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Separation of Long DNA Molecules by Quartz Nanopillar Chips under a Direct Current Electric Field

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We have established the nanofabrication technique for constructing nanopillars with high aspect ratio (100−500 nm diameter and 500−5000 nm tall) inside a microchannel on a quartz chip. The size of pillars and the spacing between pillars are designed as a DNA sieving matrix for optimal analysis of large DNA fragments over a few kilobase pairs (kbp). A chip with nanopillar channel and simple cross injector was developed based on the optimal design and applied to the separation of DNA fragments (1−38 kbp) and large DNA fragments (λ DNA, 48.5 kbp; T4 DNA, 165.6 kbp) that are difficult to separate on conventional gel electrophoresis and capillary electrophoresis without a pulsed-field technique. DNA fragments ranging from 1 to 38 kbp were separated as clear bands, and furthermore, the mixture of λ DNA and T4 DNA was successfully separated by a 380-μm-long nanopillar channel within only 10 s even under a direct current (dc) electric field. Theoretical plate number *N* of the channel (380−1450 μm long) was 1000−3000 (0.7×10^6−2.1×10^6 plates/m). A single DNA molecule observation during electrophoresis in a nanopillar channel revealed that the optimal nanopillars induced T4 DNA to form a narrow U-shaped conformation during electrophoresis whereas λ DNA kept a rather spherical conformation. We demonstrated that, even under a dc electric field, the optimal nanopillar dimensions depend on a gyration radius of DNA molecule that made it possible to separate large DNA fragments in a short time.

Many microdevices for a chemical and a biochemical analysis, so-called μTAS, have been developed[1] owing to their features such as design flexibility, ease of fabrication, and on-chip integration of whole laboratory procedures. These chip-based analytical systems for a size-based DNA separation have resolved the limiting factor, which is mainly caused by Joule heating from the power supply on a gel electrophoresis apparatus, owing to the large specific surface area of a micrometer-sized channel constructed on a chip, and have provided fast analysis with a very small amount of sample. But they still require filling sieving matrixes in a microchannel for DNA separation because a separation mechanism, molecular sieving, has been the same so far. Accordingly, the DNA separation range using a chip-based analytical system is almost the same as that of a conventional gel electrophoresis despite fast separation with a high resolving power. Furthermore, experimental difficulties such as filling high-viscosity sieving matrixes become increasingly difficult with the miniaturization of a microchannel. To defeat these limitations based on the separation mechanism and the experimental operation, a novel analytical method based on a completely different physical effect, so-called entropic trap, that is not obtained in a microspace but in a nanospace has been proposed.[2−6] Additionally, alternative sieving matrixes such as a superparamagnetic particles,[7] a microfabricated array of asymmetrically arranged obstacles,[8,9] or micrometer-sized pillars have been used.[10−12] However, the micrometer-sized pillars have been fabricated with the intention of separating very large DNA molecules over a few

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hundred base pairs (bp). The large DNA separation using micrometer-sized pillars has been done by applying a pulse field. No successful results applying a dc field to the micrometer-sized pillars have been reported. In this paper, we report that nanosized pillars realize large DNA separation despite applying a dc field within only 10 s. Nanosized pillars are one of the possible solutions to realize highly efficient separation, but their potentials as separation matrixes are still unclear.

Though most nanosized devices are fabricated on a silicon substrate, they have some undesirable properties. For instance, even if a silicon surface is sealed with an insulation layer, a dielectric breakdown is inevitable after applying a few kilovolt electric field, as is the general case with the separation science of biomolecules. Moreover, a glass cover or a transparent cover is required for optical detection systems such as fluorescence detection or UV absorbance. Coexistence of different surface properties may produce a complex electroosmotic flow (EOF) and influence the resolution. Considering the disadvantages of a silicon substrate for electrophoresis, we chose quartz as a chip substrate and fabricated a nanosized pillar structure as a substitution for conventional sieving matrixes and then covered it with a quartz plate. The greatest advantage of this nanopillar structure is its capability to modify "defined" space at will, aiming for a highly efficient DNA separation. Because of the simple and homogeneous structure, the nanopillar structure is expected to give precise experimental data for resolving an inconsistency between theoretical models and past experimental data mainly caused by an inhomogeneous gel structure or a complex fluid polymer. It would facilitate an integration of whole bioanalysis systems since the nanopillar structure does not require a sieving matrix. Consequently, the nanopillar chip is one of a promising analytical method to realize highly efficient DNA separation.

