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Electroformation of Giant Vesicles and Electrode Polarity

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Electroformation of giant vesicles (GVs) on the negative electrode of dc (static and pulsed) was identical to ac. The negative phase of ac mainly drives the formation. The mechanical oscillation of lipid had little effects. At higher frequency, electroformation with ac deteriorated, while dc pulses still yielded GVs.

Assisted by applied electric voltage, a lipid deposit on an electrode swells in water to form cell-sized vesicles (giant liposomes or GVs).[1,2] The procedure, now commonly known as electroformation or electroswelling,[2,3] efficiently yields GVs of good quality from wide varieties of lipids[4] and is one of the standard techniques of fast and well-controlled formation of GVs, which are used as a model membrane in biophysical/biochemical studies and in construction of nano/micro-chemical systems.[2] However, the details of the mechanism still need clarification. Several possibilities have been suggested,[3,5] and electroosmotic flow has been proposed as the major driving force of GV formation from the early years of study.[3,6]

Typical electroformation uses sinusoidal ac of low voltage and frequency (typically, 1–10 V, 0.5–10 Hz).[3,4,7–9] Nominally zwitterionic phosphatidylcholine or a negatively charged lipid mixture moves away from the electrode of negative polarity, and with ac voltage, this causes periodic movement or oscillation of the lipid layer.[7] The mechanical oscillation is so prominent that its possible contribution to GV formation by providing mechanical agitation has been suggested.[3,7] However, the actual effect of the oscillation or the alternation of the applied electric field on electroformation has not been studied explicitly.

Electroformation using static dc voltage has appeared only in a few earlier studies of lipid deposits on wire electrodes.[5,6,8] Although no longer used practically, it should provide a valuable insight into the possible effect of the oscillation. In the present study, we examined the lipid swelling on an ITO-coated glass electrode applying dc pulses and static dc voltage to investigate their effects on GV formation.

As the reference, we first examined electroformation with sinusoidal ac voltage on a planar ITO-coated glass electrode.[7] GV formations at various voltages (1.0–7.0 V_{pp}) and frequencies (0.1–200 Hz) were tested (data not shown), and the conditions used (3.0 V_{pp}, 2.0 Hz) were found optimal in the present study. During the formation, oscillation of the lipid layer synchronizing with the polarity change of the ac voltage was seen.[7] After 90–120 min, typical GV formation was observed as was in other studies (Figure 1).[3,7] The relatively large GV (20–50 μm in diameter) formed on the top of the layer at approximately 70 μm apart from the electrode surface (Figure 1, left). GV formation was evaluated semiquantitatively by determining the percentage of the lipid deposit surface occupied by GVs larger than 10 μm. In the present case, 80% of the top area was covered. Under the large GVs, smaller GVs were seen at 30 μm apart from the electrode. An array of tightly packed small vesicular objects was present at the location closest to the electrode surface (Figure 1, right). A similar stratum of vesicles was also previously reported by others and is characteristic of standard electroformation on a planar ITO glass electrode with ac voltage.[7]

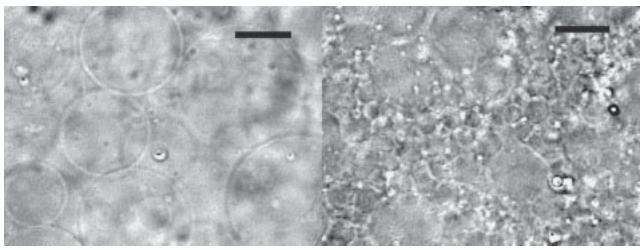

Figure 1. Electroformation of GVs on a planar ITO-coated glass electrode with sinusoidal ac voltage (3.0 V_{pp}, 2 Hz) applied for 100 min. Images are lipid layers approximately at 70 μm apart from (left) and close to (right) the electrode. Bar = 20 μm.

