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DNA Size Separation Employing Quartz Nano-Pillars with Different Allocations

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1. Introduction

Fast DNA sequencing is necessary for the tailor made therapy and the genomics drug discovery in the near future. To realize the goal, the speed of DNA separation should be improved over 10^9 times than the present. Recently a channel filled by nano-pillars of 500 nm diameter and 500 nm spacing instead of an usual gel enabled us to achieve electrophoresis separation in 25 sec for λDNA (48.5 kbp) and T4 DNA (165.6 kbp), and furthermore the tandem arrangement of nano-pillars regions allowed to separate smaller DNA of 10 kbp [1].

This report shows single molecule behavior of DNA in pillars, and reveals an important indication of DNA separation mechanism by applying different allocations of nano-pillars for DNA electrophoresis..

2. EXPERIMENTAL

The fabrication method of nano-pillars was reported already by our group [1]. Briefly, 500 nm-diameter holes pattern was delineated by a EB lithography on a posi-type resist spin-coated on a quartz plate. Ni was electroplated at holes and then after resist removal, pillars were fabricated by a dry etching using Ni mask. The diameter and the height of the nano-pillar were 500 nm and 4 μm, respectively. Four pillar regions that the length of each regions was 500 μm (the total pillar region length was 2 mm) were lined up in a channel via 35 μm pillar-free spacing as shown in Fig. 1.

For acquiring the electrophoresis behaviour of single molecule DNA in nano-pillars, T4 DNA with 166 kbp was stained with intercalating fluorescence dye of YOYO-1 and the behavior within nano-pillars was observed by a fluorescence microscope, where the applied electric field was 25 V/cm. Electrophoresis separation of 38 and 10 kbp DNA fragments (obtained by digesting λDNA by ApaI) was investigated by cross-injection into the nano-pillar regions, where the applied electric field for electrophoresis was 50 V/cm. The fluorescence intensity was observed at a point where was 2000 μm distant from the entrance to the pillar region.

3. RESULTS AND DISCUSSION

The allocations of pillars were fabricated at square and tilt types, shown in Fig. 2. A center-to-center distance between pillars was 800 nm. That is, the gap between the 500-nm pillars is 300 nm.

Series of photographs showed conformation changes of DNA in pillars with square (Fig. 3) and tilt allocations (Fig. 4). In square allocation, DNA repeated the cycles of extending and shrinking, and moved straight without the disturbance of pillars. In the case of the tilt allocation, however, DNA is hooked over pillars and repeated the cycles of hooking, extending and shrinking, and did not move straight. Based on the single molecule observation, we compared the relative mobility of DNA in pillars ($\mu_{\text{in pillars}}$) and that in pillar-free regions ($\mu_{\text{in pillar-free}}$). The relative mobility (μ) of DNA, $\mu_{\text{in pillars}} / \mu_{\text{in pillar-free}}$, is shown in Fig. 5. The smaller gap is, the relative mobility is higher. When the gap between pillars is 100 nm, the mobility in square allocation is extremely higher than that in tilt allocation.

We expected that the high speed DNA separation was obtained employing nano-pillar with 100-nm gap, because the electric field in pillars becomes strong when the gap between pillars is small. But we did not clearly observe the DNA size separation in both of square allocation and tilt allocation. Opposed to our expectation, it was considered that the excessive speed hindered nano-pillars from separating DNA by size. Therefore, we employed the nano-pillar with the gap of 300 nm. As shown in Fig. 6, the tilt allocation separated DNA fragments by their size, but the square allocation did not separate DNA fragments.

4. CONCLUSIONS

We considered that the nano-pillars separate DNA according to the sieving effect. Our result indicates that nano-pillars with tilt allocation show the good sieving effect, comparing with square allocation. Considering a distinctive behavior that is shown in nano-pillars in tilt allocation, one of the reasons why nano-pillars have sieving effect is that DNA repeats the cycles of hooking, extending and shrinking in nano-pillars. It is concluded that allocation of nano-pillars is an important factor for the fast DNA size separation.

Acknowledgements

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References

[1] N. Kaji et.al., Anal.Chem.,**76**(1) (2004), pp.15-22.

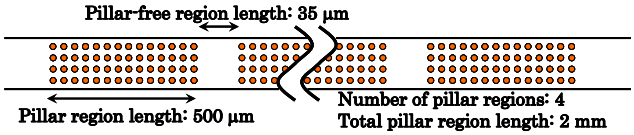

Fig. 1. Dimension of nano-pillar regions.

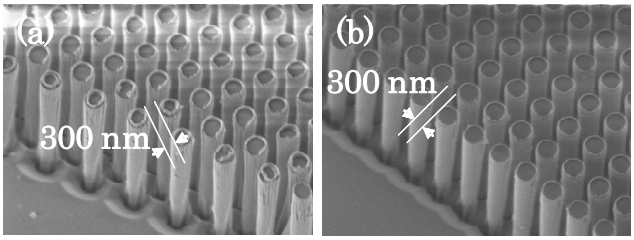

Fig. 2. Nano-pillars with tilted allocation (a) and nano-pillars with square allocation (b). The gaps between pillars are 300 nm.

