ON-SITE MANIPULATION OF SINGLE DNA MOLECULES USING OPTICALLY-DRIVEN MICROCHOPSTICKS

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ABSTRACT

This paper reports a novel DNA manipulation technique for the analysis of single DNA molecules under a fluorescence microscope. We successfully manipulated single DNA molecules using two microstructures driven by two laser beams, where we handled them like "chopsticks". We evaluated the dimensions and the shapes of microstructures, intended for grasping single DNA molecules efficiently. We have found that the geometry of the structure is dominant parameter for effective handling of single DNA molecules. This manipulation technique allows the imaging of single DNA molecules with its intact state, which will lead to the nanotechnology-based DNA analysis.

INTRODUCTION

DNA analysis plays a key role in molecular biology. Some groups have reported DNA analysis techniques by employing extended fiber FISH [1] (fluorescence in situ hybridization), DNA-protein interaction assays [2] and DNA sequencing [3]. The standard techniques for chromosomal DNA molecules, however, are based on the handling them in bulk solution, which inevitably cause fragmentation of DNA strands, because hydrodynamic shear during handling readily breaks a DNA molecule. This results in loss of positional information along the DNA strand and the three dimensional chromosomal conformations. Consequently, handling DNA molecules in their intact states is necessary for realizing next-generation DNA analysis. Although several techniques for manipulating single DNA molecules have been proposed, including those based on optical tweezers [4] and nanotweezers [5], these techniques limit the manipulatable DNA length to less than 100 kbp. To overcome it, previously we have proposed a DNA manipulation technique [6] using electroosmotic flow [7] (EOF) and microstructures captured by optical tweezers that allow the manipulation of a chromosomal DNA molecule of Mbp size. However, the technique still limits the manipulation of DNA, particularly in the elongation of DNA. To broaden the possibility in the DNA handling, we developed novel microstructures (i.e. microchopsticks) to capture a DNA molecule and demonstrated the DNA manipulation using them (Figure 1). Two microstructures are captured by two laser beams and manipulated to grasp single DNA molecules between them and manipulate it like "chopsticks".

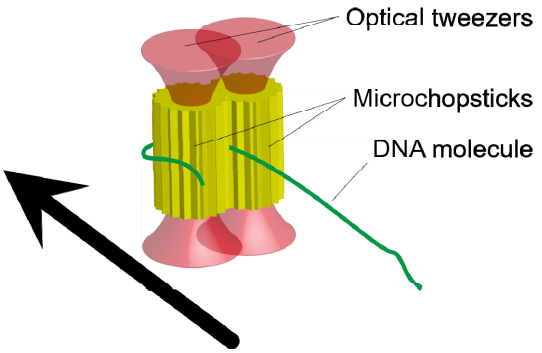

Figure 1: DNA manipulation technique using a set of optically-driven microstructures, namely microchopsticks.

MATERIALS AND METHODS

Optical tweezers

We constructed dual-beam optical tweezers system which has two trap points in the focal plane of an optical microscope (Figure 2). An ytterbium fiber laser (wavelength: 1064 nm CW, IGP Photonics) was introduced into an inverted fluorescence microscope (IX-71, Olympus). The laser beam was divided into two paths through a wave plate, WP1 (WPQ10640-4M, SIGMAKOKI Co., LTD.) and beam splitter, BS (PBS-10-10640, SIGMAKOKI Co., LTD.). Each laser beams were reflected by M1, M2 and were coaxially combined by beam combiner, BC (PBS-10-10640, SIGMAKOKI Co., LTD.). The laser beam reflected by M2 was polarized by WP2 (WPQ10640-2M, SIGMAKOKI Co., LTD.). Two laser beams were reflected by DM2 and were introduced into an oil immersion objective lens (100x, N.A. = 1.40) to focus on the observation plane. Fluorescence image was obtained through an EM-CCD camera (ImagEM, Hamamatsu Photonics), when DNA molecules were fluorescently labeled.