EXPERIMENTAL SECTION

Device Fabrication. The nanopillar chip was fabricated on a quartz plate by electron beam (EB) lithography, Ni electroplating, and neutral loop discharge (NLD) etching, followed by bonding of a quartz cover plate. The fabrication process is illustrated in Figure 1A. Thin (∼100 Å) Cr film was sputtered on the quartz substrate. Thick (∼12 000 Å) posi-EB resist (ZEP-520, Zeon Corp.) was spin-coated, and the pillar pattern was delineated by EB lithography. To produce high aspect ratio nanopillars, Ni (∼10 000 Å) as a mask for SiO_2 dry etching was electroplated into the pattern using the Cr (∼100 Å)/ Pt (∼100 Å) film as cathode, in a 60 °C hot bath, which contained nickel(II) sulfate hexahydrate (500 g/ L; Kanto Chemical Co.), boric acid (35 g/ L; Kanto Chemical Co.), saccharin (2 g/ L; Kanto Chemical Co.), and sodium dodecyl sulfate (0.2 g/ L; Wako Chemical Industries, Ltd.). The rest of the flow path in the chip was covered with thick (∼20 000 Å) photoresist (OFPR-800, Tokyo Ohka Kogyo Co., Ltd.) employing photolithography. Nanopillar patterns were dry-etched employing a planar-type neutral loop discharge (NLD) with a mixture of CF_4 and C_3F_8. A mixed solution of sulfuric acid and hydrogen peroxide removed the Ni mask on the quartz plate. The surfaces of a quartz cover plate and the patterned plate were treated with a mixed solution of ammonia and hydrogen peroxide and left overnight in contact with each other under pressure at room temperature. After that, they were directly bonded at 1100 °C for 3 h without applying pressure.

Electrophoresis and Fluorescence Measurement. Various sizes of DNA were used for DNA separation. One kilobase pair DNA fragments (Gensura Laboratories, Inc., Del Mar, Spain), bacteriophage λ DNA (Takara, Shiga, Japan), T4 DNA (Nippon Gene, Tokyo, Japan), λ-DNA digested by *Hind*III (Nippon Gene, Tokyo, Japan), and λ-DNA digested by ApaI (Takara, Shiga, Japan) were all stained with an intercalating fluorescence dye YOYO-1 (Molecular Probes) for fluorescence observation. The dye-to-base pair ratio of the final working solution was ∼1:10. The final DNA concentrations were 20−100 μg/ mL for DNA separation and ∼50 ng/ mL for a single DNA observation in the 0.5× TBE electrophoresis buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0) containing 4%(v/ v) 2-mercaptoethanol to reduce photobleaching. Prior to the electrophoresis, a glass surface in a microchannel was dynamically coated by immersing 1%poly(vinylpyrrolidone) (M_w 1 300 000, Aldrich) to reduce EOF,[13] and then, the solution was substituted with 0.5× TBE electrophoretic buffer. DNA separation and a single DNA molecule observation were done on an inverted microscope (Axiovert 135TV, Carl Zeiss, Tokyo, Japan), illuminated by a 100-W mercury arc lamp and using a 10×/ 0.3 NA and a 100×/ 1.3 NA objective lens (Carl Zeiss) for DNA separation and a single DNA observation, respectively. A No. 9 filter set (Carl Zeiss) was used for the fluorescence imaging. DNA bands and motions of an individual DNA molecule were captured by a SIT camera (C2400-08, Hamamatsu Photonics, Hamamatsu, Japan), recorded on a DV tape (DSR-11, SONY, Tokyo, Japan) and then analyzed later by an image-processing software (Cosmos32, Library Inc., Tokyo, Japan).

Safety Consideration. Fabrication of nanodevices requires extensive cleanroom processing, which includes chemical and plasma etching and both electron beam and photolithography. All the necessary safety precautions should be followed for this processing. YOYO-1 dye is a possible mutagen, and 2-mercaptoethanol is toxic, so gloves should be worn when the solutions are handled. In applying high voltage for electrophoresis experiments, standard electrical safety precautions should be taken.

