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Preconcentration of lipid vesicles using concentration polarization in a microfluidic chip

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ABSTRACT

This paper presents the first lipid vesicle preconcentrator using concentration polarization in a microfluidic chip. Concentration polarization is a well-known electrokinetic phenomenon, occurring near a micro/nanochannel interface. The preconcentrator is composed of a straight microchannel and a nanoporous membrane (Nafion strip) in it, which serves as a preconcentrating zone. We visualized the concentrated lipid vesicles near the Nafion strip and characterized the concentrating performance for different applied voltages. The maximum performance is the concentration ratio of 160 with 100 V in 10 min. This performance is at least comparable with the previous work and we believe that it has the advantages of lab-on-a-chip system compatibility and a relatively easy fabrication. Our concentration polarization platform on a PDMS chip is versatile so that the same device can be applied to other biomolecule preconcentration.

1. Introduction

Lab on a chip technology has been increasingly applied for biomolecule separation, detection, and analysis in the past decades [1–4]. Detecting a low quantity of biomolecules from abundant samples is one issue in lab on a chip technology. To enhance the detection efficiency, a preconcentration technique is often performed in a preparation step before a main analysis. Rapid and precise preconcentration of biomolecules from samples is important in diverse applications including a drug delivery system, a DNA analysis device, and a cell diagnostic device. Recently, many papers have reported on enhancing preconcentration of biomolecules and reducing operation time in micro/nanofluidic devices. Such preconcentration methods include solid-phase extraction, temperature gradient focusing, and isotachophoresis (ITP) [5,6].

Preconcentraion in a platform with nanochannels has been also reported by several groups [5,7–12]. One method utilizing a so-called concentration polarization effect at a micro/nano channel interface has some advantages including easy applicability to micro/nano fluidic devices and rapid detection of concentration changes [13–15]. Concentration polarization occurs upon the application of electric fields across nanochannels or nanoporous membranes in microfluidic devices (see Fig. 1). Electric double layers (EDLs) overlap in nanochannels or nanoporous membranes and it enables ion perm-selectivity as selected ions are more efficiently transported through them. As a result of selective ion transport, an ion enrichment zone is formed on one side of a nanochannel/pore and an ion depletion zone is formed on the other side. Nafion strip selectively transports cations from the anodic side to the cathodic side. The flux of cations transported by the Nafion strip is much higher than the electroosmotic flow and electrophoresis. Moreover, anions are hardly to be transported by the Nafion strip but transported by electrophoresis and also affected by the electroosmotic flow. And for the electroneutrality, there is regional ion enrichment zone formed at the depletion zone boundary. This ion enrichment zone can be utilized for biomolecule preconcentration. The increased concentration level of biomolecules in this ion enrichment zone during microfluidic analysis has been measured in a high-precision microscopy [15–19].

Various biomolecules such as protein, DNA, blood cells have been tested in preconcentrator research [1,7,9,11,15,16,20–22]. Lipid vesicle is one important example of biomolecules to be preconcentrated. Lipids are the compounds having three major components: phospholipids, glycolipids, and cholesterol. They are the basic entities of a lipid bilayer, which forms most of cell membranes. A lipid bilayer membrane is formed as the hydrophilic phosphate head of a lipid molecule points toward ambient water and its hydrophobic tail points toward the interstitial space

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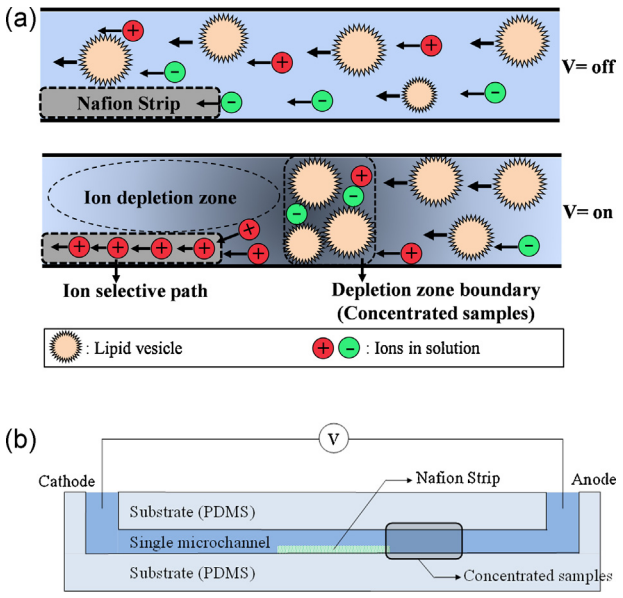

Fig. 1. (a) Mechanism of the lipid vesicle preconcentrator (side view) around a nanoporous membrane (Nafion strip in this study). (b) A plane section of device. We used a single-open-straight microchannel with a patterned Nafion film on the bottom. The region we focused on is the anodic side of Nafion strip.

