Dynamic Microfluidic Channels for Active Nanotransport Driven by Kinesin Motor Proteins

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ABSTRACT
In this paper, we describe some of our recent studies for motor protein assays using micro/nano fabrications. We fabricated two microfluidic polymer devices that enable to study active transport of kinesins and tau protein detection, respectively. The former device eliminated diffusional transport of target molecules in a single micrometer-scale channel to evaluate only the motor's active transport, which was dynamically formed by a pneumatic poly(dimethylsiloxane) (PDMS) membrane valve. The latter device enables to detect a microtubule associated protein (MAP), tau protein, for differentiating its isoforms and mutants. These devices will be further integrated with motor protein assays to explore their applicability in bio-inspired nanosystems.

Keywords
Kinesin, Microtubule, Micro/nano fabrication, Microfluidics.

1. INTRODUCTION
In vivo function of a motor protein, kinesin, is known to carry cargos on microtubules according to their polarities. It supports cellular vital functions. Over the last decade, motor proteins have been focused by engineering aspects due to their motility in addition to their chemomechanical functions. Thanks to genetic engineering, motors can be modified to have engineered functions such as the reverse motor direction [1] or accelerated motility [2]. When they are integrated with micro/nano fabrications, their motility has been further utilized as a nano-scale actuator: Motor moving directions were controlled by micro/nano structures, electrical, magnetic, or fluid forces. Target molecules loaded were transported for concentration and detection.

To further expand this research direction, I introduce our recent results: One is a microfluidic device to evaluate motor active transport. So far, researchers considered that target molecules can be transported by motor activity rather than free diffusion. However, when a flow cell (~100 µm in height) is used to construct a molecular system (~100 nm) for molecular transport, the bulk solution also can be a mediator to carry target molecules. To segregate the active and passive transports, we developed a microfluidic device that enabled easy buffer introduction to the assay area and dynamic formation of single-micrometer-scale channels on demand [3].

The other is a tau detection device. Many demonstrations have been reported for molecular transport, concentration, and detection to replace conventional micro total analysis systems (MicroTAS). However, many of them incorporated target molecules through avidin-biotin and/or antigen-antibody bindings into the molecular system, and resulting in difficulties in keeping intact motor functions. Motors may not work properly. Moreover, assay condition optimized for motor activity limits selection of target molecules. Here, we considered if this motility degradation can be used as a sensor. As a tau protein is known to hinder motor activities at single molecule level on a microtubule, we examined if this can be reproduced in vitro at multi-motor level [4].

2. RESULTS AND DISCUSSION
2.1 Dynamic Microfluidic Channels
2.1.1 Device Design
The device was fabricated by two PDMS layers and a bottom glass cover slip. Pneumatic (control) channels in the top PDMS layer (Fig. 1) actuate thin PDMS membranes in the middle layer to control the direction of buffer solution flow. Pneumatic channels have two unique functions in our device. First, one of the control channels serves to dynamically create the channel array on demand by compressing the PDMS membrane on the microtrack array fabricated on the cover slip. Second, nitrogen gas (N_2) is supplied to the assay channel area, which extends the assay time by reducing photobleaching and protein deactivation via reaction with oxygen radicals.

2.1.2 Device Fabrication
The top PDMS layer was fabricated by a standard PDMS soft-lithography. A mold for control channels was fabricated by UV photolithography of an SU-8 3050 resist on a silicon substrate. A PDMS prepolymer was cast on the mold with a thickness of ~10 mm. Middle PDMS layer with fluidic channels sealed by a thin PDMS membrane. An AZP4903 photoresist patterned on a silicon substrate was employed as the mold for fluidic channels. Following the photolithography process, the photoresist was reflowed at 150 °C for 2 h to produce round cross-sections. The PDMS mixture was spin-coated on the mold at 2000 rpm for 60 s to obtain a 60 µm-thick PDMS membrane that was then cured at 80 °C for 1 h. Bottom cover slip with a microtrack array. An aluminium layer was deposited on a borosilicate cover slip after cleaning in piranha solution. An SU-8 2000.5 resist was then spincoated and patterned using a double-sided mask aligner. An array of microtracks was fabricated by developing an SU-8 layer in an SU-8 developer and etching the aluminum layer.

