Using the Eppendorf Multiporator® to master vesicle electrofusion

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Introduction

The fusion process of cell membranes is of significant importance, as it is involved in vital functions such as endo- and exocytosis, fertilization and signaling in nerve cells.

In real cells fusion involves the participation of a number of so-called fusogenic proteins, which are thought to bring two membranes together, disrupt the lipid bilayers and eventually assist in the lipid mixing.

Establishing experimental tools for the controlled fusion of membranes is essential for optimizing fusion applications, such as those performed in drug development, *in vitro* fertilization and gene transfer.

Achieving control over the fusion process has been the driving force for initiating experiments on model membranes.

One of the most convenient systems where fusion can be observed directly using optical microscopy is giant unilamellar vesicles (GUVs) with a cell-size diameter of ~10 µm.

Using electric fields (A/C fields followed by D/C pulses) to initiate fusion is a straightforward approach, where fusion can be induced in the absence of membrane proteins. The function of fusion proteins in bringing the membranes together is fulfilled by an A/C field, which approaches the vesicles and aligns them in a pearl chain (analogous to the effect observed with cells [1]).

The role of fusion proteins in assisting the fusion of the two bilayers is completed by strong D/C pulses, which perforate the vesicles in the contact zone and thus induce electrofusion. By using the Multiporator with carefully selected field characteristics, one can control and induce fusion at the desired moment.

Fusion is a surprisingly fast process. Thus far, direct optical microscopy observations have been limited by the frequency of video recording to timescales of about tens of ms (typical recording frequencies are on the order of 25 Hz, corresponding to an image acquisition performed every 40 ms).

For the first time we were able to observe the opening of the fusion pore with high temporal resolution using high-speed digital imaging [2].

With the Multiporator controlling fusion initiation we recorded the evolution of the fusing membranes with an unprecedented time resolution of approx. 50 µs.

Materials and methods

Giant vesicle preparation
GUVs were prepared according to the electroswelling protocol described in [3] (see also [4]), which resulted in vesicles of an average size of 10 µm and large polydispersity after about 4 h.

Electrofusion protocol
The vesicle solution was removed from the electroswelling chamber and diluted 40 times into a 0.2 M glucose solution.

The osmolarities of the sucrose and glucose solutions were measured with a cryoscopic osmometer, Osmomat 030 (Gonotec GmbH, Berlin, Germany), and carefully matched to avoid osmotic pressure effects.

The resulting solution was transferred into the Multiporator's 0.5 mm Micro fusion chamber and sealed with a glass cover slip using silicone grease.

Then the vesicles were subjected to a weak A/C field (about 3 V, 10 s).

This was repeated until a couple of vesicles were aligned in the direction of the field and in contact.

To induce fusion a strong D/C pulse (about 150 V, 150 µs) was applied.
The observation was done with phase-contrast microscopy.

Due to the sugar asymmetry across the membrane (the vesicles contained sucrose solution, and the vesicle exterior was glucose solution), the vesicles appear as dark objects on a brighter background.

The fusion dynamics were recorded with a fast digital camera HG-100 K (Redlake Inc., San Diego, CA, USA) that was mounted onto the microscope and connected to a PC.

Results

When exposed to a weak A/C field, vesicles aligned in the direction of the field. This can bring two vesicles into contact.

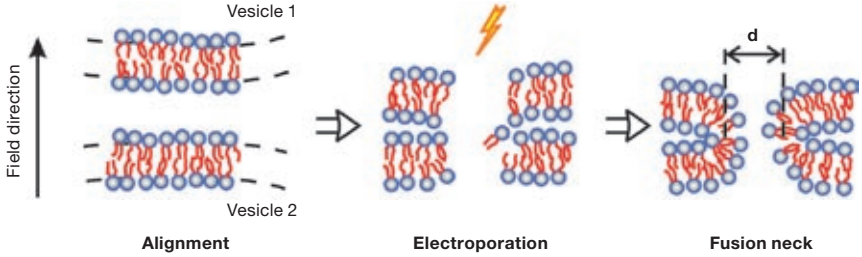

Fig. 1: Possible steps in electrofusion

(i) Two lipid vesicles are brought into contact via an A/C field (the field direction is indicated with an arrow); only the membrane area of the contact zone of the vesicles is sketched.
(ii) Perforation of the two membranes by a short electric pulse
(iii) The lipids from the opposing bilayers mix, initiating the opening of the fusion neck of diameter "d".