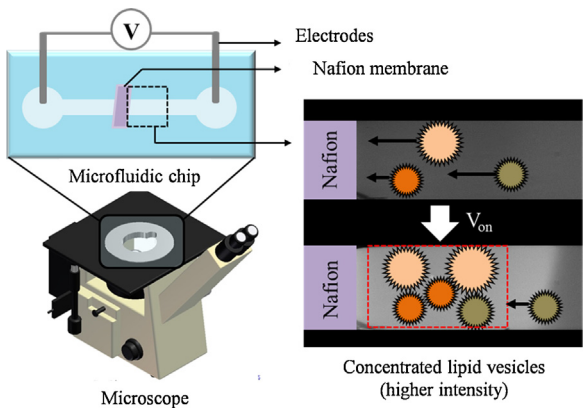

Fig. 2. Schematic of the lipid vesicle preconcentrator (top view) and the experimental setup. The red rectangle indicates the selected area for Figs. 5 and 6.

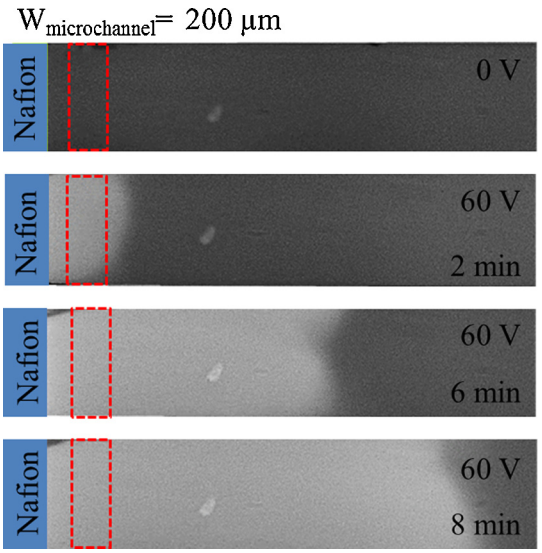

Fig. 3. Fluorescent images of lipid vesicle preconcentration. The applied voltage is 60 V. The red rectangle indicates the selected area for Figs. 5 and 6.

between the bilayers. Important characteristic functions of a lipid bilayer include storing energy and serving as a component of bio-mimicked nanoporous membrane [23–26]. Due to the interesting nature and potential applications, research on lipid bilayer membranes is now actively being conducted by many groups [23,25–29]. Several lipid vesicles preconcentration techniques have been reported accordingly [20,21,30]. Lipid preconcentration is important in synthesizing lipid bilayer membranes in a chip level and analyzing the characteristics of lipid vesicles with a concentrated sample. There is no lipid vesicle preconcentrator based on the concentration polarization effect in a micro/nanochannel platform. A concentration polarization-based preconcentrator is expected to enable the rapid detection of low-concentration species while it can be easily integrated with on-chip biomolecule analysis devices.

In this paper, we demonstrate a lipid vesicle preconcentrator using the concentration polarization effect. We visualized the preconcentration of lipid vesicles and we calculated the concentration ratio with varying applied voltages. The results show that the proposed preconcentrator is competent with other lipid vesicle preconcentration methods [20,21,30]. We expect that it can be beneficial for integration into lab-on-a-chip system due to its simple design and relatively easy fabrication.

2. Experimental

Fig. 2 shows the schematic of the lipid molecule preconcentrator (top view) and the experimental setup in this study. We fabricated a straight microchannel preconcentrator using the typical PDMS fabrication process (the details can be found in, e.g. ref. [18,31]) The dimension of the fabricated PDMS microchannel is 200 μm in width, 60 μm in depth, and 40 mm in length. A Nafion strip, formed with a 5 wt.% Nafion 117 solution from Sigma–Aldrich, serves as an ion perm-selective membrane. In preparing lipid molecules, we used the standard vesicle fusion technique: 1 mg of lipids (DOPC and Rh-DPPE in a 99.5/0.5 mol.% ratio) was dried and rehydrated in a Tris–HCl buffer (10 mM Tris–HCl, 150 mM NaCl, 2 mM $CaCl_2$, adjusted with 1 M HCl to pH ≈ 7.5) to the concentration of a 20:1 volume ratio [24,32,33]. DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and Rh-DPPE (1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)) was purchased from Avanti Polar Lipids. Trizma hydrichloride (Tris buffer) was from Sigma–Aldrich.

We used a sourcemeter (Keithley 2410) to apply DC voltages. We varied the applied voltages of 25 V, 60 V, 80 V, and 100 V to generate the concentration polarization effect. We performed fluorescent imaging with an inverted microscope (Olympus IX51) for detecting and recording images. We measured the fluorescent intensity from the lipid molecules inside the straight microchannel in every 30 s. We evaluated the preconcentration performance in terms of three factors: a concentrated area ratio, a fluorescence intensity averaged over the selected area, and the normalized concentration ratio there. We show the overall microchannel area in Fig. 3 and the selected area, where preconcentration mainly occurs, are indicated with the red rectangular window near the Nafion strip inside the microchannel. We calculated, for example, the concentration area ratio with the formula given in Fig. 4. Detailed explanations of all these calculations are discussed below.