When dc pulses (3.0 V, 2 pulses s^{-1}, 50% duty) were used in place of sinusoidal ac, the swelling lipid layer on the electrodes moved in synchronization with the switching of the voltage. On the negative electrode, the lipid layer moved away from the electrode during the on period as it did in the negative phase of ac voltage. And during the off period, the lipid almost restored its position, indicating that the lipid was under tension during the presence of the voltage. This resulted in periodic vibration or swing of the swelled lipid layer. The movement apparently resembled that with sinusoidal ac voltage but was slightly less smooth, probably due to the abrupt change of the voltage and/or possibly uneven speeds of the driving and the relaxation. GV formation proceeded in a manner similar to ac voltage (Figure 2). After 100 min, GVs were seen at the top of the lipid layer 30–50 μm thick (Figure 2A). Approximately 80% of the lipid layer was covered by GVs. The stratum, the larger GVs at the top and the closely packed vesicular objects attached on the electrode (Figure 2B), was also observed. The conditions (3.0 V, 2 pulses s^{-1}) resulted in the optimum GV formation among various dc pulses examined (2.0–6.0 V, 1–200 pulses s^{-1}; data not shown).

On the positive electrode, the periodic movement of the lipid layer was also seen but the direction of the movement was opposite; the layer was attracted to the electrode during the on period and was relaxed in the off time. The thickness of the layer remained at approximately 10–20 μm, thinner than that on the negative electrode, probably because the layer was pressed against the electrode surface. Large spherical GVs formed only sparsely; less than 1% of the top area of the lipid layer was covered with GVs. Instead, most of the lipid layer became packed membranous objects (Figure 2C).

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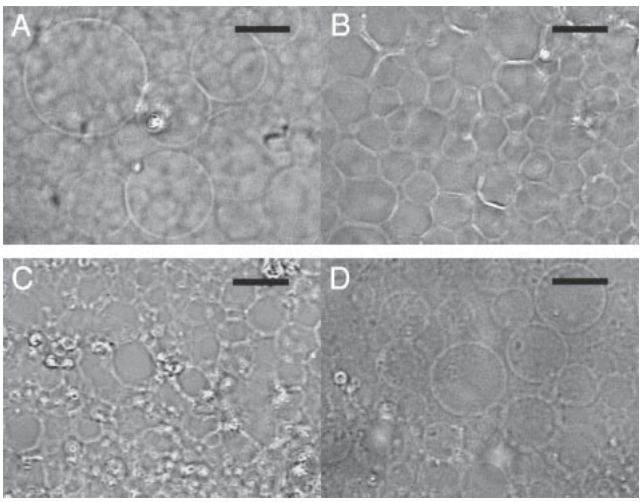

Figure 2. Electroformation with dc pulses (3.0 V, 2 pulses s^{-1}, 50% duty). Images are lipid layers at 50 μm apart from (A) and close to (B) the negative electrode, on the positive one (C) after 100 min, and at 100 min after switching the polarity from positive to negative (D). Bar = 20 μm.

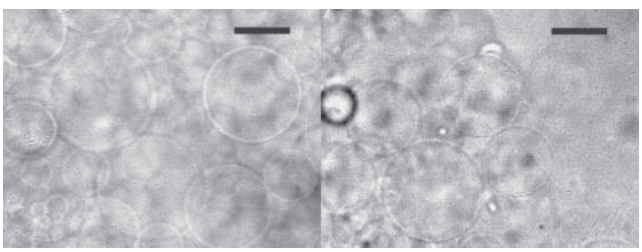

Figure 3. Electroformation with various electric voltage forms. Images are GVs on the negative electrode after static dc voltage (3.0 V) was applied for 95 min (left) and on the electrode after square-wave ac voltage (3.0 V_{pp}, 2 Hz) was applied for 95 min (right). Bar = 20 μm.

Switching the electrode polarity at this point (120 min after the primary application) turned the membranous objects on the previously positive (presently negative) electrode into GVs. The lipid layer started to increase in thickness to 40–60 μm after 30 min. And after 90 min, GVs were seen on the top of the lipid layer at 50–70 μm (Figure 2D). However, the GV formation was less extensive than the primary formation on the negative electrode (Figure 2A). The number of the formed GVs was less than half, and the typical diameter was also slightly smaller (20–30 μm) than the latter (20–40 μm). This suggests that a part of the swelled lipid layer should have become unsuitable for GV formation during the primary prolonged application of the positive voltage.