RESULTS AND DISCUSSION

A nanofabrication technique for constructing nanopillars in the range of 100−500 nm inside a microchannel on a quartz chip was established. Panels B−E of Figure 1 show nanopillar structures with different aspect ratios before sealing by a quartz cover plate. The Ni electroplated mask technique enabled us to construct a high aspect ratio nanopillar on a quartz substrate without a rugged side wall. In our first attempt to fabricate nanopillars on a quartz substrate, a Cr layer, which is conventionally used for semiconductor fabrication, was used as a mask for SiO_2 dry etching. In this fabrication process, however, an upper side of the Cr mask was gradually damaged proportional to the etching time employing NLD and become a cone shape on the quartz substrate. This deformed mask converged ions, which are generated by NLD, to the middle of nanopillars and resulted in rugged sidewall nanopillars as shown in Figure 1F. So the Ni electroplated mask is a core technique to control the sidewall inclination and fabricate such a high aspect ratio nanopillar. For the use of electrophoresis chips, perfect sealing of a microchannel is required because buffer leakages, as little as it may be, give rise to nonuniform fields and

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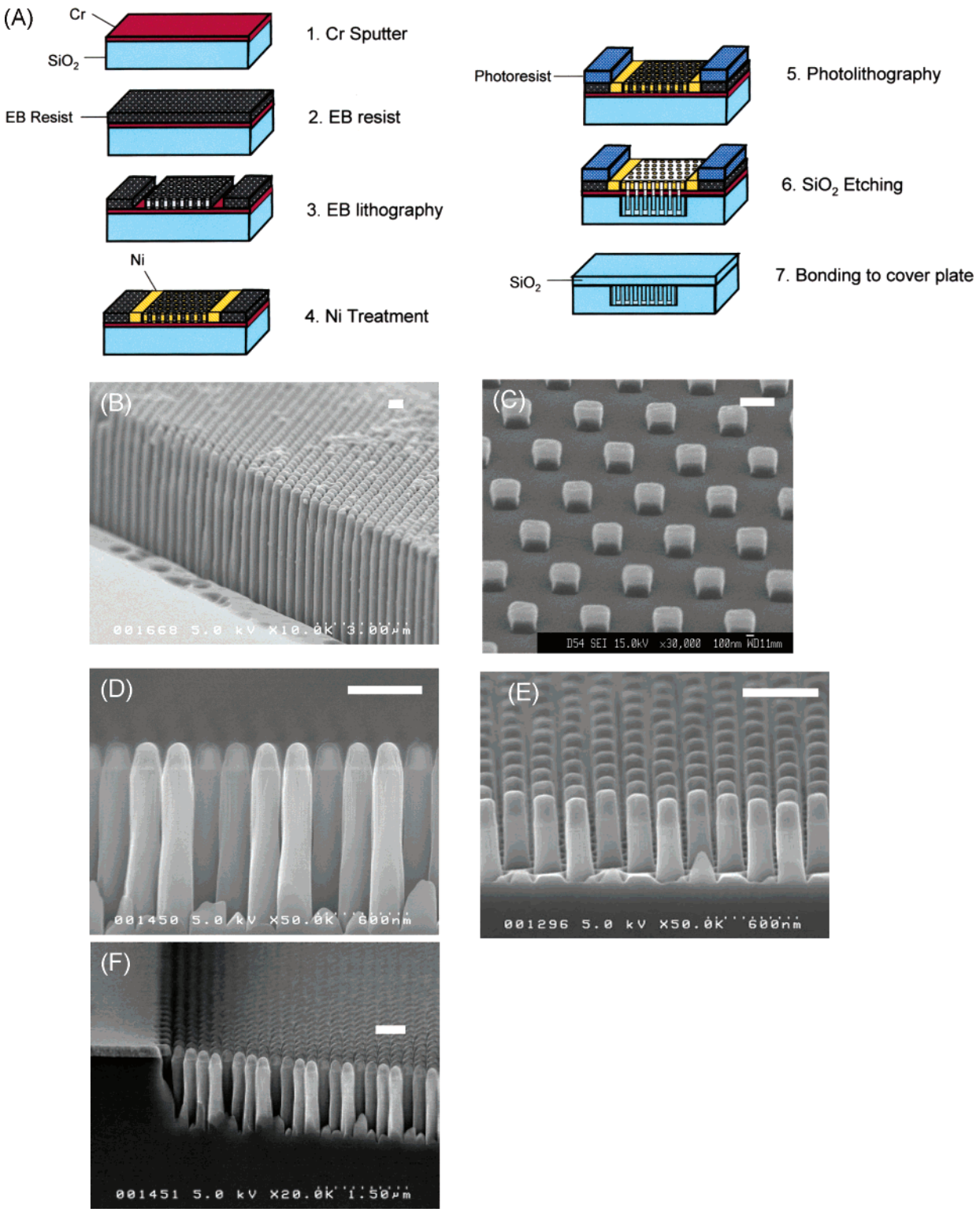

