THE RAPID SYNTHESIS OF CELL-SIZED LIPOSOMES BY CENTRIFUGE-BASED MICROFLUIDIC DEVICE

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ABSTRACT

We developed a simple production method of monodisperse cell-sized liposomes by centrifuge-based droplet shooting device (CDSD)[1]. This device does not require specialized equipment compared to other microfluidic techniques, and furthermore, this device needs a very small volume of encapsulated solutions (0.5-5 μL). We hope that this method will allow to make cell-sized liposomes for a given biological application.

KEYWORDS: Microfluidic device, Vesicle, Droplet-transfer

INTRODUCTION

Recently, cell-sized liposomes have widely been used to mimic the cell-membrane in the fields of biology, biochemistry, biophysics, and synthetic biology.[2] Many production methods of cell-sized liposomes, such as gentle hydration (or natural swelling) methods,[3, 4] droplet transfer methods,[5-8] pulsed jetting methods[9, 10] have been reported. To use liposome for biological application, the production methods are required to control unilamellarity and size monodispersity. However, gentle hydration method cannot be achieved to the above properties. Droplet transfer methods and pulsed jetting methods realize the membrane unilamellarity, but the size monodispersity cannot be achieved. Here we report a simple production method for forming monodisperse cell-sized unilamellar membrane liposomes by centrifuge-based droplet shooting device[1].

EXPERIMENTAL

Dioleoylphosphatidylcholine (DOPC) dissolved in chloroform/methanol (2:1, v/v) were poured into a glass test tube. The organic solvent was then evaporated under an air flow and dried under vacuum to make a dry film at the bottom of the test tube. The glass tube was placed in a desiccator for 120 min to remove the organic solvent. 400 μL of mineral oil was then added to the glass tube prior to ultrasonication for 60 min at 40°C and vortex mixing. The final lipid concentration was 1 mM. MilliQ water (50 μL) was introduced at a bottom of the microtube and covered with 125 μL of oil containing lipids. The microtube was then incubated for about 90 min at room temperature (24°C). We injected the 2 μL of MilliQ water into glass capillary and set the polyacetal holder. We installed the holder in the microtube and set the desktop small centrifuge (ATT101, HITECH Co., Ltd.) and then centrifuged the microtube for 5 min at 1380 rpm.

RESULTS AND DISCUSSION

First, we demonstrated the generation method for monodisperse cell-sized liposomes (Figure 1A, B). The method was accomplished using a capillary-based microfluidic device, called a centrifuge-based droplet-shooting device (CDSD)[1]. The device composed of a glass capillary, a capillary holder, and a microtube (Figure 1A). Precursor water microdroplets for generating liposomes continuously drip off from a tip of the capillary by a centrifugal force[1], and then the microdroplets simultaneously transfer from the oil phase to water phase through the oil-water interface (Figure 1B).

As shown in Figure 1C, we successfully obtained the monodisperse cell-sized liposomes (5-15 μm). In a single centrifuge-based shooting experiment, we succeeded in generating around 400 numbers of cell-sized liposomes from 2 μL sample solution. This suggests that a very small amount of volume (0.5-5 μL) was sufficient to generate a large number of liposomes.

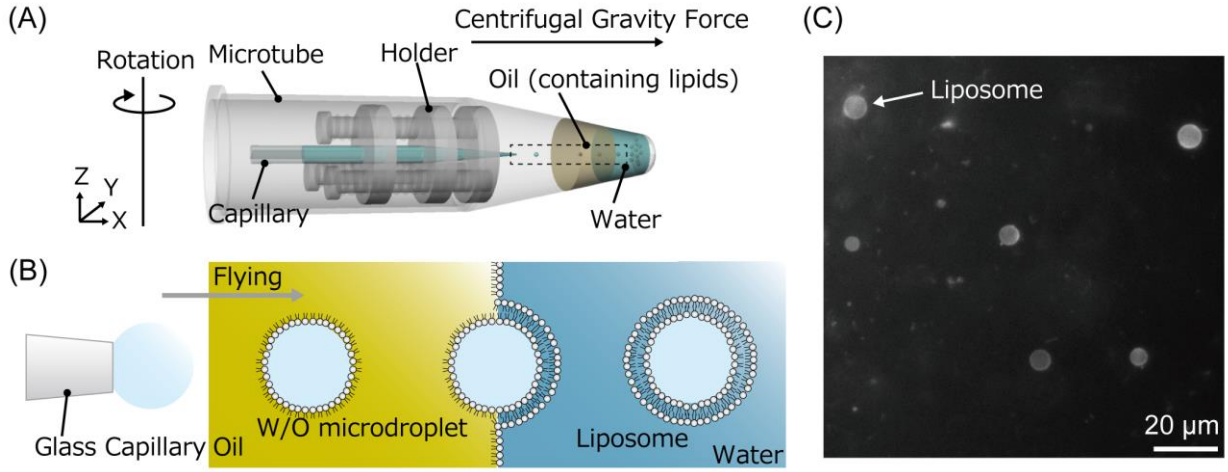

Figure 1: (A) Schematic depicting the experimental liposome synthesis setup. (B) The process of making cell-sized liposomes by centrifuge-based droplet shooting device (CDSD). Solutions pumped out from the capillary form pendant drops. Transfer process from W/O droplets into liposomes through an oil-water interface. (C) Fluorescence microscopic image of cell-sized liposomes.

CONCLUSION

We achieved an simple production method of monodisperse cell-sized liposomes by centrifuge-based droplet shooting device technique. We believe that this method is easier to implement for preparation of monodisperse cell-sized liposomes than other microfluidic methods.

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