FORMATION OF CELL-SIZED VESICLES WITH ASYMMETRIC LIPID BILAYER USING PULSED JET FLOW

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ABSTRACT

This paper describes the preparation of asymmetric cell-sized lipid vesicles from a planar lipid bilayer by using pulsed jet flow. First, the cell-sized vesicles (diameter of 5 μm in over 1 day) were stably formed using an improved double-well device wherein a separator with a small hole was mounted between the wells (Fig.1). Moreover, asymmetric vesicles of phosphatidylserine lipids were successfully formed on each leaflet using this device. The asymmetric cell-sized lipid vesicles will create new opportunities for artificial cell research model such as the interactions between lipid membranes and proteins.

KEYWORDS

Liposomes, vesicles, jet flow, artificial cells

INTRODUCTION

Giant liposomes or giant vesicles are composed of phospholipid membranes similar to those commonly found in living cells. Giant liposome is typically ~10 μm in diameter, which is sufficiently large for observation using an optical microscopy [1]. Therefore, such an artificial liposomal system has played an important role in the biochemical and biophysical studies such as the interactions of lipid membranes with cytoskeletal proteins, and the microencapsulation of gene expression systems [2,3]. It has been considered that an assay based on a single giant liposome may help to clarify biochemical properties that can be missed in the analysis of an ensemble of many conventional small liposomes [4], and this is known as a single giant liposomes assay. Unsymmetrical membrane structures have also been visualized on giant liposome [5], which suggests that they may be useful as platforms for the reconstitution of intricate cell membrane functions. Recently, the giant vesicles have also been used in the study of membrane proteins function [6]. In the new membrane-protein-integrated giant vesicle method, membrane fusion between liposomes and glycoprotein 64 (gp64) displayed on recombinant budded viruses (BVs) of baculovirus (Autographa californica nuclear polyhedrosis viruses (AcNPV)) is analyzed under acidic conditions. The human nicotinic acetylcholine receptor a-subunit (AChRa) [7,8]; the connexin [9], which forms cellular gap junctions; the adrenergic receptor [10]; and cadherin [11] were successfully prepared by using this method. There are two classical giant liposome preparation methods: gentle hydration and electroformation. The liposomal morphology and size depend on the preparation method that is employed, chemical nature of the lipids, and other conditions (buffer, pH, ionic strength, and osmolality). Moreover, the preparation of asymmetric lipid vesicles and the encapsulation of large biological molecules into giant vesicles are difficult to perform using the current preparation methods.

To effectively form uniformly sized giant vesicles and encapsulate biological molecules into giant vesicles, Weitz and co-workers transferred a lipid-coated water-in-oil (w/o) droplet from an oil phase to an aqueous phase [12,13]. The cell-free protein synthesis of Green Fluorescent Protein (GFP) into giant vesicles was succeeded by this droplet transfer method. Microfluidics devices can also be used to prepare giant vesicles [14,15].

In our previous study, we created giant vesicles by the microfluidic-jetting-induced deformation of a planar lipid bilayer including an organic solvent [16]. However, this method has limitations; for instance, the giant vesicle is a W/O/W emulsion having a thin shell of an organic solvent. Further, it is unstable and very large (over 300 μm in diameter). In the present study, we improved double-well device wherein an acrylic separator with a small hole, was mounted between the wells. Cell-sized vesicles were stably formed using this improved double-well device. Moreover, we prepared asymmetric lipid vesicles to emulate asymmetric lipid leaflets of plasma membrane.

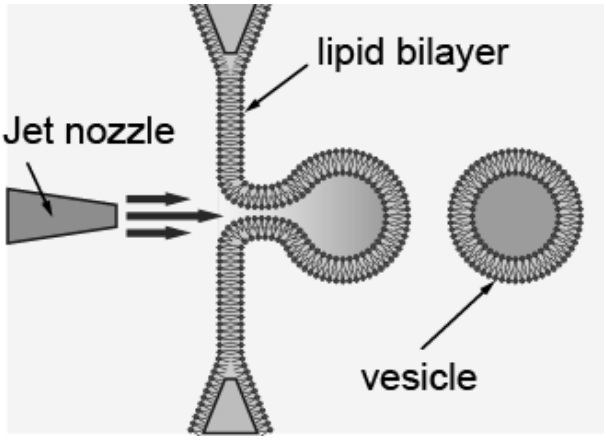

Fig.1 *Illustration of cell-sized vesicle formation from planar lipid bilayer using pulsed jet flow.*

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EXPERIMENT

The double-well chamber was fabricated by machining a poly methacrylate (PMMA) plate using an automated CAD/CAM modeling machine. Fig.2 shows the preparation process of cell-sized liposomes by pulsed jet flow. A planar lipid bilayer membrane was formed in the double-well chamber [17], wherein a thin acrylic film, having a small hole, was placed between the chamber wells. To form the planar lipid bilayer membrane, the lipid solution (1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and Rhodamine-conjugated DOPE (Rh-DOPE) dissolved in *n*-decane) was added to each well. Next, aqueous solution was added to each well and a bilayer was easily formed. Giant vesicles were formed by impinging the pulsed jet flow against the lipid bilayer. The micro jet nozzle was fabricated by pulling a 1mm glass capillary tube with a micropipette puller. The capillary tube was bent using a microforge to adjust its shape for obtaining an access point near the planar membrane. The pulsed jet flow was generated by opening an electromagnetic valve between a glass capillary nozzle and an air compressor (Fig.2). The giant vesicles were observed by confocal laser scanning microscopy (LSM).