Figure 1. (A) Schematic description of nanofabrication processes. (B−E) Nanopillar structures fabricated on a quartz glass plate before sealing by a cover plate. The nanopillars dimensions were 200 nm wide and 4000 nm tall (aspect ratio, 20), 500 nm wide and 500 nm tall (aspect ratio, 1), 200 nm wide and 1200 nm tall (aspect ratio, 6), and 200 nm wide and 600 nm tall (aspect ratio, 3) in (B−E), respectively. (F) Nanopillar channel fabricated with Cr mask for SiO_2 dry etching. Damaged Cr mask during NLD caused a nonuniform etching and results in rugged sidewall nanopillars. The scale bars are all 500 nm.

disturb DNA migrations. Additionally, each pillar head must adhere to a cover plate to perform as a sieving matrix. The bonding method used here meets these requirements and enable us to seal nanopillar arrays inside a microchannel perfectly.

We measured the migration velocity of a single DNA molecule in a nanopillar channel[14] and predicted that a nanopillar channel

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with 500-nm spacing was more efficient than one with 300-nm spacing for a large DNA separation. In addition, the pore diameter of an agarose gel at a concentration of 1%, which is applicable for a size range of about 250−12 kbp[15] was directly determined as ~500 nm by AFM imaging.[16] So, in this paper, a quartz-made nanopillar chip, which consists of nanopillars (500 nm diameter and 2700 nm tall) with 500-nm spacing inside a 25-μm-wide and 2700-nm-deep microchannel, was employed. To exclude an entropic effect obtained from a vertical confinement, the nanopillar height and microchannel depth (2700 nm) were adopted for being a larger space than the gyration radius of DNA used here. Owing to this vertical space, we can simplify the discussion about a molecular sieving effect. The reasons why we adopted a 25-μm-width microchannel are the following: The first reason is attributable to the technical problem. When the width is larger than 25 μm, it takes an extremely long time to delineate so large area by EB lithography. Second, when the width is smaller than 25 μm, a quantity of injected sample becomes smaller and our detection system would not detect such a small amount of sample. From these two points of view, we thought that a 25-μm-width microchannel is reasonable for this experiment and was adopted. As shown in Figure 2, short nanopillar-free regions were placed at regular intervals as a detection point because nearly the same size order of nanofabricated structure and a fluorescence wavelength was presumed to cause

When DNA molecules intruded into the nanopillar channel, they migrated at different velocities according to their molecular weights. The mixture of a 1-kbp fragment and λ-DNA digested by *Apa*I (10.1 and 38.4 kbp) were separated in ~170 s as shown in Figure 3. In this run, a migration distance of 380 μm was sufficient to resolve λ-DNA digested fragments, 10.1 and 38.4 kbp, whereas both of the 1- and 10.1-kbp fragments were still observed as a single band. As the migration distance was extended, the fragments of 1 and 10.1 kbp appeared at their own migration velocity and separation was achieved at the third detection point (1450 μm below from the nanopillar entrance). Each peak can be assigned to the labeled size in Figure 3 for the following reasons: First, the peak height and area had a direct correlation with the sample DNA concentration. Second, in this optical setup using a microscope, we can roughly distinguish a band that consists of small fragments or large fragments. Small DNA fragments up to a few kilobase pairs could not be observed as their single molecule shapes inside the band through 40-power objective lens. But in the case of large DNA fragments, shallow channels (~2700 nm) prevent large DNA molecules from a vertical overlapping and enable us to observe a single molecule shape inside the band. From the above two points, it was confirmed that DNA molecules migrate in ascending order. These results indicate that the nanopillars produce a molecular sieving effect and can work as a DNA sieving matrix as well as conventional gels or polymers. To validate the quality of the separation, we calculated the resolution, which is defined by the ratio of the distance between two peaks to the average peak width: $R_s = \Delta X / {}^1/_2(W_1 + W_2)$. The resolution in the range of 1−10.1 kbp at 1450-μm migration distance was 1.45. For DNA fragments of 10.1−38.4 kbp, the resolution was