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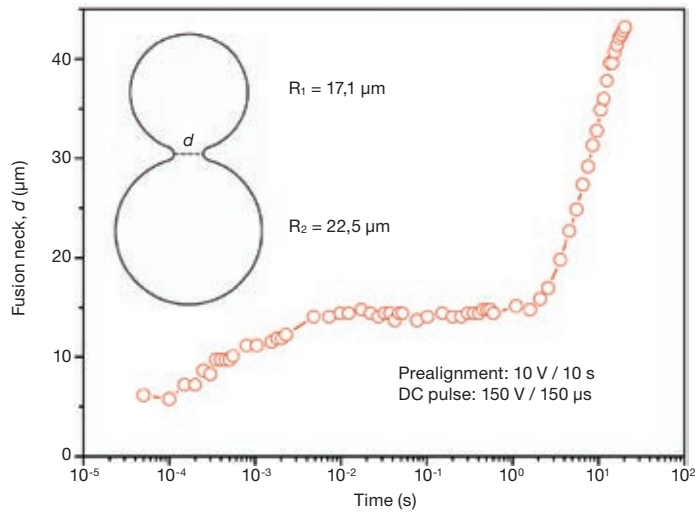

Fig. 2: Time evolution of the opening of the fusion neck "d" as sketched in the inset and in Fig. 1 (last diagram).

The two vesicles were aligned via an A/C field (10 V, 10 s) that was applied prior to the D/C pulse (150 V, 150 µs).

The vesicle radii are indicated in the inset.

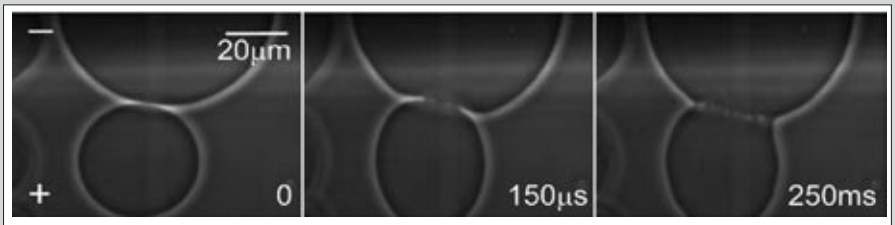

Fig. 3: Snapshot sequences of electrofusion of a vesicle couple. The time after the pulse start is indicated on each snapshot. First, the two vesicles were aligned via an applied A/C field (first snapshot). A D/C pulse (90 V, 150 µs) was then applied, which leads to fusion of the vesicles at several locations (second and third snapshots). The electrode's polarity is indicated with a plus (+) and a minus (–) sign.

Subsequent D/C pulses created a transmembrane potential, which was enhanced at the vesicle poles (facing the electrodes). The corresponding compression of the membrane effectively induced tension. D/C pulses can lead to perforation of the membrane in two cases [3]: (i) when the transmembrane potential exceeds a critical value of about ~ 1 V; or (ii) when the total membrane tension approaches the lysis tension of the membrane (~ 5 dyn/cm). When poration is induced in the contact area of two vesicles, fusion is expected to occur. Fig. 1 illustrates a possible mechanism.

Using intensity profile image analysis, we are able to follow the evolution of the opening of the fusion neck diameter "d" (see last picture in Fig. 1). One example, as observed with a fast digital camera and phase-contrast microscopy, is presented in Fig. 2. Time t = 0 corresponds to the beginning of the D/C pulse, which was applied after aligning two vesicles via an A/C field. Close inspection shows that fusion occurred within the first 100 µs. Two characteristic times are revealed, presumably corresponding to two different processes: molecular rearrangement of the lipid bilayers, related to the relaxation of the edge curvature of the fusion pore (~ 1 ms); and the hydrodynamics that occur when the fluids of the fusing vesicles mix (~ 1 s).

In certain cases, multiple fusion events were observed. The reason for such behavior is that the fusing vesicles were porated at several places in the contact zone. This leads to reclosure of the membrane inside the product vesicle and to the formation of smaller, internalized vesicles (see the example in Fig. 3 at t = 250 ms, where the internal vesicles appear as brighter spots inside the fusion product).

Conclusions

We achieved controlled fusion through the electrofusion of giant unilamellar vesicles. The Eppendorf Multiporator and the tools available in our lab allowed us to reach unprecedented time resolution of the fusion process.

Literature

[1] Zimmermann, U. Electric field-mediated fusion and related electrical phenomena. *Biochim. Biophys. Acta.* 1982; 694: 227-277.

[2] Riske, K. A., Lipowsky, R., Dimova, R. High temporal resolution of electroporation, fusion and deformation of giant vesicles. "Squaring" the vesicles. *Biophys. J.* 2004; 86: 518a.

[3] Riske K. A., Dimova, R. Electro-deformation and poration of giant vesicles viewed with high temporal resolution. *Biophys. J.* 2005; 88: 1143-1155.

[4] Electrofusion Protocol – Lipid Vesicles (No. 4308 915.911); see www.eppendorf.com

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