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Short communication

Mechanoformation of neutral giant phospholipid vesicles in high ionic strength solution

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ABSTRACT

We present new and simple method for formation of giant unilamellar vesicles (GUVs) in high ionic strength solutions, such as phosphate buffered saline (PBS). Mechanoformation method is an alternative method to electroformation method. The advantage of the mechanoformation procedure is that there are no limitations with respect to the ionic strength of the aqueous solutions, because there is no applied electric potential thus no current flow through the formation cell and no electrolysis is induced.

1. Introduction

Nowadays there are many different methods for formation of GUVs, as described in recent review paper (Walde et al., 2010). The method of the electroformation is well known and the preferred technique for the formation of giant unilamellar vesicles (Angelova and Dimitrov, 1986; Dimitrov and Angelova, 1988). The main drawback of electroformation method is the inability to form GUVs in high ionic strength solutions (PBS, saline). These physiologically relevant solutions are used to achieve stable and well-controlled conditions. Such conditions are of great interest in research fields, like medicine, physical chemistry and biophysics.

Some different techniques have been developed for high ionic strength solutions, such as sonication or extrusion techniques (Lapinski et al., 2007). Though some attempts to form GUVs in high ionic strength solutions has been performed (Pott et al., 2008; Pavlič et al., 2010; Montes et al., 2007; Estes and Mayer, 2005; Akashi et al., 1996), still there is no easy and suitable method that does not depend on the ionic strength of the aqueous solution.

In this work we propose a new formation method that is independent of the aqueous solution used, during GUV formation and does not induce chemical changes to the vesicle's membrane environment due to hydrolysis caused by applied voltage.

2. Materials and methods

For vesicle preparation we used 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) lipid (Avanti Polar Lipids, Alabaster, Alabama, USA). The lipid was dissolved in chloroform in concentration of 1 mg/ml. SOPC lipid was applied in small drops over the thin coverglass (coverslip) and subsequently subjected to vacuum for 15–20 min to evaporate the organic solvent (chloroform). After the entire evaporation of the organic solvent, the cell was filled with phosphate buffer saline (PBS) solution with osmolarity of 0.28 osmol/l. The PBS tablet (Sigma–Aldrich Chemie GmbH, Buchs, Switzerland) was dissolved in double distilled water. The cell was assembled from thin coverglass, a rubber spacer taken from perfusion chamber (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) and an objective glass as seen on the left side in Fig. 2.

To assure that the used rubber spacers do not cause any artifacts, we measured the time dependence of the resistivity of double distilled water, in which the spacers were immersed: (i) a brand new rubber spacer (without both glass slides), and (ii) a thoroughly washed rubber spacer (washed in methanol and immersed in double distilled water for 72 h). The results, obtained (cf. Fig. 1) were made using the experimental set-up explained in details in (Popkirov and Schindler, 1992; Vitkova et al., 2010). The value of

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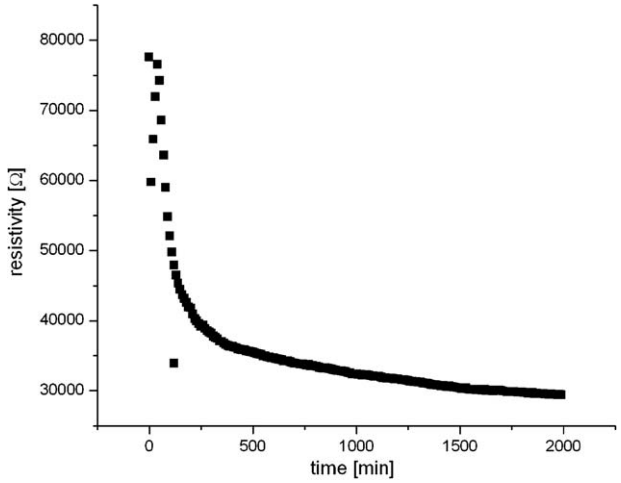

Fig. 1. Resistivity vs. time for non-washed rubber spacer. The aqueous solution was CO_2 saturated double distilled water.

the resistivity at frequency of 219 Hz was used. The time interval between two subsequent measurements was 10 min.

