Extended Abstracts of the 2011 International Conference on Solid State Devices and Materials, Nagoya, 2011, pp404-405

Quantitative characterization of guided motion of dynein-microtubule system

Norihiko Ashikari[12], Yuji Shitaka[2], Hiroyuki Sakaue[1], Takayuki Takahagi[1],
Hiroaki Kojima[2], and Kazuhiro Oiwa[2], Hitoshi Suzuki[1],

[1] Hiroshima Univ.
1-3-1 Kagamiyma, Higashi-hiroshima,
Hiroshima 739-8530, Japan
Phone: +81-82-424-7645 E-mail: hitoshi-suzuki@hiroshima-u.ac.jp
[2] National Institute of Information and Communications Technology.
588-2 Iwaoka, Nishi-ku, Kobe, Hyogo 651-2492, Japan

1. Introduction

Using motor proteins as nanometer actuators in micro- and nano-scale devices is attractive idea for many researchers in the field of nanotechnology and biophysics. Motor proteins are essentially tens nm in size and have a function to generate force with chemical reaction. Dynein used in this study is one of the motor proteins that generate linear motion as well as myosin and kinesin. It interacts with protein filament (microtubule) and adenosine triphosphate (ATP), resulting in generation of the force. In *in vitro* motility assay of dynein-microtuble system, dynein molecules were immobilized on a substrate surface and fluorescently labeled microtubules moved by them were observed with an epi-fluorescence microscope [1]. To use the motion generated by dynein in micro- and nano-scale devices, it is necessary to develop the techniques to control the direction of the motion. Since the first attempt to regulate the direction of the motion by a lithographically patterned surface and myosin [2], many techniques to regulate the motions by kinesin and myosin have been reported [3]. However, the useful structure to regulate the motion generated by dynein has not yet been quantitatively analyzed.

In this study, we fabricated walls made of resist polymers to guide the microtubles' motion generated by dynein. The efficiencies of the regulation of the motion were characterized by calculating the ratio of the microtubules that climbed over the wall.

2. Experimental section

Substrates and microstructure

The patterned structure, a wall, was made with negative resist polymer (SAL601) or positive resist one (OEBR-1000) with electron beam lithography on SiO_2 surface. The wall height was varied from 200 nm to 1600 nm.

The pattern of the wall made of the resist polymer is shown in Fig. 1. It consists of two symmetric regions surrounded with the resist polymer wall. The shape of the region was designed to make the microtubules collide to the central part of the wall, as shown with arrows. The surface for dynein immobilization was SiO_2.

Motility assay

The details of the preparation of dynein and microtubule was similar to the previous report [4]. We used axonemal dynein that is extracted from outer-armless mutant of chlamydomonas.

A flow cell was made of the Si substrate having the walls and a coverslip with two double-sided tape as spacers. The basic procedures of motility assay are as follows. The flow cell was filled firstly with the dynein solution, incubated for 5 min in order to adsorb dynein molecules on the surface and then the solution was washed by bovine serum albumin (BSA) solution to remove excess of dynein molecules. After that, the solution was replaced with the microtubule solution and subsequently ATP solution were introduced into it.

Fluorescently labeled microtubules were observed with epi-fluorescence microscope (BX-51, Olympus) using Cy3 filter set and an EMCCD camera (Hamamatsu Photonics).

2. Results and discussion

A typical snapshot of the microtubules' motion regulated by SAL601 is shown Fig. 2(a). Since SAL601 has weak fluorescence, the pattern of the wall is clearly observed. The white filaments are fluorescently labeled microtubules. The accumulated image (Fig. 2(b)) showing the trajectories of the moving microtubules indicates that some microtubules moved along the wall and collided with the central part of the pattern perpendicularly as designed.

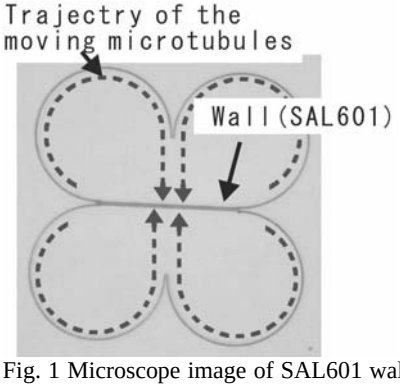

Fig. 1 Microscope image of SAL601 wall. Arrows indicate the direction of moving microtubules.