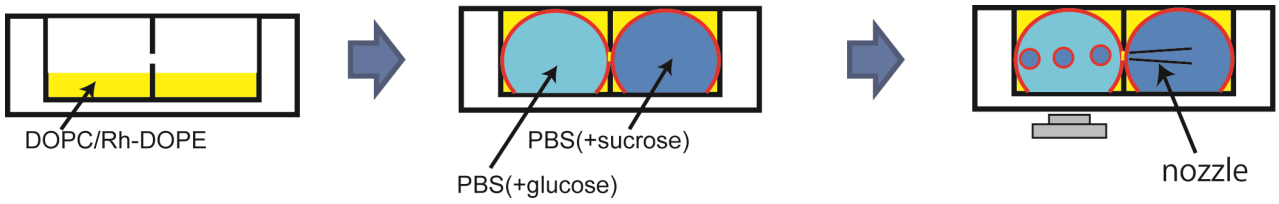

Fig. 2 *Preparation of cell-sized vesicles using pulsed jet flow. To form planar lipid bilayer membrane, lipid solution (DOPC and Rh-DOPE dissolved in n-decane) was added to each well. Next, sucrose in* Phosphate buffered saline *(PBS) buffer was added to the inner phase well, and glucose in PBS was added to the outer phase well. Vesicles were formed using pulsed jet flow for 4.0 ms (dispensing time) at 300 kPa (pressure at the dispenser).*

RESULTS AND DISCUSSION

When the jet was applied for 4 ms at 300 kPa, vesicle formation could be observed using a high-speed camera. Vesicles of two different sizes were formed by the jet flow. The vesicles with a diameter of over 100 µm immediately collapsed in the 500 mM glucose solution. However, the vesicles with the diameter of approximately 5 µm sank in the 500 mM glucose solution and their shape was maintained. The average diameter of the vesicles formed immediately and after 24 h was 4.4 µm (Fig.3a). Thus, stable cell-sized vesicles were produced. To form asymmetric vesicles of phosphatidylserine (PS) on the inner or the outer leaflet, we performed annexin V staining by performing LSM. The fluorescence of annexin V was detected on cell-sized vesicles containing PS on the outer leaflet (Fig.3b,c). Therefore we could form the symmetric cell-sized PS vesicles on each leaflet using this method.

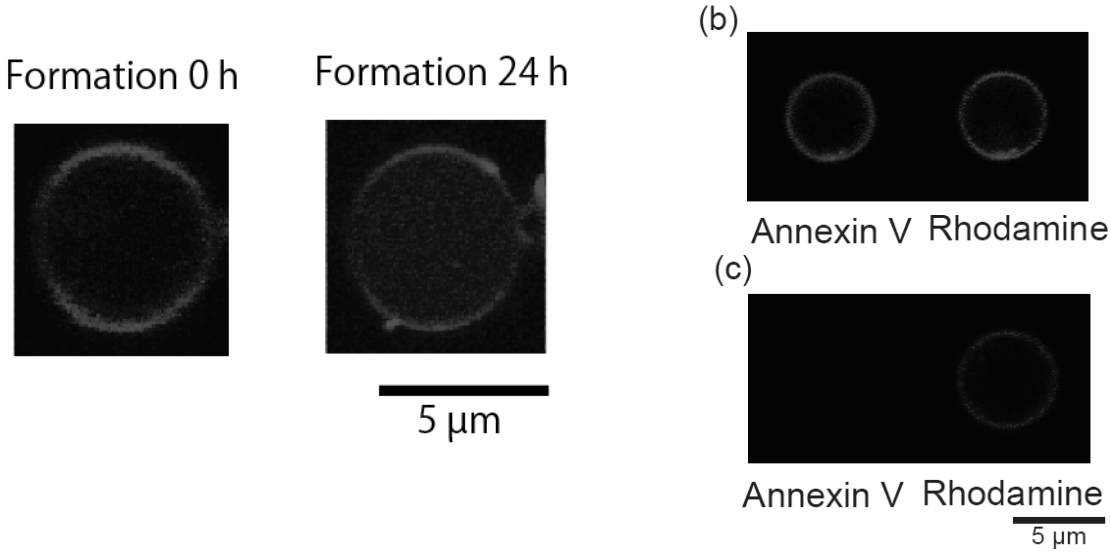

Fig. 3 *The fluorescence images of DOPC-vesicles formation after 0 h and 24 h (a). Preparation of asymmetric vesicles of phosphatidylserine (PS) lipids. The asymmetric droplet of PS lipids was arranged and the asymmetric vesicles were prepared using pulsed jet flow. Annexin V binds only to PS. The fluorescence of annexin V was detected on vesicles containing PS in the outer leaflet (b). But no fluorescence of annexin V was detected on vesicles containing PS in the inner leaflet (c).*

CONCLUSION

We produced a device that generates cell-sized giant vesicles in physiological buffer solutions. Furthermore, we demonstrated the formation of asymmetric cell-sized lipid vesicles using this method, and could successfully emulate the asymmetric lipid leaflets of plasma membrane. These asymmetric cell-sized vesicles that can be used as a research tool may create new opportunities for biochemical and biophysical studies.

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