MICROFLUIDIC VALVE TECHNOLOGY PRODUCES MICROCHANNEL ARRAY TO EVALUATE KINESIN-DRIVEN MOLECULAR TRANSPORT

K. Fujimoto, H. Shintaku, H. Kotera and R. Yokokawa

Department of Micro Engineering, Kyoto University, JAPAN

ABSTRACT

In this paper, we propose a microfluidic device that enables the controlled introduction of microtubules (MTs) into a designated region in the fluidic channel and transport of MTs by gliding motility on a kinesin-coated surface. The proposed device includes a pneumatic valve to control the flow direction and microchannel formation. MTs introduced into a defined area of the device glided into a microchannel connected to a second area, resulting in a directed change in the distribution of MTs from the former to the latter. This is the first report to realize and analyze gliding-based transport between two geometrically separated areas.

KEYWORDS: Microtubule, Kinesin, Motorprotein, Autonomous transport, Pneumatic valve

INTRODUCTION

Promising characteristics of motor proteins that autonomously exert mechanical forces encouraged their use in vitro to contribute to the further miniaturization of microfluidic devices. The gliding motility of microtubules (MTs) on surfaces coated with kinesin is widely used. The establishment of autonomous sample processing by the MT gliding has been long sought as a goal in developing a gliding-based device [1]. Thus far, various reports have intensively addressed issues toward the goal such as the application of external forces, guiding by surface patterns, and selective coating of kinesin to control the MT gliding direction [2]. However, since those techniques have been examined in a flow cell, numerous MTs are freely suspended in a bulk solution. It resulted in contamination of target molecules, although MT gliding areas are segregated by patterned kinesin. One large gap toward the goal is to define multiple areas without contamination of target molecules including in the bulk solution, where molecules are carried by MTs only. Once one determines areas for molecular loading, concentration and detection without contamination of molecules and MTs among areas, MTs glide those different areas to achieve their functions.

Here, we developed a microfluidic device in which two assay areas connected by a microchannel array are accessible with a kinesin-driven gliding assay. The device is equipped with a pneumatic valve [3], which controls the fluidic flow and formation of microchannel to achieve gliding assay-based transport. Introducing MTs into one of the two separated areas resulted in directed transport of MTs through the microchannel to the other.

Fig. 1: Schematic illustration of integrated microfluidic valve and microtrack array. A polydimethylsiloxane (PDMS) device and microtrack array patterned on a cover slip are bonded to form a fluidic device. The enlarged cross-sectional view shows three layers: top PDMS slab for a control channel, PDMS membrane for a flow channel, and bottom cover slip with the microtrack array.

EXPERIMENTAL

Device fabrication: The pneumatic valve device was constructed with three layers: a coverslip at the bottom, a polydimethylsiloxane (PDMS) membrane with fluidic channels, and a PDMS slab with control channels for pressurized valves (Fig. 1). The flow and control channels were fabricated by molding. The microtrack array was fabricated with the following dimensions: 2 μm in width, 700 nm in depth, and 200 μm in length. Tubing was connected to the N₂ gas line to add pressure controlled by external solenoid valves operated by a PC. The valves were completely enclosed when 100 kPa of pressure was applied (Fig. 2b).
Thus, the constructed pneumatic valve structure realized the control of flow direction and dynamic formation of microchannel array by enclosing the top of the microtrack. After the microchannel array was formed, 1 nM of Q-dot 655 was introduced and the trajectories of individual Q-dots were observed to evaluate confinement of the microchannel.

Motility assay: A MT gliding assay was implemented in the device. Solutions were introduced by using differences in water head pressure. A kinesin solution of 70 μg/ml was flowed for 10 min and incubated for 3 min after the flow was stopped by valve actuation. After the kinesin solution was flushed out by a buffer solution flowed for 3 min, a MT solution (containing 120 μg/ml of MT, 1 mM ATP, and an oxygen scavenging system) was introduced to perform gliding motility assay. The flow direction was controlled by the valves to selectively introduce the MT solution into area located on left end of microchannel array in Fig. 3a (Area 1), and MT solution was not introduced into opposite area (Area 2). Once the solution flow reached the Area 1, gliding MTs were fluorescently observed, maintaining the MT solution flow. Time course change of MT densities were measured in microchannel area and outside of microchannel at 3-min intervals to quantitatively evaluate the change of MT distribution in the device.

RESULTS AND DISCUSSION
Confinement evaluation: Individual trajectories of Q-dots were analyzed to evaluate the microchannel array. In the region where the microtrack array was not sealed by the PDMS membrane, Q-dots rapidly left from the range of focus depth (2.68 s in average) owing to free Brownian motion perpendicular to the substrate, whereas Q-dots in a microchannel were observed for longer periods (4.97 s). In addition, the trajectories obtained from inside the microchannel array were confined in the shape of the channel (Fig. 2c). The maximum distance travelled perpendicular to the channels, calculated from over 300 trajectories, showed a distinct peak at 1–2 μm. In contrast, the distances travelled parallel to the channels were distributed between 0 and 11 μm (Fig. 2d). This indicates that Q-dot Brownian motion is confined in an enclosed channel with a width of 2 μm.
In this paper, we demonstrated control of the location and gliding of MTs by manipulating the flow and dynamic formation of single-μm scale channels in a PDMS microfluidic device. Experiments showed directed gliding of MTs between two areas separated by a microchannel array. The transport of MTs by gliding was demonstrated and qualitatively evaluated. The proposed experimental system constitutes an important advance toward the development of micro devices in which samples are autonomously manipulated by motorproteins.

ACKNOWLEDGEMENTS
This work was supported by Grant-in-Aid for JSPS Fellows Grant Number 25·1836 to K. F.

REFERENCES

CONTACT
Kazuya Fujimoto. 81-0753-3559 or fujimoto.kazuya.78m@st.kyoto-u.ac.jp