

# **Development of new device for manipulation** of giant plasma membrane vesicles 巨大細胞膜小胞をマニピュレートするための新規デバイスの開発

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## Introduction

Results

Giant plasma membrane vesicles (GPMVs) isolated directly from cultured cell membranes are known for the useful model membranes. A protocol we reported previously produces efficient yields of large GPMVs (up to 10 µm). Recently, we have developed new device for manipulation of the GPMVs to observe specific GPMVs for a long time by an optical microscopic. We prepared micro-hole quartz plates with diameters of 2 µm and succeeded in manipulating the GPMVs on the micro-holes by suction of the solution.





#### **Preparation of the GPMVs**

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum at 37  $^{\circ}$ C in the presence of 5  $^{\circ}$  CO<sub>2</sub>. After removed DMEM,5 ml of 5  $\mu$ M DiO was added to the cells and incubated for 15 min at 37 °C. DiO Labeled cells washed three times with buffer 1, added buffer 2 and incubated for 30 min at 37 °C. Then, the media was replaced buffer 1 and incubated for 30 min at 37 °C. Finally, GPMVs were isolated into the supernatant by vortex for 10 min. Buffer 1:100 mM NaCl, 2 mM CaCl<sub>2</sub>, 50 mM Tris-HCl pH 7.5. Buffer 2:20 mM ZnCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 100 mM NaCl

# [Micro-hole quartz plates and suction pump system for microscopic observation of GPMVs]

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#### [ Manipulation of styrene micro-beads ]





Time-course observation of manipulated styrene micro-beads on the micro-holes

Suction pressure 30 kPa	• •		a a a a a a b a b	
20 kPa				
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Conclusion

In this study, we developed new suction devices for manipulation of GPMVs to observe specific GPMVs. The devices were micro-hole quartz plates and suction pump system (suction unit). The micro-styrene beads can be manipulated on the micro-holes by using this system and the beads can be observed by fluorescence microscope. We tried to manipulate GPMVs on the micro-holes. Then, we found that moderate suction pressure for manipulation of GPMVs was about -3 kPa. Moreover, structure of GPMVs was not collapsed on micro-holes. These devices enable easily manipulation of GPMVs and observation of the GPMVs for a long time by optical microscope. We will use this system to reveal the physical properties of membrane proteins and lipids in GPMVs.