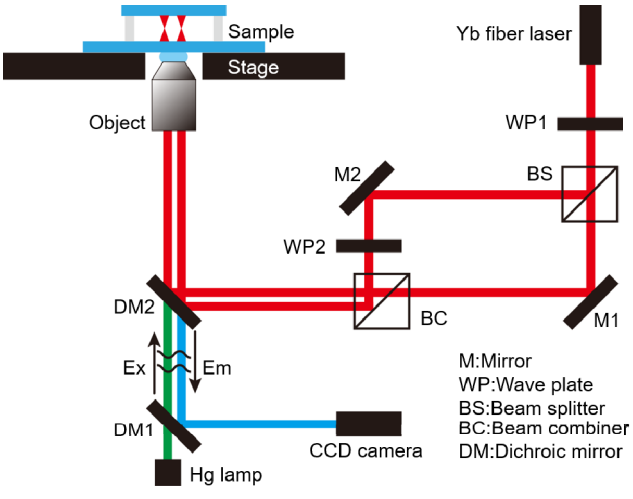

Figure 2: Optical setup of dual beam optical tweezers.

Fabrication

We fabricated microchopsticks as optically-driven manipulation tool [8]. Microchopsticks were fabricated with standard UV lithography of SU-8 (Figure 3). Cr on a glass substrate was patterned by standard lithography. Then, PVA (Polyvinyl Alcohol) solution was coated as sacrificial layer. After that, SU-8 was coated, exposed to UV light from the backside of the substrate using the Cr pattern as a mask and developed. Finally, PVA was removed in DI water on a hotplate at 120ºC, by which 240,000 pieces of microchopsticks were released.

We developed two types of microstructures to evaluate the size of microchopsticks for effective DNA capture. We fabricated microstructures of two sizes (large one and small one) as shown in A2 and B2 of Figure 4. The design value of the large block is 10 µm × 5 µm, the small block is 5 µm × 2.5 µm as shown in A1 and B1 of Figure 4. The measured value of the large block was 9.88 µm × 5.04 µm and the small block was 5.06 µm × 2.88 µm, which have the same thickness of 3.71 µm.

Next, we developed microstructures of various geometries as shown in C2-G3 of Figure 5, to evaluate the shape effect in DNA handling. C1-G1 show the mask pattern. The microstructures were slightly expanded due to light diffraction in exposure process. E2 microstructure shows the most different shape from mask pattern (E1). This also might be due to the diffraction and interference of exposure light.

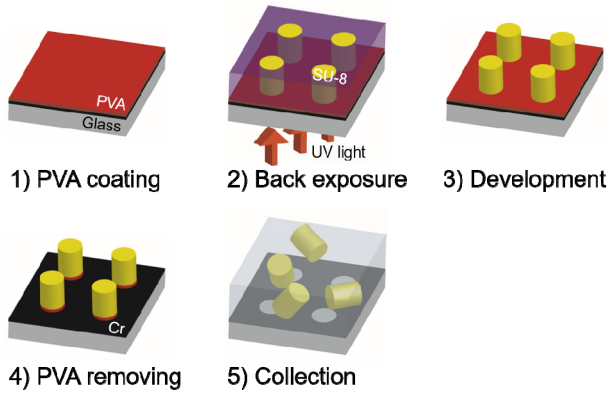

Figure 3: Fabrication process of microchopsticks.

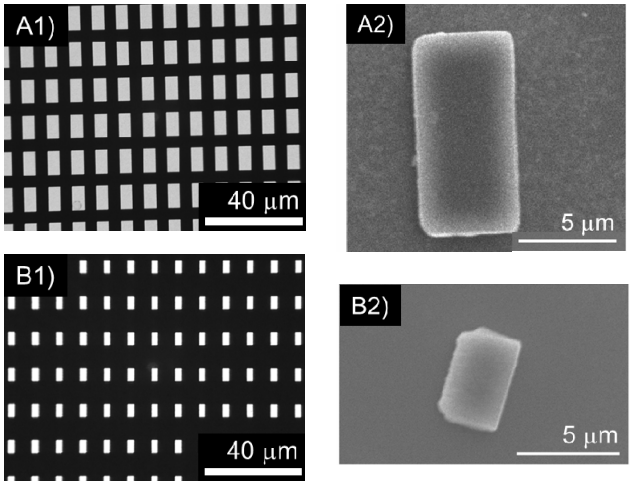

Figure 4: Two types of rectangular parallelepiped microstructures. A1 is mask pattern of the large block. A2 is a SEM image of the large block. B1 is mask pattern of the small block. B-2 is a SEM image of the small block.

