BIOMOLECULE AND ORGANELLE TRAP USING ELECTRIC AND HYDRODRAG FORCE FIELDS IN TAPER SHAPED MICROCHANNEL

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Abstract
In this paper, trapping of cell organelles such as nucleus and mitochondria, and chromosome was reported using a taper shaped polydimetylsiloxane (PDMS) microchannel. The trap conditions for each biosubstance were investigated. In addition, trapping of biomolecules such as denatured RNA and protein (BSA) was also demonstrated using a silica glass chip, which has fine taper structure compared with PDMS ones.

Keywords: Biomolecule and organelle trapping, extraction, purification, molecular filter

1. Introduction

Extraction of a specific molecule or organelles from cells is one of the important issues for the understanding the biological phenomena from the point of embryology, tissue engineering, aging, and carcinogenesis. However, current microfluidic device technologies are not sufficient for extraction and purification of DNA, RNA and organelles from cells. We reported earlier a selective DNA trapping employing mutually reverse electric force and hydrodrag force field in a taper-shaped channel [1-2]. This time, we attempted to develop the trapping system for cell organelles (mitochondria and nuclei), chromosome and biomolecules of RNA and Protein (BSA) by the same principle. This technique can trap biomolecules selectively by tuning the ratio of the two forces with mild electric condition and relatively large structure compared to the case of dielectrophoresis [3]. The trapping technology using microfluidic devices has many advantages in bio-analysis. Our trap system needs no machine element. The size of microchannel is over 10 times larger than that of target biomaterials. For this reason, our channel is not easily blocked.

2. Preparation of biomaterials

The sample solution of nuclei was prepared by following steps. At first, cell density of HeLa was adjusted to 1×10^6/ml. The nuclei were extracted using Nuclear Extraction Kit (Chemicon International Inc). The nuclei were loaded with a fluorescent dye, YOYO-1 (Ex. Peak 491 nm, Em. Peak 509 nm). The mitochondria solution for a trap experiment was

prepared by following steps. A cell density of HeLal was adjusted to 2×10^7/ml. The mitochondria were extracted using Mitochondria Isolation Kit for cultured cells (PIERCE). The nuclei were loaded with a fluorescent dye, Mito Red (Ex. Peak 560 nm, Em. Peak 580 nm). The sample solution of chromosome was prepared as follows. Chromosomes were extracted from BALL-1 cell. The chromosome were dyed with YOYO-1.

BSA staining solution was prepared as follows. Sodium dodecyl sulfate (SDS) of 1gm was taken into a 200ml beaker and 100ml of 0.1M Tris-HCl (pH= 6.8) was added in it. In an another 50ml beaker, 0.87 gm of bovine serum albumin (BSA) and 10 ml of distilled water was taken. From each of the above solutions, 0.5ml was taken into a test tube and boiled for one minute. 1µl SYPRO orange protein gel stain (Ex. Peak 470 nm, Em. Peak 570 nm) was taken into another test tube and 5ml of 7.5% acetic acid was added in it. Finally, from the content of each of these test tubes, 5µl was taken into a sample bottle and 80µl of 7.5% of acetic acid and 0.03mg of polyvinylpyrrolidone were added in it.

3. Experimental set up

A sample solution was introduced into a microchannel with triangle shape tapered structures. When sample biomolecule or organelle was driven by electric force and hydro drag force simultaneously in opposite directions, target biomaterials were then trapped near the triangle shape tapered structure. The trapping motion of each samples were observed under a fluorescence microscope. The observed fluorescence was intensified by the CCD camera. The fluorescence image was stored in PC as digital data (Fig 1.). The change of intensity of fluorescence was analyzed using SimplePCI software (Leeds Instruments).

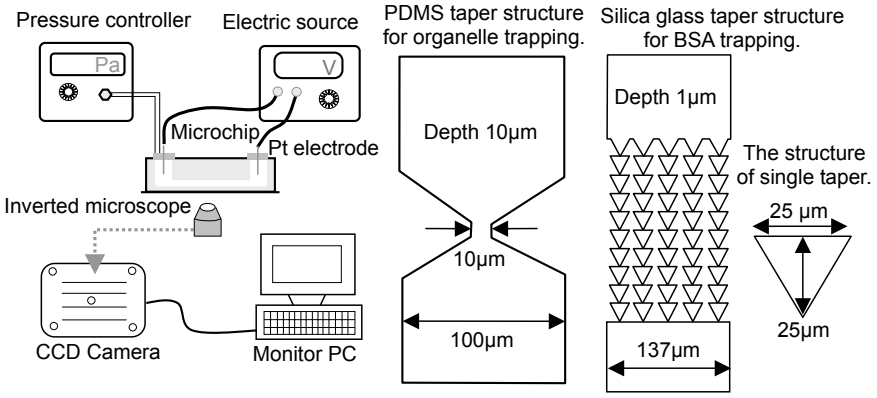

Fig 1. Experimental set up (left) and structure of the taper shape microchannel (right).

4. Results and discussion

The trap condition for nucleus, mitochondria and chromosome were investigated using a taper microchannel which was made form PDMS (polydimetylsiloxane) (Fig 2.). The trap condition of each organelle obtained as results of each experiment were shown in Fig 2 (right). We discovered that higher voltage and higher current were needed, in order to trap the smaller organelle. Addition to these, denatured RNA and Protein (BSA) samples were also successfully trapped (Fig 3) in the silica glass taper microchannel which has a more fine structure compared with PDMS microchannel.

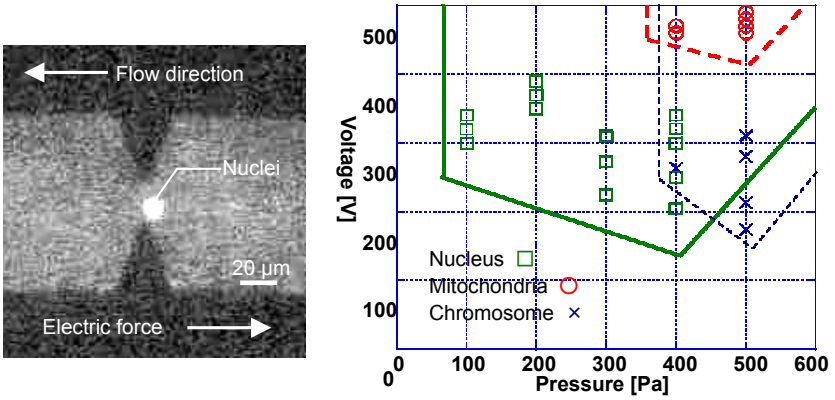

Fig 2. Fluorescence image of nuclei trapping (left) and trapping condition of organelles (right).

5. Conclusions

We successfully develop the trapping system for organelles (mitochondria and nuclei) in the cells, chromosome, RNA, and Protein (BSA). Our trap has a great potential for extraction and purification of target biomaterials from the cells in microfluidic chips. We consider that the trapping technology is a novel method for single cell analysis, single molecule detection, gene expression, and continuous PCR etc in the future.

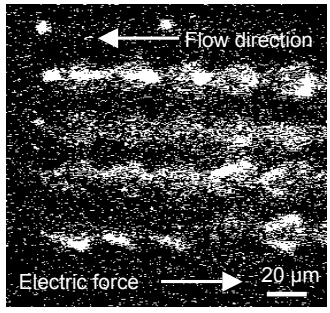

Fig 3. Fluorescence image of protein trapping.

References

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