As can be seen in Fig. 1, the resistivity of the solution fell drastically. The possible explanation for the observed resistivity drop could be the presence of (conductive/ionic) impurities on the rubber spacer, which could be soluble in water.

However, for thoroughly washed spacers the measured resistivity of the aqueous solution was much higher, i.e. in the range between 50 and 80 kΩ, and it was not changing (falling) with time. In conclusion, for precise experiments investigating the properties of lipid membranes and in order not to alter the system characteristics, it is necessary to use thoroughly washed spacers only.

The mechanoformation method is very simple and inexpensive method. At first, we used a regular door bell (buzzer) as a source for mechanical agitation of the thin coverglass on which the lipid was applied (see the left side of Fig. 2). The buzzer was connected to a function generator (HP 33120A, Hewlett-Packard, Palo Alto, California, USA). The cell was laid on the buzzer's hammer, which induced mechanical perturbations while kicking the thin coverglass. For the case of non-amplified mechanoformation (see Fig. 3), we applied the sine voltage signal at the resonant frequency of the system (see the right side of Fig. 2): buzzer – formation cell – weight (between 680 and 685 Hz). The purpose of the weight was to fix the formation cell in place on the buzzer. The generator's effective output sine voltage was set to 4 V with an offset voltage of 4.3 V. With this offset voltage we set the optimal distance between the kicking hammer and the coverslip.

As it can be seen on Fig. 3 the perturbations of the thin coverslip surface were not high enough to produce appropriately sized vesicles for further experiments.

To induce more intensive kicking of the coverslip, we connected an audio amplifier (K4001 from Velleman, Gavere, Belgium) with a higher current throughput (up to 0.5 A) to the buzzer (see Fig. 5). Amplifier's sine voltage was set to a frequency of around 110 Hz. The duration of the mechanical agitation was from 180 to 240 min for both cases, which is the same time duration as in the case of the electroformation method.

In this case, we managed to mechanoform larger and nicer vesicles (predominantly unilamellar), since more intense perturbation of the lipid deposits was induced (see Fig. 5).

We also tried to mechanoform vesicles at frequencies below or above the resonant frequency, for the case when only function generator was connected to the buzzer; we did not observe any vesicles to form. For the case when the buzzer was connected to the amplifier, we had to be careful not to induce too vigorous kicking of the coverslip because the lipid deposits were washed away from the glass and no lipid vesicles were formed. The signal's frequency was selected so that the buzzer's hammer kicking was out of resonance not to induce so vigorous kicking. We chose approximately two-times the resonant frequency (f_0) of the system (see the right side of Fig. 2): buzzer – formation cell – weight ($\approx 2 \cdot f_0$), since at resonant frequency (between 50 and 60 Hz) the kicking was too intense, even at the minimal amplified sine voltage.

3. Results and discussion

A mechanoformation method for preparation of giant unilamellar vesicles in high ionic strength solutions was developed. The parameters of the mechanoformation could be adjusted as to obtain high yield of large enough vesicles without visible defects.

The process of formation with time of SOPC vesicles in 0.28 osmol/l PBS solution using the mechanoformation technique described in details in the previous section without using an amplifier is shown in Fig. 3. As it can be seen from Fig. 3 some vesicles are formed already at 120 min. The best population of vesicles is seen at 240 min of mechanoformation, after that the situation does not improve in quality, quantity or size of the vesicles. In the negative control experiment (hydration of SOPC deposits in PBS solution) no vesicles were formed, as can be seen in Fig. 4.

