ELECTROFORMATION OF GIANT LIPOSOMES FROM DENSELY MICRO-PATTERNED LIPID FILMS

Kaori Kuribayashi¹, Andrew Utada¹ and Shoji Takeuchi¹, ²
¹CIRMM-IIS, The University of Tokyo, Tokyo, Japan
²PRESTO, JST, JAPAN

ABSTRACT

We describe a method for controllably generating giant balloon-like liposomes through electroformation of densely micro-patterned lipid films. These balloon-like liposomes are still tethered to the substrate through a thin lipid tube. We found that the patterns that were more densely spaced generated the balloon-like liposomes, whereas patterns that were sparsely spaced generated hemi-spherical dome shaped liposomes. Utilizing this feature, we patterned lipid patches closely so that the growing hemi-spherical dome shaped liposomes begin to touch and then push each other; this causes their shape to change from domes to the balloon shapes, which are more easily detached from the substrate.

KEYWORDS: Liposome, Lipid films, Electroformation, Micro-patterning

INTRODUCTION

Giant liposomes are cell-sized lipid bilayer membranes, and are widely used as artificial cells or biological reactors [1]. Using the electroformation method [2, 3], we generate giant liposomes from dried lipid films by first rehydrating the lipid films and then by applying an external electric field (Fig. 1a, b). During electroformation, we observe that the lipid films form hemi-spherical dome shaped liposomes (phase 1, Fig. 1c). As the domes grow larger, they begin to push one another and this squeezing by the surrounding liposome-domes cause them to undergo a shape transition to a balloon-like shape. This balloon-like liposome is still attached to the substrate through a narrow lipid tube (phase 2). Eventually, the balloon-like liposome detaches and becomes a free-floating vesicle (phase 3).

Recently, it has been reported that doing electroformation on micro-patterned lipid films has been able to generate monodisperse giant liposomes [4, 5]. In this process, while individual giant liposomes were generated from each patterned lipid film, smaller liposomes were able to aggregate and form a cluster of liposomes.
patch, most were, however, the dome-shaped vesicles, which are difficult to detach from the substrate. Here, we develop an efficient way to prepare the balloon-like liposomes since these are more easily released from the substrate than are the hemi-spherical dome-shaped liposomes.

Our idea is that we pattern lipid films closely using a micro-patterned Parylene shadow mask (Fig. 2a-b). The electroformed vesicles generated from these patterns are highly crowded; this crowding leads to the shape transition from the dome shapes into the balloon-like liposomes (Fig. 2c-d).

MATERIALS AND METHODS

We deposited a 2 μm thick Parylene (poly-paraxylylene, Parylene C, Cookson Electronics Equipment, USA) film onto an indium tin oxide (ITO) glass slide. We patterned the Parylene film using standard photolithography and etched it with O₂ plasma. A phospholipid solution was then deposited onto the patterned Parylene film and the organic solvent was evaporated under N₂ gas. After that, we removed the Parylene film by peeling it off with tweezers, which leaves behind the patterned lipid films. We used egg yolk L-α-phosphatidyl choline (Sigma-Aldrich) at concentration of 0.8 mg/mL dissolved in methanol with fluorescent dye DiI (ex/em: 549/564 nm, Molecular Probes).

We formed the giant liposomes using electroformation in degassed, deionised water. A second ITO glass was used as a counter electrode; this electrode was placed 0.5 mm away from the patterned lipid films using a silicone rubber spacer. Applying a 10 Hz, 0.5-1.0 V pp AC signal between two ITO electrodes generated giant lipid liposomes from the patterned lipid films.

RESULTS AND DISCUSSION

We easily generated micro-patterned lipid films patches with different spacings. After applying an electric field, the patterned lipid films became swollen and eventually formed a dome shape (Fig. 3a, b). In the wider spacing patterns (Fig. 3b), the hemi-spherical dome shaped liposome grew without any restriction. In the narrower spaced patterns (Fig. 3a), on the other hand, the liposomes were tightly constrained due to the restriction caused by the spacing size.
Further applying the electric field to the hemi-spherical dome shape vesicles in the narrower spacings led to intra-vesicle crowding at $t = 20$ min (Fig. 4-i). The center liposome is displaced due to the neighbouring liposomes at $t = 90$ min as shown in Fig. 4-ii and Fig. 5. Since the focal point of the center liposome B (Fig. 5a-i) is at a higher position than that of the liposomes A and C (Fig. 5a-ii), we see direct evidence that liposome B was squeezed upwards and away from the substrate, becoming a balloon-like liposome. The diameter of the liposome A (Fig. 5b) was restricted in the spacing size, while the diameter of the liposome B increased without restriction.

**CONCLUSIONS**

In this research, our results imply that the electroformation with densely micro-patterned lipid films is useful for efficient detachment and collection of the giant liposomes.

**REFERENCES**