With static dc voltage (3.0 V), no visible vibration of lipid layer was present but GV formation occurred on the negative electrode (Figure 3, left). The GVs were indistinguishable from those observed in the electroformation using ac or dc pulses. Approximately 80% of the lipid area was covered by GVs. The stratum of vesicles was also present, the larger GVs (20–50 μm) at the top and the smaller ones (20–30 μm) below. As was with the pulses, GV formed poorly on the positive electrode, covering only 16% of the lipid area.

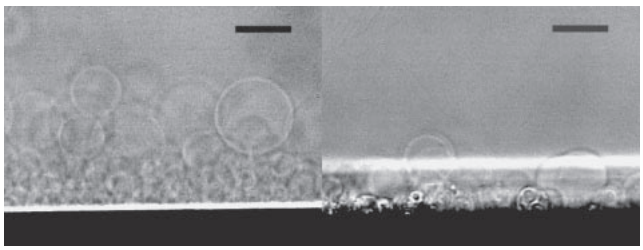

Figure 4. Electroformation on Pt wire electrodes with dc pulses (3.0 V, 2 pulses s^{-1}, 50% duty) at 105 min. Images are lipid layers on the negative (left) and positive (right) electrodes. The electrodes appear as black shadows at the bottom. The bright white lines were artifacts produced by reflection from the electrode surface. Bar = 20 μm.

One notable observation with dc voltage is rapid movement of the top part of the deposited lipid layer to approximately 300 μm apart from the negative electrode surface at the moment of the voltage application. Similar rapid movement of swelling lipid was also reported in an experiment using a thin wire electrode and attributed to electroosmotic flow.[8] Later, the moved lipid formed irregular aggregates and gradually returned toward the negative electrode and covered the top of the formed GVs. This was observable only once immediately after the voltage application, and frequently occurred with static dc voltage and also occasionally with dc pulses but rarely with sinusoidal ac voltage. The prevention of the contamination with the irregular aggregates is one advantage of using the ac voltage.

Using square-wave ac (3.0 V, 2 Hz) in place of sine-wave ac resulted in similar GV formation (Figure 3, right). Approximately 60% of the lipid layer was covered with GVs (20–30 μm). The edges of the pulses had no appreciable effect on the formation.

In addition, electroformation with dc pulses was examined on wire electrodes. GV formation on the negative electrode (Figure 4, left) was the same as that with ac or static dc (data not shown).[4,8,10] On the positive electrode, irregular membranous objects were mainly formed (Figure 4, right). The result is consistent with that for planar electrodes. Also, in our previous study of electroformation on a nonelectroconductive poly(ethylene terephthalate) mesh, GV formation with static dc voltage proceeded as with sinusoidal ac.[10]

Although there is subtle difference in the size and yield of GVs, the electroformation with the three different types of electric voltage, ac, pulsed and static dc, was essentially identical, and the three cases should have the fundamental part of the mechanism in common. As demonstrated by dc pulses, the lipid layer is under tension when electric voltage is applied. And, on the negative electrode, this inflation of the lipid layer should be the main driving force of electroformation. The effect may be electrokinetic and affected by the membrane charge under the conditions as it was dependent on the polarity of applied voltage. On the other hand, there is little evidence that the mechanical oscillation of lipid layer, however prominent, could provide significant promotion to electroformation. The poor GV growth on the positive electrode indicates that for sinusoidal ac voltage, the negative part of the electric wave should mainly be effective for driving electroformation.

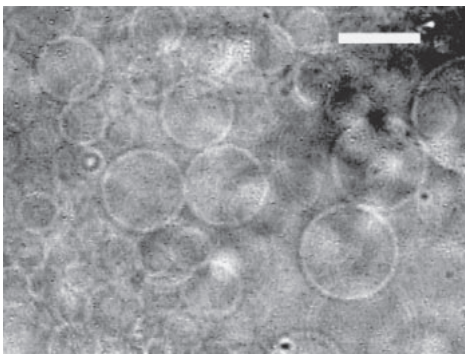

Figure 5. Electroformation with fast dc pulses (6.0 V, 200 pulses s^{-1}, 50% duty) on the negative electrode at 110 min. Bar = 20 μm.

