FABRICATION OF BIO-MIMETIC EXTENDED NANOSPACE AND INVESTIGATION OF THE UNIQUE LIQUID PROPERTY: pH SHIFT

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ABSTRACT

We for the first time successfully realized a nanometer-sized bio-mimetic device (so-called bio-mimetic extended nanospace), and report pH shift of solution in the space. These results suggest correlation between the size of extremely small cellular space and its acidity.

KEYWORDS: extended nanospace, bio-mimetic, pH

INTRODUCTION

10-100 nm inter/intracellular space, such as synaptic gaps, plays an important role in biological functions and activities. In this space, unique liquid properties were suggested [1]. It is required to reveal correlation between unique liquid properties and biological function, but there has been no experimental tool even in vitro. In our laboratory, we constructed the extended nanospace (10-100 nm) on glass substrates and found unique liquid properties, such as higher viscosity, higher proton concentration and faster proton transfer [2]. Inspired by similar properties between the extended nanospace and intercellular space, we made bio-mimetic extended nanospace by modifying lipid bilayers onto the space (Figure 1). In μTAS2013, our laboratory reported that solution in bio-mimetic extended nanospace had higher viscosities, but could not achieve to confirm monolayer modification of lipid bilayer due to difficulty of surface-analysis for extremely small enclosed space. In this study, we verified that bio-mimetic extended nanospace was successfully constructed with monolayer using separable micro-nano chip, and measured the pH which was important for many chemical reactions.

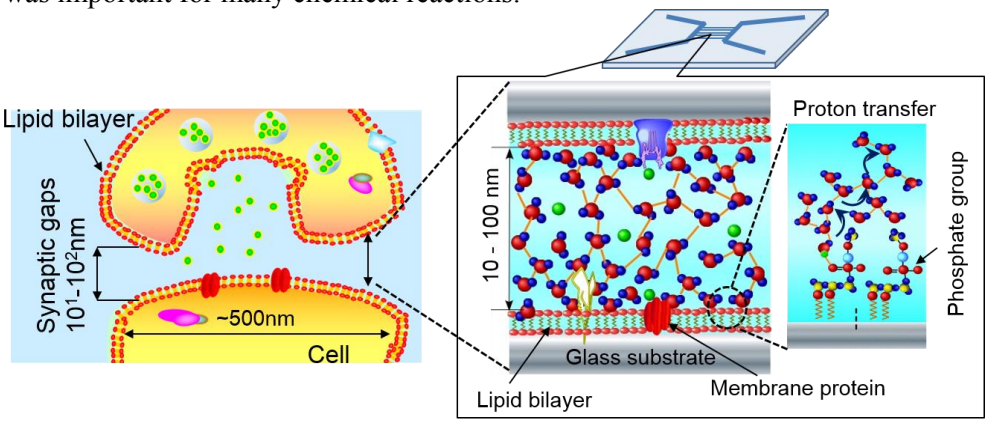

Figure 1: Concept of this study.

EXPERIMENTAL

The extended nanochannels were fabricated on a fused-silica substrate by electron beam lithography and plasma etching. The microchannels and holes for sample introduction were also fabricated on another substrate. Then, micro-nano chip was made by bonding the substrates with each other.

We utilized vesicle fusion method to make bio-mimetic extended nanospace. The procedure of modification was shown in Figure 2. First, vesicle solution was introduced into extended nanochannels. Vesicle solution was composed 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) as lipids and buffer solution (ion strength was about 100 mM). Then, vesicles interacted with surface of glass substrate to rupture and lipid bilayer was modified. After about 30 minutes, we washed out suspended vesicles by buffer solution.

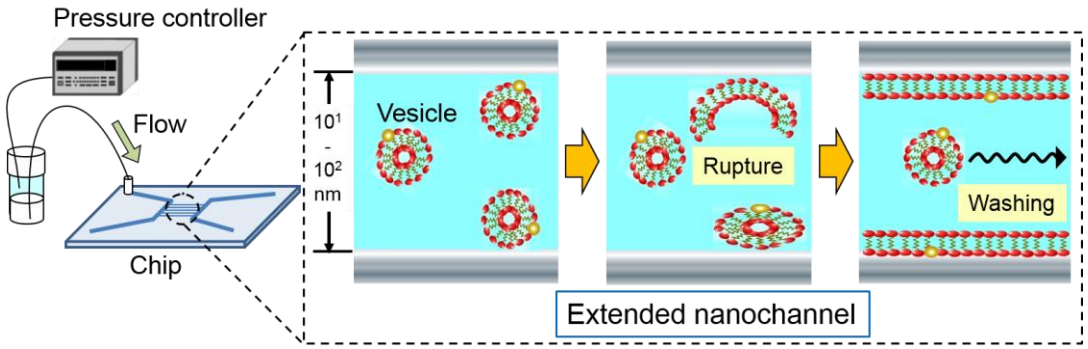

Figure 2: Procedure of lipid bilayer modification.

First, we confirmed that lipid bilayer is modified with monolayer and covers channel surfaces sufficiently. We made a separable chip and modified extended nanochannels (width: 3μm, depth: 400 nm). Then, we separated the micro-nano chip and measured thickness of lipid bilayer by fluid atomic force spectroscopy (Figure 3).