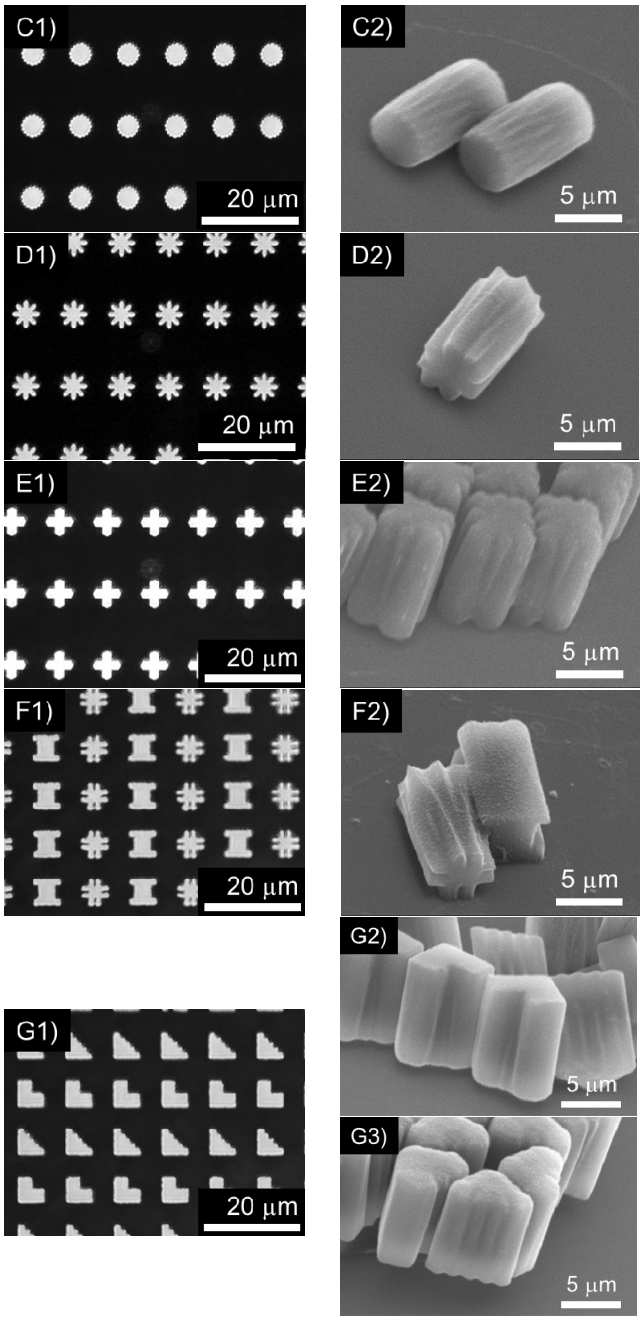

Figure 5: Six types of microstructures. A1-G1 show mask patterns of the microstructures. A2-G3 are SEM images of the structures.

DNA sample

Commercially available yeast chromosomal DNA molecules (CHEF DNA Size standard S. pombe, Bio-Red) were used in our experiments. DNA molecules are embedded in a gel block, which were extracted by heating the block at 70ºC. To visualize DNA molecules, 1 µM YO-PRO-1 (YO-PRO-1 iodide, Molecular Probes)/ DI water was used together with 1.4 mM dithiothreitol (DTT, TAKARA) and 0.05 % TWEEN20. Then, 8 µL of the suspension containing the microstructures was dispensed onto the DNA sample solution on the microscope stage to

be 10 μL in total.

DNA manipulation

We examined DNA manipulation with each pair of microstructures (the large blocks and the small blocks). Two microstructures are trapped by two focused laser beams and handled them like 'chopsticks'. Some groups have reported DNA handling with optically-driven microspheres, involves chemical modification and electrostatic interaction on their surfaces [4]. Our approach is purely mechanical, requiring no chemical modification and no electrostatic interaction. Single DNA molecules were captured between two microstructures and translocated them to elongate the molecules by moving the x-y microscope stage at the speed of 50 μm/s. DNA molecules sometimes escaped from the space between the microstructures due to the flow induced by their motion during the approach. Thus, we evaluated the capture rate of each pair of microstructures by counting the number of trials without escaping DNA from the structures.