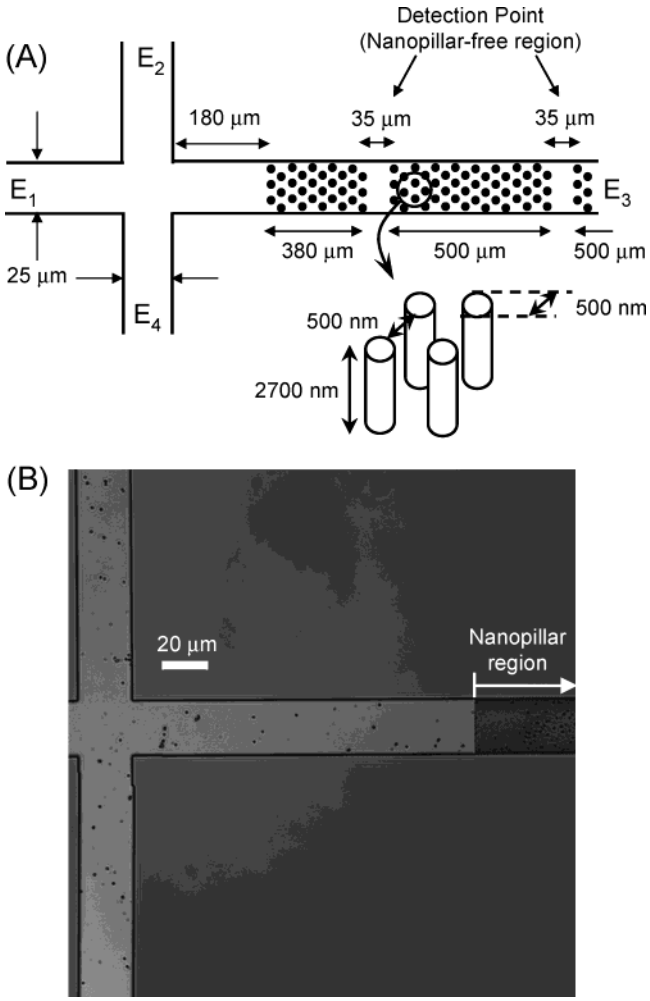

Figure 2. (A) Schematic representation of a microchannel equipped with nanopillars for a large DNA separation. Each nanopillar (a black dot) diameter, spacing, and height used here was 500, 500, and 2700 nm, respectively. Short nanopillar-free regions were placed at regular intervals to avoid a fluorescent light scattering. (B) Optical image of nanopillar chip around the cross channel.

2.69. Though a resolving power of the nanopillar chip was basically the same or less compared with gel electrophoresis or capillary electrophoresis, a high-speed separation was achieved as a microchip electrophoresis. Theoretical plate number N, which characterizes the efficiency of a molecular separation system, for this nanopillar channel (380−1450 μm long) was 1000−3000 (0.7×10^6−2.1×10^6 plates/m). Though the run-to-run and the chip-to-chip reproducibility of the relative mobility was better than 5 and 10%, respectively, the reproducibility of migration time was ~20%. In the nanopillar experiment, a DNA migration velocity is likely to vary with each experiment despite applying the same field. It is attributable to the extremely small amount of buffer solution (~30 pL in this case) because, with such a small amount of buffer in the nanopillar chip, the buffer composition could be easily changed when a field is applied for a long time. In consequence, the current in the nanopillar channel is affected by the buffer composition change and it is not surprising if the migration velocity is different under the same separation condition. This susceptibility to a buffer composition change is an inevitable factor for the nanopillar chip.

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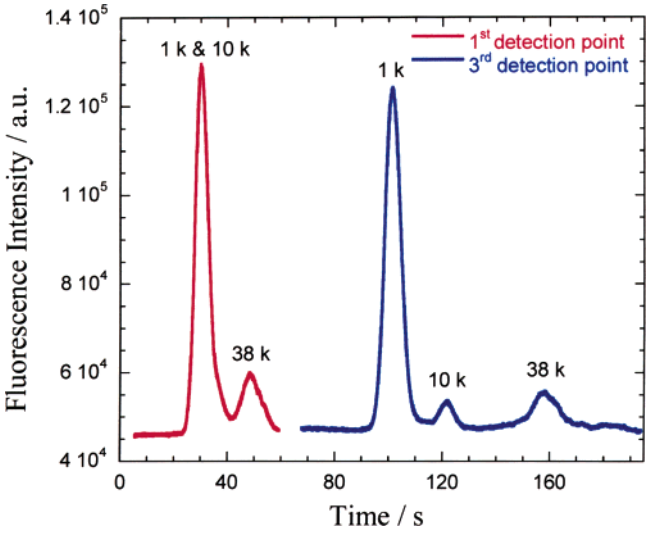