To achieve more dense population of larger vesicles we connected the mechanoformation device (buzzer) to an audio amplifier. In such a way the agitation of deposited lipid on coverglass of the formation cell was increased. Some micrographs of vesicles formed in such a way are given in Fig. 5. As it can be seen from the micrographs there is a significant difference in size (much

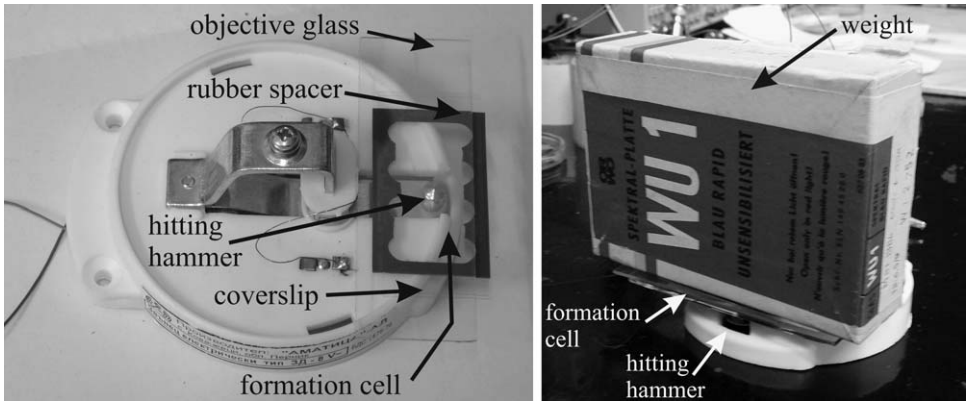

Fig. 2. Left side shows buzzer and formation cell; the weight is omitted due to clarity. Right side shows the system; i.e. buzzer – formation cell – weight.

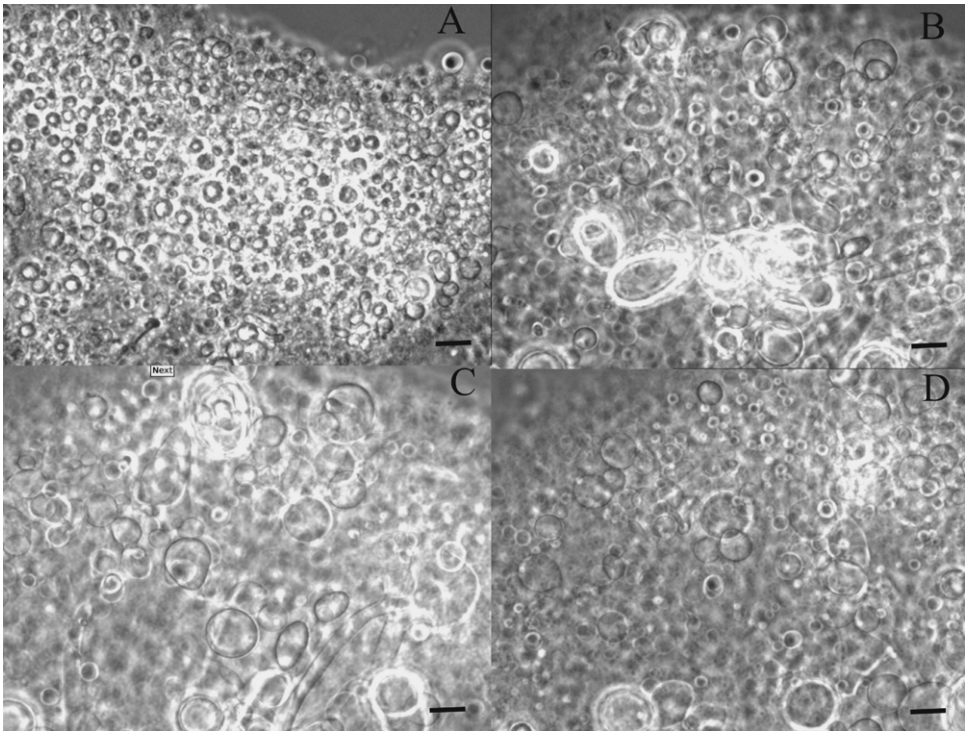

Fig. 3. Mechanoformation process of forming SOPC vesicles in PBS at 0 min (A), 120 min (B), 240 min (C) and 390 min (D). The buzzer was connected to function generator, only. All figures show the same spot of deposited lipid. Bar is 10 μm, optical magnification is 63×.

