NANOPILLAR PARALLEL-ARRAY STRUCTURE WITH DNA TRAPPING AND TORQUE-ASSISTED ESCAPE MODE FOR DNA SEPARATION

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ABSTRACT

We revealed that the nanopillar parallel-array structure has two modes for DNA separation; DNA trapping and torque-assisted escape mode. Single DNA molecule observation revealed that the separation of DNA molecules could be achieved by two modes in the wide range of DNA molecules; 166 kbp to 100 bp.

KEYWORDS

DNA separation, DNA trapping, Torque-assisted escape

INTRODUCTION

Recent developments of nanofabrication techniques have been able to construct various nanometer-sized structures. These highly ordered nanostructures have been used to experimentally elucidate DNA dynamics in confined spaces. On the other hand, for the practical use of these nanofabricated structures as an alternative to conventional separation matrices such as gels or polymers, nanopillar arrays [1], nanowall arrays [2], nanofilter arrays [3], nanofence arrays [4], nanochannels [5], and nanoparticles [6] have been developed. The separation processes using these nanofabricated structures were less time-intensive and fewer manual operations comparing with other conventional methods, however, these separations were all fundamentally based on single separation mode. In this paper, we demonstrated that the nanopillar parallel-array structure could realize DNA separation in torque-assisted escape and DNA trapping mode.

THEORY

Recently, Laachi et al. has pointed out non-equilibrium DNA transport from a viewpoint of theoretical and computer simulation, *i.e.*, torque-assisted escape [7]. In all chromatographic separations, it is commonly believed that there is an intrinsic trade-off between the throughput and the resolution. If we move the system away from equilibrium to increase the throughput, it reduces the resolution. However, non-equilibrium DNA transport could achieve a good balance between the throughput and the resolution. For an estimation of quantitatively non-equilibrium DNA transport, the rotational Péclet number was proposed. The rotational Péclet number Pe_r is expressed as follows

$$Pe_r = \left(\frac{1-\delta}{1+\delta}\right)\left(\frac{\hat{q}E_{av}L^2}{k_BT}\right) \quad (1).$$

At the above equation, δ is the depth ratio, k_BT is Boltzmann factor, q is the effective charge per unit length, E_{av} is an average electric field, and L is DNA contour length.

EXPERIMENTAL

Nanopillar parallel-array structures were fabricated on a quartz substrate using the same procedures as described elsewhere [1,2]. A Pt/Cr layer about 20 nm in thickness was sputter-coated on a 0.5 mm fused silica substrate. Upon this layer, a positive electron beam (EB) resist was spin-coated, and then, a pattern of nanopillar structures was delineated by EB lithography. Ni was electroplated into the hole of the pattern in the EB resist to provide strong resistance in the following etching process. After the EB resist removal, photoresist was spin-coated and a microchannel pattern was transferred by photolithography. The substrate was etched by neutral loop discharge (NLD) plasma using CF_4. Inside 25 μm wide microchannel, a 500 nm wide and 4 mm high nanopillar was fabricated. After removal of the remaining metal and resist layers on the quartz substrate, both of the substrate and a 130 μm fused silica cover plate were dipped into H_2SiF_6 and bonded under pressure of 5 MPa at 65 °C for 12 h.

RESULTS AND DISCUSSION

Figure 1 shows the overview of the nanopillar parallel-array structure. We fabricated the nanopillar parallel-array structure with various spacings. There were two separation modes in the nanopillar parallel-array structure; DNA trapping at the entrance of the nanopillar structure (red-dotted box) and torque-assisted escape mode inside it (blue-dotted box).

In DNA trapping mode, the large size DNA, which gyration radius is greater than nanopillar spacing, was trapped at the entrance of the nanopillar structure, and therefore DNA separation was achieved by the difference of the trapping time associated with DNA length, resulting in short length DNA (still its gyration radius is larger than the nanopillar spacing) migrated faster. In the torque-assisted escape mode [1], the small size DNA, which gyration radius is smaller than nanopillar spacing (rod-like shape), was affected by the external electric field gradient, and longer rod-like DNA molecules (still its gyration radius smaller than the nanopillar spacing) migrated faster than smaller ones due to thermal diffusion. By combining DNA trapping with torque-assisted escape, DNA molecules ranging from 166 kbp to 600 bp were separated; DNA molecules, which have larger gyration radius than the nanopillar spacing, trapped and separated in DNA trapping mode, and then, untrapped DNA molecules were separated in torque-assisted escape.

We measured the trapping time of 20, 48.5 and 166 kbp DNA molecules at the entrance of the nanopillar structure, and time-traced trajectories of 600 bp DNA inside it by observing single DNA molecule. The trapping and the tracking behaviors were summarized in Figures 1(b) and 1(c), respectively. The most trapped time of each size DNA was 0.3, 3, over 1.2 s for 20, 48.5, and 166 kbp DNA, respectively. The straightforward and random-walked trajectories of DNA molecules were shown at 70 and 20 V/cm, respectively.