Next, we measured proton concentration utilizing Fluorescein. It is known that the ratio of fluorescent intensities of Fluorescein at 488 and 458 nm excitations changes depending on the pH of the surrounding liquid [3]. Experimental setup is shown in Figure 4. Extended nanochannels were modified with lipid bilayer by vesicle fusion method. Fluorescein solution (10^{-6} M) was injected into bio-mimetic extended nanochannels through a micro channel with 300 kPa. We used two kinds of solvent: water and PBS. Then, we measured fluorescent intensities at 488 and 458 nm excitations in the channels and calculated the pH by using a calibration curve obtained in bulk. We confirmed adaptability of probe in bio-mimetic extended nanospace, and measured the pH in various sized bio-mimetic extended nanochannels (equivalent diameter: 220-1200 nm).

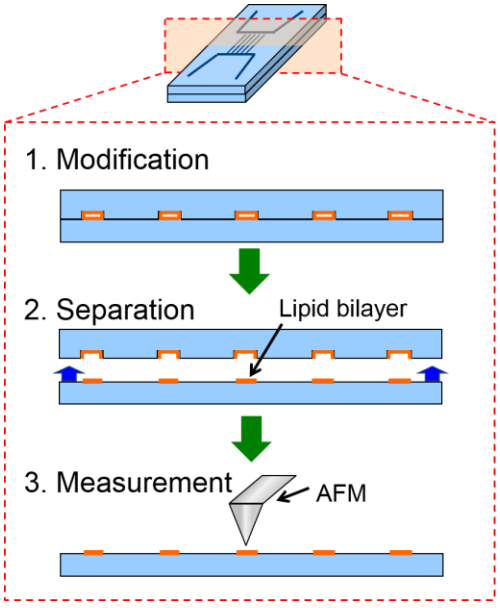

Figure 3: Protocol of lipid bilayer confirmation.

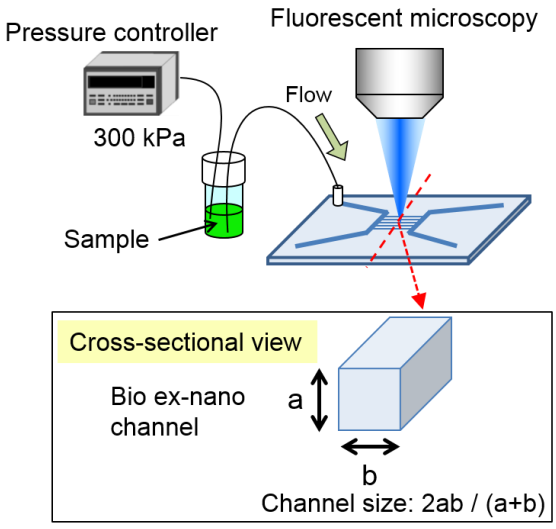

Figure 4: Experimental setup of pH measurement.

RESULTS AND DISCUSSION

In confirmation experiment, we found for the first time that the thickness was ~5 nm and same height as single layer of lipid bilayer [4]. We also confirmed that the coverage was sufficient. We verified that bio-mimetic extended nanospace was successfully constructed with monolayer.

The result of pH measurement is shown in Figure 5. Vertical error bars show the standard deviation of the measurement of five nanochannels. With scaling down to 400 nm or less, pH of water and PBS decreased from bulk value as well as glass surface [3]. In 220 nm nanochannels, the proton concentration of water is 25 times larger, and the proton concentration of PBS is 2.5 times larger than bulk one. It is suggested that proton dissociation from phosphate group of lipid bilayer contributes to decrease of pH in bio-mimetic extended nanospace. Especially, the result of pH decrease of PBS suggests that buffer mechanism is unrevealed in ultra-small space.

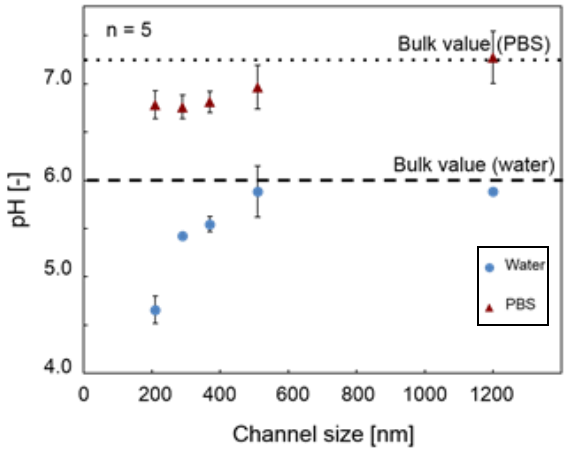

Figure 5: Dependency of pH on size of bio-mimetic extended nanochannel.

CONCLUSION

We successfully realized a nanometer-sized bio-mimetic device (so-called bio-mimetic extended nanospace), and report higher proton concentration of solution in the space. This result will contribute to clarify correlation between the size of extremely small cellular space (such as endosome) and its acidity.

ACKNOWLEDGEMENTS

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