larger) and quality (unilamellar) of the mechanoformed vesicles using an amplifier. But we note that the agitation of the lipid deposits should not be increased over some critical level, especially one has to avoid the buzzer's electromagnet resonant frequency, where the agitation is so vigorous, that all the lipid deposits are washed away.

Perfusion chambers are very suitable spacers for different types of experimental cells and are used by different research groups all

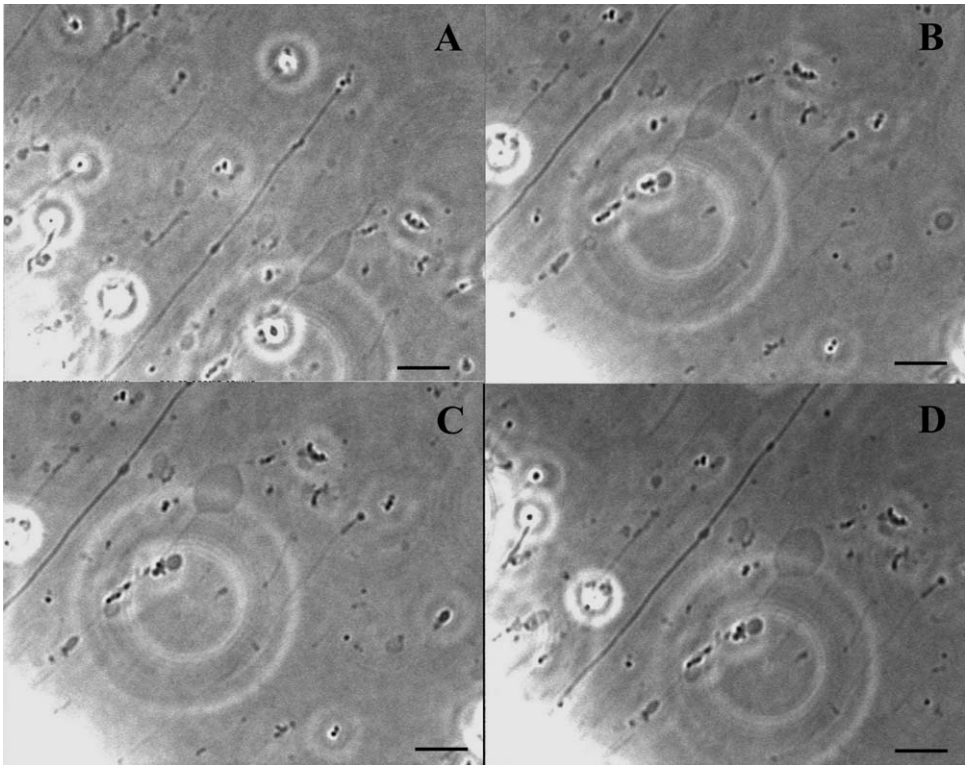

Fig. 4. Negative control experiment at 0 min (A), 120 min (B), 240 min (C) and 390 min (D). The buzzer was not connected to function generator. All figures show the same spot of deposited lipid. Bar is 10 μm, optical magnification is 63×.

