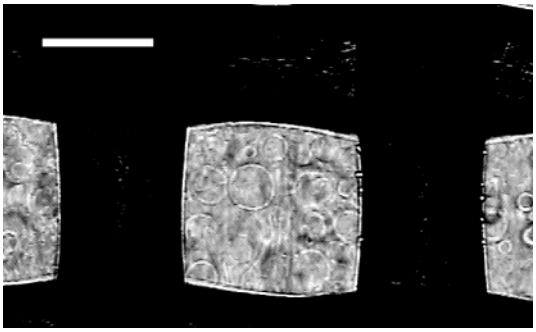

Figure 4. Electroformation on PET mesh. Giant vesicles were formed from an eggPC deposit on poly(ethylene terephthalate) mesh after application of AC voltage (60 min, 5 V, 2 Hz). The threads appear as the dark shadow. The bar indicates 50 μm.

by the glass substrate. Also, the adherence of the lipid layer onto the glass seemed lower than that onto Pt, possibly due to the electronegative charge on the glass surface.

Our third experiment showed that the substrate does not have to be strongly hydrophilic and further that the electroformation on a non-electroconductive substrate occurs in a chamber with ITO coated glass electrodes. EggPC (1.0 μL, 10.0 mg/mL) was deposited in a 6 mm square on a piece of poly(ethylene terephthalate) mesh (PET mesh, 0.05 mm thread diameter and 0.08 mm opening width) and placed between two parallel ITO coated glass electrodes (0.46 mm apart from both electrode surfaces). Upon application of AC voltage, GVs were formed after 60 min (Figure 4) similar to the electroformation on an ITO glass electrode.[4b] The GV formation occurred on the part of the lipid layers that were over the opening of the mesh. In fact, such opening was essential for the successful formation. When a piece of borosilicate glass plate or PET sheet was used in place of the PET mesh, the lipid layer only moved weakly and sporadically at the perimeter of the spread lipid deposit by the AC voltage, and no appreciable GV formation was observed. Apparently, these materials prevent the swinging of the lipid layers.

With the PET mesh, the electroformation was also observed using static DC voltage (3 V). The formation was similar to that with the AC voltage, although no periodic vibration of the swelled lipid layer was visible.

The present results provide some important insights into the mechanism of the electroformation. Our study revealed that a phenomenon that requires proximity to the electrode surface could contribute little to the electroformation. The GV formation from the lipid layers over the opening of the mesh suggests that the contact with a substrate surface might not even be essential since the layers could be fixed in water. On the other hand, it is crucial for both sides of the layers to be either in contact with or at least in close proximity to the bulk water; otherwise, no vibration of the layer under AC voltage would occur. The separation of a membrane layer is essential for GV formation. For that, the constraint of movement of the membrane layers by the adhesion to a substrate and appropriate attenuation of the movement possibly by the interaction with the neighboring layers and by the applied electric field could be crucial. The presence of the optimal lipid layer thickness[6] and the vertical heterogeneity in the lipid layers at the end of the GV formation (Figure 2)[4b] are consistent with this view. With the GV formation on the substrates so similar to that on the electrode, the two should share the fundamental part of the mechanism. For the latter, a theoretical examination concluded electrostatic and osmotic ones as the basic driving forces for the membrane separation,[4a,d] which is considered to be enhanced by the electro-osmotic flow previously reported.[4e]

The present study liberates the electroformation from one of the restrictions in the conventional protocol, which could limit the design of the formation cell and confine the substrate material to an electroconductive one. The findings should also help further examination and development of the electroformation by allowing various unconventional setups and substrate surfaces, including those with modified chemical characteristics and/or with micro- or nanostructures.

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Supporting Information Available: Pictures of the lipid deposits on the Pt wire and the glass tube without the applied AC voltage, the scheme for the formation cell with ITO glass electrodes, and the picture of the GV formation with static DC voltage. This material is available free of charge via the Internet at http://pubs.acs.org.

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