NANOWALL ARRAY CHIPS FOR DNA SEPARATION
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ABSTRACT
A 25 s separation of DNA fragments (48.5 and 1 kbp fragments) was achieved by a nanowall array structure, although the separation of 1 kbp and 100 bp DNA fragments was not. Direct observation of a single DNA molecule indicates that it takes over 20 s for an elongated DNA molecule to relax inside the nanowall array structure. Numerical fitting of DNA molecular dynamics reveals that the balance between times for the transverse of an elongated DNA molecule and the relaxation process of a DNA molecule inside the nanowall array structure governs the separation of DNA.

KEYWORDS: Nanowell, DNA Separation, Direct Observation of a Single DNA Molecule, DNA Molecular Dynamics

INTRODUCTION
From a practical standpoint, it is important to integrate micro- and nanostructures into micro total analysis systems (µTAS) as separation matrices and to apply size fractionation of biomolecules based on electrophoretic techniques. Recently, we fabricated two types of nanopillar array patterns on quartz substrates, including tilted and square array patterns along microchannels. Separation capability has been demonstrated for tilted array pattern along microchannels, and even large DNA molecules that were difficult to separate under direct current (DC) electric fields could be separated in only 20 s [1]. We found that even the square array pattern along microchannels, which resulted in fewer physical interactions between DNA molecules, could generate a molecular sieving effect, and we achieved DNA separations [2]. Based on the ratio of the radius of gyration (Rg) of a DNA molecule to the pore size a of a matrix gel, which is corresponding to the nanopillar spacing, the separation of DNA molecules could be classified into Ogston sieving (Rg/a < 1), entropic trapping (Rg/a ~ 1), or biased reptation (Rg/a > 1) [3]. Taking into consideration of these regimes as they have been established for gel electrophoresis, it can be said that each migrating DNA molecule in the square array pattern underwent a different molecular sieving effect leading to separation. However, the separation mechanism is still not clear, because physical collisions between DNA molecules and nanopillars are far fewer in number than in the tilted array pattern. Thus, it is unlikely that interactions in the nanopillar region are essential for separation. Unlike the demonstration of the entropic trapping technique [4], the small number of interfaces between nanopillar-free regions and the nanopillar region might not play any role in DNA separation. Therefore, in this study, we fabricated multiple nanowalls, which are defined as the partition-like structures of which thicknesses are less than 1,000 nm and their heights and lengths greater than their thicknesses, into an array structure in order to focus on the contribution of nanospace to DNA separation and to reveal a separation mechanism [5].

THEORY
A DNA molecule is introduced and confined in a cylindrical tube of diameter D (D ≪ Rg), and then the DNA must be elongated to a certain end-to-end length. This confined elongation keeps the DNA stretched in its equilibrium configuration without any external forces, such as hydrodynamic flow or electric fields. Physical properties of DNA, considered as a random walk polymer, have been extensively studied using the nanospace formed by nanofabricated structures, which resulted in verification of the scaling law of self-avoiding confined polymers developed by de Gennes [6]. Although the the spacing between nanowalls, fabricated in this study (as below mentioned), is of sufficient size to confine DNA molecules larger than ca.15 kbp (Rg ~ 200 nm) one-dimensionally, DNA molecules can be relaxed to their equilibrium length along perpendicular axes (x and z) to the electric field (y axis) even under electrophoretic migration because the nanospace formed by the nanowall array structure is 200 nm spacing, 5000 nm high, and 215 µm long. The time to achieve the equilibrium length is termed by the relaxation time τ, and an understanding of this relaxation time τ directly leads to understanding the behavior of DNA molecules in the spacing between nanowalls for electrophoresis, and as a result the effect of the nanowall array on the separation of DNA fragments can be estimated.

The relaxation time τ in a cylindrical tube of diameter D can be numerically estimated from the following equation [7] involving de Gennes’ arguments [6]:

\[ \tau \approx \frac{D^2}{2kT} \]
\[ \tau = \frac{8\pi \eta L^2}{5 k_B T} \left( pw \right)^{2/3} \]  

(1)

where \( \eta \) is the viscosity of the solvent, \( L \) is DNA contour length, \( k_B \) is the Boltzmann constant, \( p \) is DNA persistence length and \( w \) is the width of DNA molecule. Using the relaxation time, the end-to-end length of the DNA molecule \( L(t) \) during the relaxation process at the arbitrary time \( t \) can be roughly estimated by an exponential fitting function described by Mannion et al. [8]:

\[ L(t) = L_e + \left( L_0 - L_e \right) \exp \left( -\frac{t}{\tau} \right) = L_e + \left( L_0 - L_e \right) \exp \left( -\frac{5tk_B TD^{1/3}}{8\pi \eta L^2 \left( pw \right)^{2/3}} \right) \]  

(2)

where \( L_e \) is the equilibrium length in the nanowall spacing and \( L_0 \) is the measured initial length.