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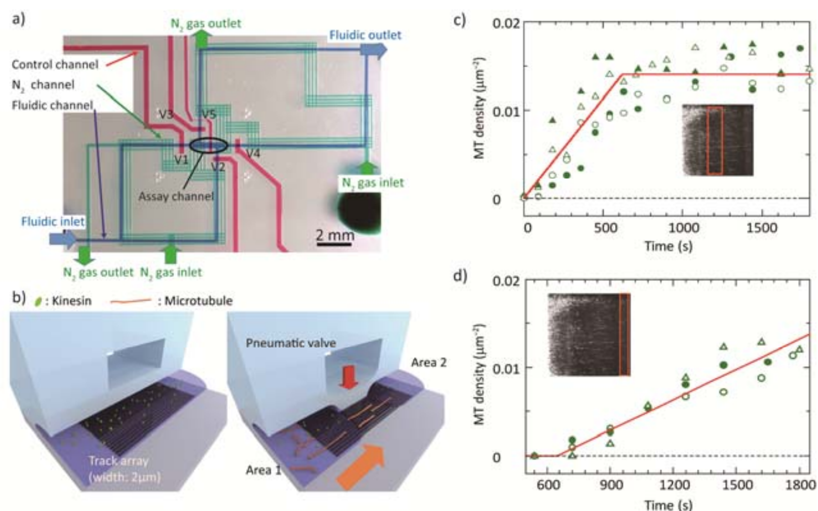

Fig. 1. Fabricated device and its functions. a) Overview of the device. Control channel, fluidic channel, and N_2 channel are colored red, blue, and green, respectively. The microtrack array patterned on the glass cover slip is located at the center (enclosed by a circle). Scale bar is 2 mm. b) Schematic of dynamic formation of a microchannel array. After kinesin molecules are immobilized on the glass surface, the top PDMS membrane is pneumatically actuated, resulting in the formation of the closed channel array. c) MT density in the microchannel area. MTs were counted within the area enclosed by the orange rectangle. Data in the plots were obtained from four experiments, and curve fitting yielded the red line. d) MT density in area 2 was measured by counting the number of MTs within the area enclosed by the orange rectangle. Data were obtained from three experiments and were fitted as noted for panel c.

2.1.3 Protein Preparation

The kinesin construct consisted of sequence-encoding human kinesin amino acid residues 1–573 with a 6-residue N-terminal histidine tag. Tubulin was purified from porcine brain, and fluorescently labelled by tetramethyl rhodamine. Labelled MTs were polymerized by mixing non-labelled tubulin and labelled tubulin (10: 1) at 37 °C for 45 min in the presence of 0.5 mM $MgSO_4$ and 0.5 mM GTP.

2.1.4 Results and Discussion

We measured the time-course change in MT density in the two regions demarcated by orange rectangles in the insets of Fig. 1c and d. The former corresponds to the area where the microchannel array was formed, and the latter is at the exit of the channels. The MT density was nearly zero (t = 0 s) and increased until it reached a maximum. The increase can be explained by the fact that the MTs moved into the microchannel array where MTs were not initially present. Once the microchannel array was filled with MTs and the numbers of incoming and outgoing MTs were balanced, the MT density was maximal. Fig. 1d shows the time course of the MT density in area 2. MTs were not observed in area 2 until t = 700 s. Results demonstrated the directional active transport by suppressing free diffusion of MTs.

2.2 Tau Detection Device

2.2.1 Design of Tau Detection Device

We designed a microfluidic device comprising of a MT reservoir and an arrowhead shape MT collector, both of which are connected by a microchannel. The microchannel acts as a guiding structure to channelize tau-bound MTs from the reservoir towards the collector. Therefore, the velocity difference is amplified during gliding in the channel, resulting in the difference in fluorescent intensity (FI) increase at the collector. The arrowhead shape collector serves as a rectifier and a concentrator. It rectifies the MT gliding direction to make them glide within the collector until FI was measured. In addition to functions of three regions, an overhang structure was designed circumscribing the entire assay region to prevent MTs from leaving from the kinesin-coated surface. In a single microfluidic device, six assay units were located radially such that all the collectors points towards the center. This arrangement allows the simultaneous observation of six collector regions to measure FIs.

2.2.2 Results and Discussion

We could measure FI increase according to tau protein decorated on MTs. In addition to tau isoforms, we were able to differentiate some of major mutants, which will possibly lead to early diagnosis of neurodegenerative diseases.

3. CONCLUSION

I introduced two device concepts that help to understand kinesin motility and to explore application of kinesin-microtubule system. Our group will continue working on what micro/nano fabrications contribute to understand motor proteins and/or develop motor protein-based nanosystems.

4. ACKNOWLEDGMENTS

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