We counted the number (N_{all}) of the microtubules colliding with the central wall and that (N) of the microtubules climbing over the wall. Here, we define the ratio (N/N_{all}) to estimate the efficiency of the wall on regulation of the motion. The ratio is shown in Fig. 3. In the case of SAL601,

approximately 20% of microtubules were climbing over the wall whose height was lower than 500 nm. On the other hand, in the case of OEBR-1000, the ratio was less than 10%. These ratios decreases with increase of the height of the wall. Interestingly, both ratios decreases rapidly around 1000nm.

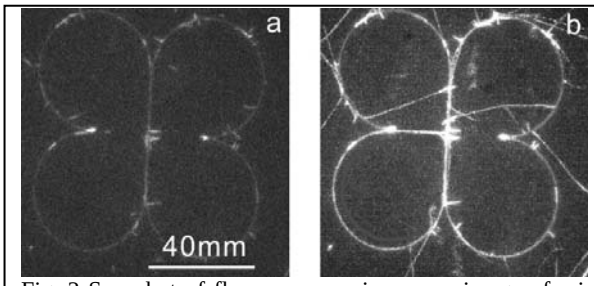

Fig. 2 Snapshot of fluorescence microscope image of microtubules' motion (a) and accumulated image (b).

The difference in the ratio between the resist materials at lower wall height suggests that the function of dynein depends on the surface materials. It is consistent with the observation of the microtubules on dynein immobilized on the resist polymers. Namely, dynein on SAL601 surface can move the microtubules but that on OEBR-1000 does not produce such motion.

When a microtubule collides with a wall, its head part should be bent and elevated by the pushing force and its own rigidity. Such bending part detaches from dynein molecules on the surface. The higher wall makes the bending part long because of the rigidity of the microtubule and pushing force from its tail part. Such a long part is easily fluctuated and falls sideways, resulting in change of its moving direction. In the case of the lower wall, the bending part is short and sufficiently long part of microtubules are supported and propelled by dynein. Thus, this part moves with less fluctuation and climb over the wall.

3. Conclusions

The microtubules' motion generated dynein molecules immobilized on SiO_2 surface can be regulated with the walls made by lithography technique. The efficiency of the regulation of microtubules' motion can be calculating the ratio of the number of microtubules climbing over the wall. The material and height of the wall effect on the guiding motion along the wall.

Acknowledgements

We would like to express sincere thanks to Prof. Y. Kadoya of Hiroshima University for collaboration on e-beam lithography.

References

[1] H. Sakakibara, H. Kojima, Y. Sakai, E. Katayama, K. Oiwa, Nature **400** (1999) 586.

[2] H. Suzuki, A. Yamada, K. Oiwa, H. Nakayama, S. Mashiko, Biophys. J. **72** (1997)1997-2001.

[3] Y. Hiratsuka, T. Tada, K. Oiwa, T. Kanayama, T.Q.P. Uyeda, Biophys. J. **81** (2001) 1555; A. Mansson, M. Balaz, N. Albert-Torres, K.J. Rosengren, Frontiers Biosci., **13** (2008) 5732; etc.

[4] K. Oiwa, R. Kometani, D.Y. Li, Y. Shitaka, R. Nakamori, S. Matsui, H. Sakakibara, Mater. Sci. Forum, **539-543** (2007) 3290-3296.

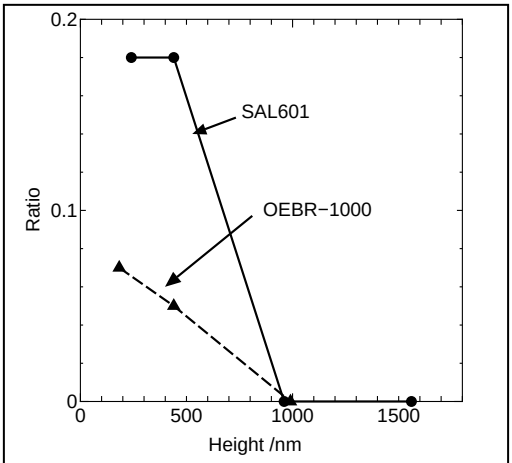

Fig.3 Ratios of microtubules climbing over the wall.