EXPERIMENTAL

The nanowall array structure was fabricated on a quartz substrate using the electron beam lithography, the conventional photolithography, and reactive ion etching. Each fabricated nanowall was 500 nm thick, 5000 nm high and 215 \( \mu \)m long, respectively. A spacing between the nanowalls in the array structure was 200 nm. DNA fragments of 100 bp, 1 kbp, and Lambda DNA (48.5 kbp) were stained with bis-intercalating dye, YOYO-1, at a dye-to-base pair ratio of 1:15 for the separation experiments of DNA fragments. T4 DNA (166 kbp) was stained with the YOYO-1 dye at a dye-to-base pair ratio of 1:5 for the observation experiments of a single DNA molecule. For the reduction of photobleaching of DNA stained by YOYO-1, a 3\( \times \)TBE buffer containing 10 mM dithiothreitol was used. All experiments were performed on an inverted microscope equipped with a high voltage sequencer and EB-CCD camera.

RESULTS AND DISCUSSION

As shown in Figs. 1(a) and (b), although the mixture of 48.5 and 1 kbp DNA fragments was successfully separated within 25 s, the mixture of 1 kbp and 100 bp DNA fragments was not. We found that longer DNA fragments migrated faster than shorter DNA ones. The features of the separations were contrary to that of electrophoresis in nanopillar chips with tilted array pattern [1]. Although our results resembled the separation of DNA by the nanopillar chips with square array pattern or the entropic trapping [4], the separation mechanism could not be fully explained by entropic trapping due to the small number of interfaces from the nanowall-free to the nanowall region.

![Figure 1](image_url)

Figure 1: (a) Separation of 48.5 kbp (20 ng/\( \mu \)L) and 1 kbp (50.5 ng/\( \mu \)L) DNA fragments. Electropherograms were taken at 3900 \( \mu \)m from the entrance of the microchannel with the nanowall array structure. (b) Separation of 1 kbp (40 ng/\( \mu \)L) and 100 bp (20 ng/\( \mu \)L) DNA fragments. Electropherograms were taken at 4115 \( \mu \)m from the entrance of the microchannel with the nanowall array structure. In both separations, the applied voltage in the separation channel was 14 V/mm. (c) Plot of contraction length of a T4 DNA molecule as a function of time for the relaxation process in the 200 nm spacing. Numerical model was fitted to the data on the basis of the contraction length of the DNA molecule in a cylindrical tube with diameter of 200 or 5000 nm, to quantify this relaxation process.
To clarify the separation mechanism in the nanowell array structures, Fig. 1(c) shows the plot of the contraction length of a T4 DNA molecule as a function of time. Counterintuitively, the DNA length in the 5000 nm diameter tube gave the best fit to the experimental value, instead of the 200 nm one. These results showed that the confined elongation in the nanowell spacing was governed by the x-axis dimension (200 nm spacing), and the relaxation process in the nanowell spacing was governed by the z-axis dimension (5000 nm height) for this time course.

One possible reason why we could separate DNA fragments in the nanowell array structure is that the migration time of the 48.5 kbp DNA fragment is shorter than the relaxation time at 14 V/mm. Because 48.5 kbp DNA should change its configuration inside the nanowell spacing, its configuration should be a rod-like or an oval sphere. In those cases, the apparent gyration radius ($R_g$) to the hydrodynamic friction is smaller than that in the nanowell-free region ($R_g$), unless the DNA has completely relaxed. This process results in a different velocity between that in the nanowell spacing and in the nanowell-free region, and the DNA velocity eventually increases to some extent inside the nanowell spacing due to the relatively small $R_g$ generated by the confined elongation (the rod-like or the oval sphere configurations). On the other hand, 1 kbp DNA does not change its configuration inside the nanowell spacing because its gyration radius is smaller than the nanowell spacing, and therefore, 1 kbp has a constant velocity during electrophoresis even if it is in the nanowell spacing. From above reasons, the velocity of 48.5 kbp DNA molecules was different from that of 1 kbp DNA molecules, resulting in the separation of these DNA molecules inside nanowell array structures.

CONCLUSION

In summary, we realized the separation of DNA fragments within 25 s using a nanowell array structure. To measure the contribution of the nanospace to the separation of DNA molecules, we carried out the direct observation of a single DNA molecule. From the findings, we identified the separation mechanism in the nanowell array structure and discussed it mainly from the viewpoint of molecular confinement in the nanowell spacing. Considering all the various factors, not only the entropic trapping, but also the balance between transverse and relaxation times might be dominant factors for DNA separation in the nanowell array structure, which allowed larger DNA fragments to migrate faster than smaller ones. Since this highly controlled nanostructure can determine the separation mechanism by the structure design, we expect it to provide a core technology for further development of μTAS.

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