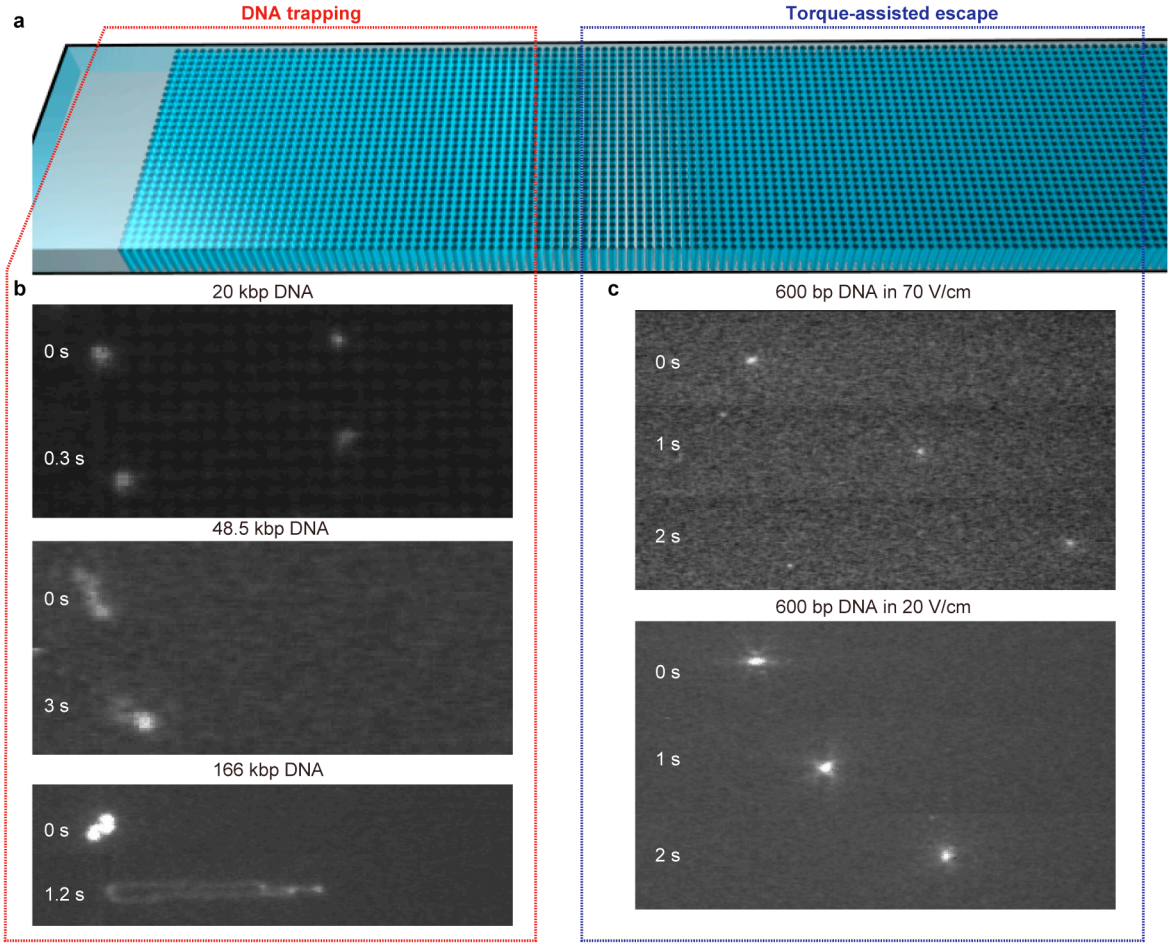

Figure 1. Overview of the nanopillar parallel-array structure with two modes for DNA separation. (a) Schematics of the nanopillar parallel-array structure. Inside the red and blue dotted-box, we showed two separation modes; DNA trapping at the entrance of the nanopillar structure and torque-assisted escape inside the nanopillar structure, respectively. (b) DNA trapping mode; trapping time at the entrance of the nanopillar structure depended on the DNA size. (c) Torque-assisted escape mode; the images showed sequences of single DNA fluorescence image with 1 s time interval.

We could separate DNA molecules based on those two separation modes, as shown in Figure 2. In DNA trapping mode, the separation of 166 and 48.5 kbp DNA by the difference of trapping time were achieved (Figures 2(a)). In torque-assisted escape mode, 300 and 100 bp DNA molecules could be separated at 2450 V/cm (Figure 2(b)). By using two separation modes, it is anticipated that the separation of wide range of DNA molecules will be achieved by controlling the two modes.

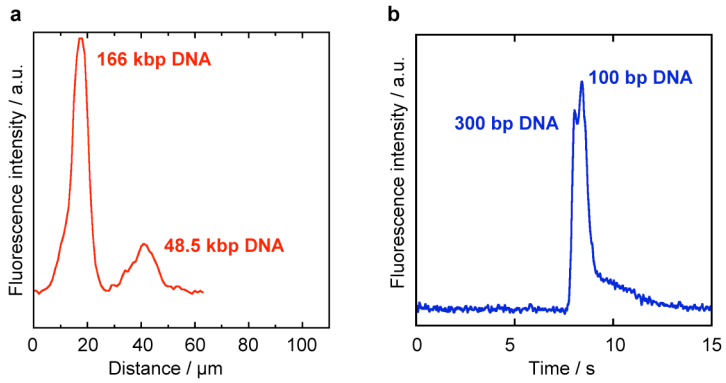

Figure 2. Electrophoretograms of DNA separation in DNA trapping mode, (a), and torque-assisted escape mode, (b). The applied electric fields were (a) 5 V/cm for 72 s, (b) 2450 V/cm.

CONCLUSIONS

In summary, we demonstrated that the nanopillar parallel-array structure has two modes for DNA separation; DNA trapping and torque-assisted escape mode. Single DNA molecule observation revealed that the separation of DNA molecules could be achieved by two modes in the wide range of DNA molecules; 166 kbp to 100 bp. The nanopillar parallel-array structure will offer a significant contribution to progress in the separation of wide-range size DNA molecules, and moves researchers towards the further integration with other nanostructures, such as nanopore DNA sequencing.

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