Figure 3. Electropherograms detected at 380 (red solid line) and 1450 μm (blue solid line) from the entrance of nanopillar channel. Separation of the mixture of 1-kbp fragments and λ-DNA digested by *Apa*I (10.1 and 38.4 kbp) was confirmed. The reservoir for sample loading had DNA concentrations of 1-, 10.1-, and 38.4-kbp fragments at 74, 4.2, and 16 μg/mL, respectively. The peak height and width showed a good correlation with DNA concentrations. Conditions: In sample loading phase, potential at E_2 well was 100 V, and E_1, E_3, and E_4 wells were grounded. In separation phase, potentials at E_2, E_3, and E_4 wells were 100, 110, and 100 V, respectively, and the E_1 well was grounded.

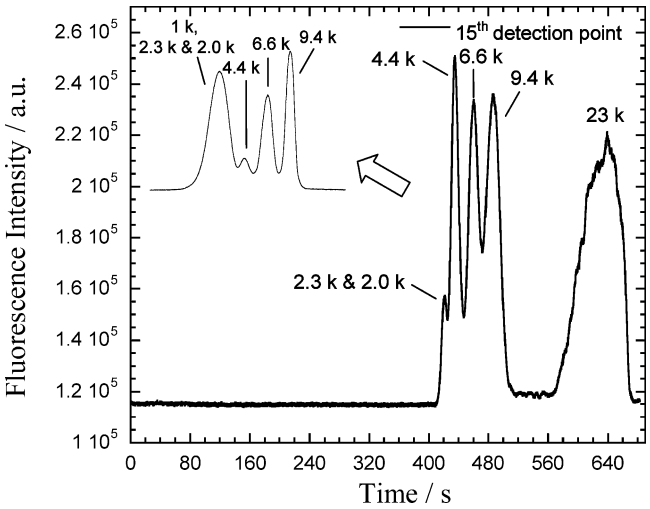

Figure 4. Separation of λ-DNA digested by *Hind*III, which consists of eight fragments of different sizes, 125, 564, 2027, 2322, 4361, 6557, 9416, and 23 130 bp, at 7870 μm from the entrance of nanopillar channel. Conditions are identical to these in Figure 3. A video clip of this separation is available with the Supporting Information to this report. Inset: Electropherogram after adding 1-kbp fragments to λ DNA digested with *Hind*III for peak identification. Read the main text for more detail.

Using this nanopillar chip, λ-DNA digested by *Hind*III, which consists of eight different fragments of DNA size, was analyzed. As shown in Figure 4, the DNA fragments were separated as five distinct peaks at the 15th detection point (7870 μm below from the nanopillar entrance) in 10 min. As mentioned in the above paragraph, each peak was assigned to the DNA band as indicated in Figure 4 based on a peak height and area and also by direct observation. Additionally, for peak identification, we analyzed a mixed sample of a 1-kbp fragment and λ-DNA digested by *Hind*III and then found that the peak of the 1-kbp fragment appeared as being overlapped around the first peak (inset of Figure 4). In this run, the shortest two fragments were missing. It can be explained by the following two possibilities: One is that the shorter four fragments from 125 to 2.3 kbp could not be separated completely under this condition. Another possibility is the shortest two fragments, 125 and 564 bp, being missing. The amount of injected DNA molecules of 125 and 564 bp in the nanopillar channel are estimated at 0.44 and 2.0 fg, respectively. The gain of the SIT camera was too low to detect such a weak fluorescence generated from a trace amount of DNA. The resolutions, R_s, were 0.93 (2.0/2.3 and 4.4 kbp), 1.08 (4.4 and 6.6 kbp), 1.04 (6.6 and 9.4 kbp), and 2.39 (9.4 and 23 kbp), respectively, with 7870-μm migration distance. Theoretical plate number N was 600−17 000 (0.07×10^6−2.1×10^6 plates/m). Though the optimal design of a nanopillar channel such as a nanopillar diameter, spacing between the pillars, and optimal separation conditions including an applied voltage is still required, these results clearly illustrated the potential of the nanopillars as a DNA sieving matrix and were expected to shorten the separation time as compared to a conventional gel electrophoresis and a capillary electrophoresis apparatus.