Generation of peroxidized lipids during electroformation on an ITO electrode with ac voltage was previously reported.[11] The present study showed sufficient GV formation on a cathode with dc, and this could be an option effective against the undesirable anodic oxidation of unsaturated lipids.

At higher frequency, the difference between ac and dc pulses appeared. Electroformation does show dependence on the frequency of applied ac voltage.[8,9] Optimum frequency seems to vary depending on circumstances but usually is lower than 10 Hz,[9] although GV formation from eggPC at 10 kHz has been reported.[12] Also in the present study, GV formation with ac voltage deteriorated at higher frequency (3.0 or 6.0 V_{pp}, 200 Hz; data not shown). The details of this frequency dependence have been one of the unsolved enigmas in electroformation.

Unlike ac voltage, fast dc pulses (6.0 V, 200 pulses s^{-1}, 50% duty) resulted in GV formation (20–40 μm, approximately 60% of the lipid area covered) without visible periodic movement of the lipid layer (Figure 5). It was similar to the formation under static dc voltage. The deterioration of electroformation at high frequency is not likely to be caused by possible sub-microscopic mechanical agitation of lipid layer because the pulse should have provided comparable vibration. This indicates that the electrokinetic phenomenon driving electroformation could not respond instantaneously to the change of the electric polarity. To be effective, the polarization induced by applied voltage should be maintained for a certain period of time. Due to the slow response, the effect seems to become "averaged" over a time around the frequency region of 200 cycles s^{-1}. The unsuccessful electroformation with high frequency ac voltage may be explained by attenuation of the electrokinetic effect by the alternation.

In conclusion, the present study demonstrated that the mechanical movement of the swelled lipid layer under ac voltage, which is a prominent feature observed in the standard protocol of electroformation, should have little promotional effect on the growth of GVs. Lipid swelling under negative voltage mainly drives electroformation, and the positive part of ac could contribute to the swelling only marginally at low frequency. At higher frequency, the positive part could interfere the electroformation by possibly attenuating the polarization that is essential for driving the formation.

Experimental

General. Phosphatidylcholine extracted and purified from egg yolk (eggPC) was obtained from Avanti Polar Lipids (Alabaster, AL, U.S.A.). ITO-coated glass was purchased from Yamakyu Special Glass Co., Ltd. (Tachikawa, Tokyo) or Sigma-Aldrich (St. Louis, MI, U.S.A.). Lipid swelling was observed with an inverted optical microscope (Olympus IX-50, Tokyo) equipped with contrast enhancement options.

Electroformation of GVs. As a formation chamber, two ITO-coated glass electrodes were stacked separated with a spacer (1.0 mm). Typically, eggPC/methanol (2.0 μL, 5.0 mg mL^{-1}) was spread as a patch of 6 mm × 6 mm on the electrode surface. After drying, the chamber was filled with Milli-Q grade ultrapure water, and appropriate electric voltage was applied.

For evaluation of GV formation, the whole area of the deposited lipid was divided into 200 sectors of 200 μm^2. For each sector, the percentage of the area occupied by GVs was determined, and an average was taken for all the sectors. Only spherical vesicles larger than 10 μm were treated as GVs. When GVs was so small that the larger magnification was necessary, smaller sectors were randomly taken (typically, 20–30 sectors of 50 μm^2) and used in the evaluation. The thickness of the swelling lipid layer was estimated from the focal distance in the observation with a microscope (Olympus IX-50, Tokyo).

For electroformation on a wire electrode, a standard formation chamber was constructed with two parallel platinum wires (diameter 0.5 mm) separated 5 mm apart.[4] A methanolic solution of eggPC (2.0 μL, 5.0 mg lipid mL^{-1}) was deposited on the electrode. After drying, the chamber was filled with pure water, and appropriate electric voltage was applied.

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