ISOLATION AND MANIPULATION OF SINGLE MICROTUBULE BY SILICON MICROTWEEZERS

M. C. Tarhan1, D. Collard1,2, C. Bottier3, R. Yokokawa1, M. Hosogi4, G. Hashiguchi4 and H. Fujita1
1 CIRMM, IIS, The University of Tokyo, JAPAN
2 LIMMS / CNRS-IIS, The University of Tokyo, JAPAN
3 Department of Micro System Technology, Ritsumeikan University, JAPAN,
4 Research Institute of Electronics, Shizuoka University, JAPAN

ABSTRACT
This paper presents capturing single microtubule between the tips of micromachined silicon microtweezers. A glass slide was used to fabricate parallel CYTOP walls where rhodamine-labeled microtubules were overhung in between. Microtweezers’ tips were coated with Poly-L-Lysine to attach microtubules on. A single microtubule, hanging between CYTOP walls, was captured in buffer solution by microtweezers connected to a micromanipulator under microscope observation.

KEYWORDS: Kinesin, Microtubule, Microtweezers, Single Molecule Capturing

INTRODUCTION
Performing assay at the molecular level is a current challenge in biological analysis to avoid the “blurring” and “averaging” effect of bulk experiments. Current molecule manipulation techniques, like optical tweezers (OT) [1] or atomic force microscopy, exhibit low throughput as the molecule has to be modified for attaching beads or the substrate. To avoid this limitation, in this paper a direct capturing and manipulation of single microtubule molecule with silicon microtweezers is proposed. Bead attachment or surface immobilization of the molecule is avoided because the tweezers can directly capture overhanging microtubule. The molecules can be easily relocated and put in interaction with other molecules; this result suggests that high throughput molecular assays can be envisioned.

PREPARATION
The single microtubule experiment was held by silicon microtweezers (figure-1) and a glass slide with parallel CYTOP (CTL-809M) walls of 4 μm high. The tweezers for capturing the microtubule were fabricated by silicon micromachining techniques [2]. The tweezers had two opposing sharp tips with a gap spacing of 12 μm. Before capturing, the microtubules were overhanging on microstructured CYTOP walls patterned on a glass slide. Walls were fabricated by spin coating (1000 rpm for 10 sec) and baking (100°C for 30 min) CYTOP for seven times (figure-2a). After the seventh time, the wafer was baked at 185°C for 90 minutes (figure-2b). Then, aluminium was evaporated (100 nm) and patterned. After aluminium etching (figure-2c), the wafer was exposed to oxygen plasma with 100W for 30 minutes (figure-2d) to dry etch CYTOP. The masking aluminium was etched and the remaining CYTOP walls were used to obtain overhanging microtubules.
EXPERIMENTAL

Experiments were observed with an inverted microscope both with optical and fluorescence setups. The tweezers were connected to a micromanipulator attached to the microscope stage. A flow cell was prepared using the glass slide with parallel CYTOP walls where flow was perpendicular to the walls. First, kinesin was injected into the flow cell (figure-3a). After 3 minutes, flow cell was washed with BRB80 solution to remove unbound kinesin molecules. Second step was to add the rhodamine-labeled microtubules. After injection, the flow cell was undisturbed for 5 minutes to let microtubules attach on kinesin molecules, overhanging between parallel CYTOP walls (figure-3b). After washing, the upper glass of the flow cell was removed adding oxygen scavengers solution (to prevented labeled microtubules from bleaching) with care not to dry the surface. The tweezers were coated with Poly-L-Lysine, a polycation binding to any negatively charged proteins, by simply dipping in a PLL drop for 10 seconds. Then the microtweezers were inserted into the liquid over CYTOP walls with the overhanging microtubules. Because of the negative charge of the microtubules, with the PLL coating, tweezers attached to microtubule strongly. As a result, a single microtubule could be captured and removed from the surface (figure-4).
RESULTS AND DISCUSSION

The results of experiments can be seen in figure 5. Tweezers were moved closed to the CYTOP walls with the overhanging microtubules (figure-5a). PLL coating on the tips resulted in strong attachment of a microtubule (figure-5b). Using a micromanipulator, the captured microtubule was removed from the surface so that the captured microtubule could be seen clearly (figure-5c&d). Then, microtubules can be easily manipulated and translocated to another location in the droplet.

CONCLUSIONS

We have fabricated walls, made of CYTOP, on a glass slide to have hanging microtubules. This hanging structure allowed us to use a microtweezer with a micromanipulator to access to a single microtubule. Coating the tips of the microtweezer gave us chance to capture the hanging microtubule. Successful capturing of a single microtubule was shown. Using microtweezers for such kind of processes gives us chance for single molecule manipulation. As an example, captured microtubules can be relocated in different chemical environments for kinesin motility analysis.

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REFERENCES