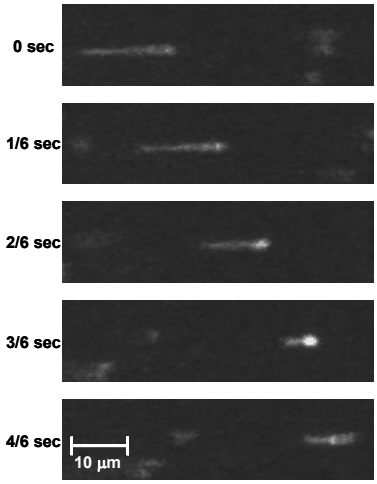

Fig. 3. Single molecule behavior of DNA (166 kbp) in nano-pillars with square allocation.

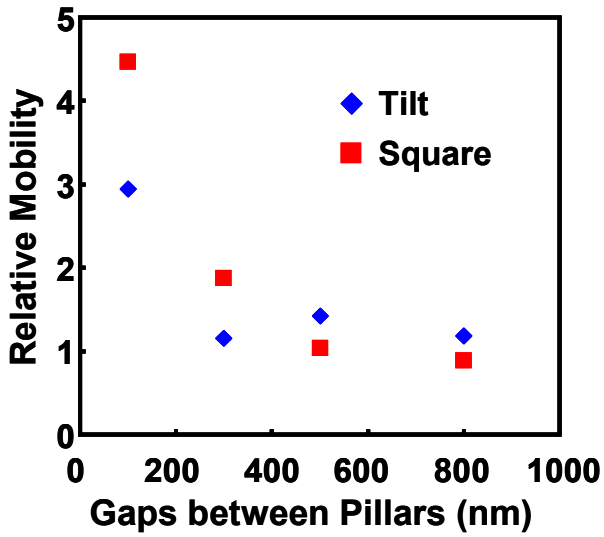

Fig. 5. Relative Mobility of DNA.

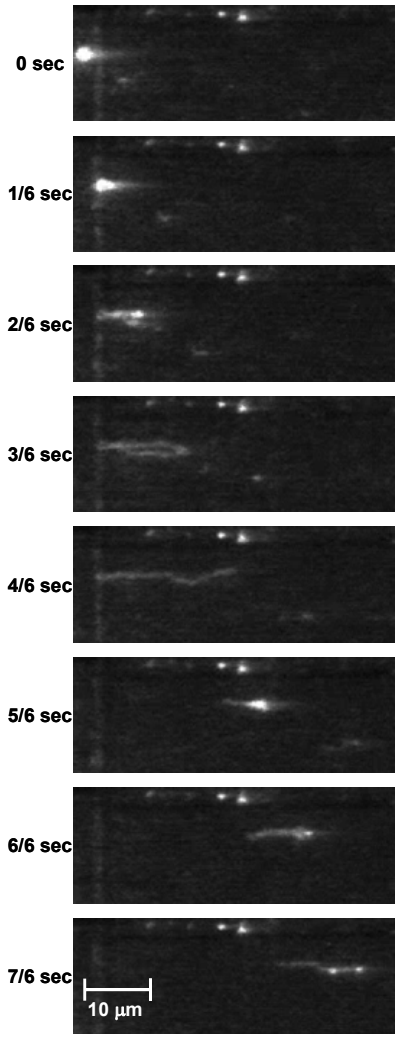

Fig. 4. Single molecule behavior of DNA (166 kbp) in nano-pillars with tilt allocation.

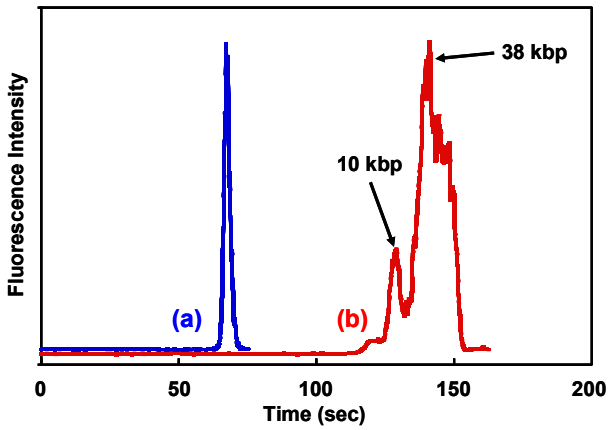

Fig. 6. Electropherograms in square (a) and tilt (b) nano-pillars for 10 kbp and 38 kbp. Intensity of fluorescence stained DNA fragments was observed after DNA fragments traveled 2-mm pillar regions.