Next, we examined DNA manipulation with various geometries of the microstructures in Figure 5 to evaluate the effect of the geometry on DNA manipulation. As described above, DNA molecules were captured between microstructures and translocated by moving the x-y microscope stage at the speed of 50 μm/s. We evaluated the distance of DNA elongation without its escaping from the space between microstructures, using various geometries of the microstructures, from which we determined the manipulation efficiency using the distance as an index.

RESULTS AND DISCUSSION

Microchopsticks dimensions

Figure 7 shows the result of the capture rate of the DNA molecule using each microstructures. We manipulated DNA molecules with commercially available ϕ10 μm microspheres as a control experiment. The capture rate was zero, indicating that the spherical shape has no function for "chopsticks" [8]. In the experiments using block structures, the small one has lower capture rate, which was 16.6%. In contrast, the large one was 37.5%. These results indicate that the large one improves the capture rate, because the area for DNA immobilization is relatively large. On the other hand, the structures larger than the size sometimes cause the interference with the substrate surface in our setup. From these results, we selected the size (10 μm × 5 μm × 4 μm) as manipulation tool of the DNA molecule.

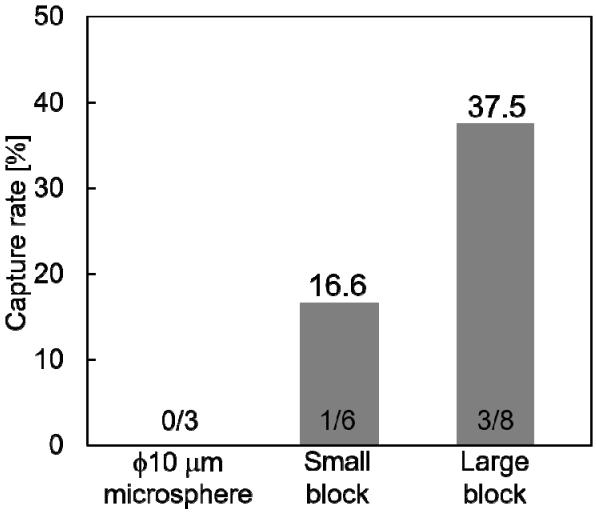

Figure 7: Capture rate of DNA molecules using ϕ10 μm microspheres, the small blocks and the large blocks.

Microchopsticks shapes

We have successfully manipulated single DNA molecules using two microstructures driven by two laser beams, where we handled them like "chopsticks" (Figure 8). Rectangular parallelepiped microstructure allows capture of a DNA molecule and its manipulation to the distance of 22.5 μm (mean value). C2, D2, E2, F2, G2 and G3 microstructures show 15 μm, 30.8 μm, 65 μm, 22.5 μm, 21.7 μm and 28.8 μm of DNA elongation respectively. C2 microstructure shapes show relatively short manipulation distance. In contrast, E2 microstructure shows the longest manipulation distance. These results indicate that the microchopsticks geometry governs the efficiency in manipulating single DNA molecules.

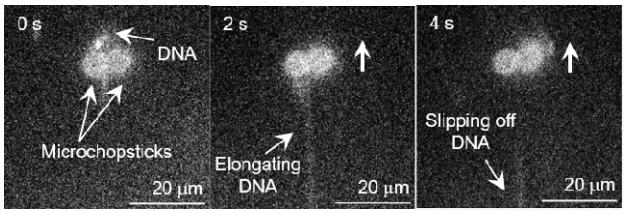

Figure 8: Time-series of fluorescence image in DNA manipulation using the microchopsticks of type E2.

CONCLUSIONS

We have demonstrated the manipulation of single DNA molecules using optically-driven microchopsticks, which are intended for the use in nanotechnology-based DNA analysis. We evaluated the microchopsticks from the view point of their dimensions and shapes. The results of DNA capture rate show that the large block size (10 μm × 5 μm) was adequate to manipulate single DNA molecules. Moreover, DNA manipulation largely depends on the geometry of the microchopsticks, and we determined the effective geometry for elongating DNA molecules without slipping off. These results indicate that the design of the geometry of microchopsticks brings more effective on-site manipulation of single DNA molecules under a fluorescence microscope.

ACKNOWLEDGEMENTS

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