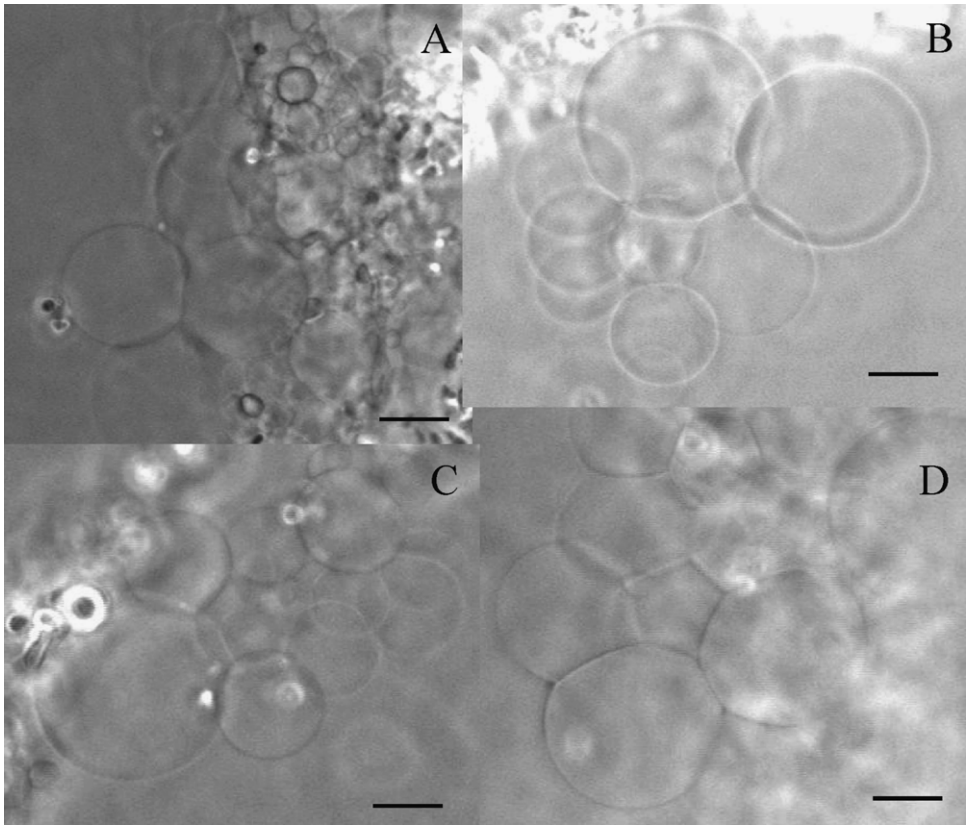

Fig. 5. SOPC vesicles formed in PBS. The buzzer was connected to an audio amplifier to increase the agitation of deposited lipid on coverslip. Letters denote different spots of lipid deposits. Bar is 10 μm, optical magnification is 63×.

over the world. We have experimentally proved (see Section 2), that for precise experiments the spacers only need to be thoroughly washed, first with methanol and then immersed for longer period of time in double distilled water to exclude any impurities in the sample.

Further, we stress that alternative set-ups using standard loudspeakers for the mechanoformation of vesicles failed in the first place. This fact gives us insight that in order to stimulate the GUV formation process, the mechanical agitation should be point-like and impulse perturbation. With the loudspeaker we induced the movement of the whole coverslip, and not just its flexing (swivelling).

All the obtained results in this work show that the mechanoformation method has a potential to become a profoundly used method for formation of GUVs in different environmental conditions. Still there are some possibilities to improve the mechanoformation set-up. An important step towards repetitive mehanoformation is to monitor the applied contact pressure to the formation (perfusion) cell, e.g. by using a pressure gauge. Another idea for future developments of the mechanoformation is with loudspeaker's membrane, which is connected to a point-like kicking head, similar in shape to the kicker (hammer) of the buzzer. This would give the possibility to form GUVs with yet another source of mechanical agitation.

4. Summary

We developed mechanoformation method for preparation of giant unilamellar vesicles. The time duration of the mechanoformation method has similar time scale as the electroformation method. The formation yield of large enough, unilamellar vesicles is comparable to that of the electroformation method. At the same time the mechanoformation method has one main advantage: the giant unilamellar vesicles can easily be formed in high ionic strength solutions.

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