To test which existing mechanism is applicable to our results, we plotted the data of DNA mobility versus DNA size as shown in Figure 5. The relationship between the logarithm values of relative mobility and those of the sizes of DNA had been first examined. From Figure 5A, it could be seen that there is no linear relationship between them, which means that under our experimental conditions, the reptation model is inappropriate to describe migration in the nanopillar channel. While, on the other hand, our result, in Figure 5B, shows great improvement in the linear regression coefficient between the logarithm values of relative mobility and the size of DNA in the range of 1−23 kbp. This demonstrates that DNA electrophoretic behavior in a nanopillar chip seems to obey the Ogston model,[17] in which the DNA molecule is assumed to be a globular particle passing through a random network with an average characteristic pore size, in this size range.

Our next interest was an applicable range of DNA size for separation in the nanopillar chip. A mixture of λ DNA (48.5 kbp) and T4 DNA (165.6 kbp) was separated in a very short time and separation distance, nevertheless applying a dc electric field. Panels A and B of Figure 6 show video images of the process of λ DNA and T4 DNA separation in the nanopillar chip and fluorescence intensity profiles corresponding to each left fluorescence image along a separation channel, respectively. As shown in Figure 6A, after a sample plug injects into a separation channel by a simple cross-injection method, the sample plug migrates in a nanopillar-free region toward a nanopillar region with a slight diffusional broadening due to self-diffusion, but any separation was not observed. Following the intrusion of a sample plug into the nanopillar region, the fluorescence intensity was apparently decreased due to light scattering. Fluorescence intensity profiles along a migration direction, shown in Figure 6B, indicate that two bands begun to separate at 4 s and each band could be confirmed after 8 s. During this separation process, only the 300-μm

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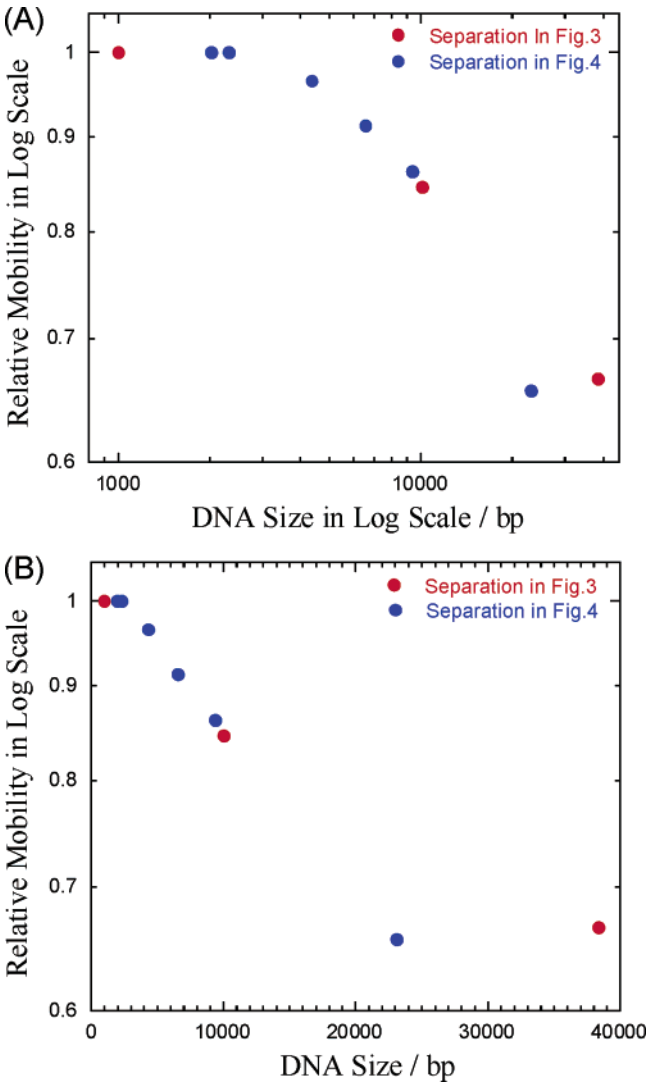

Figure 5. (A) Log−log plot and (B) semilog plot of DNA relative mobility versus DNA size obtained in the separations shown in Figure 3 and Figure 4.

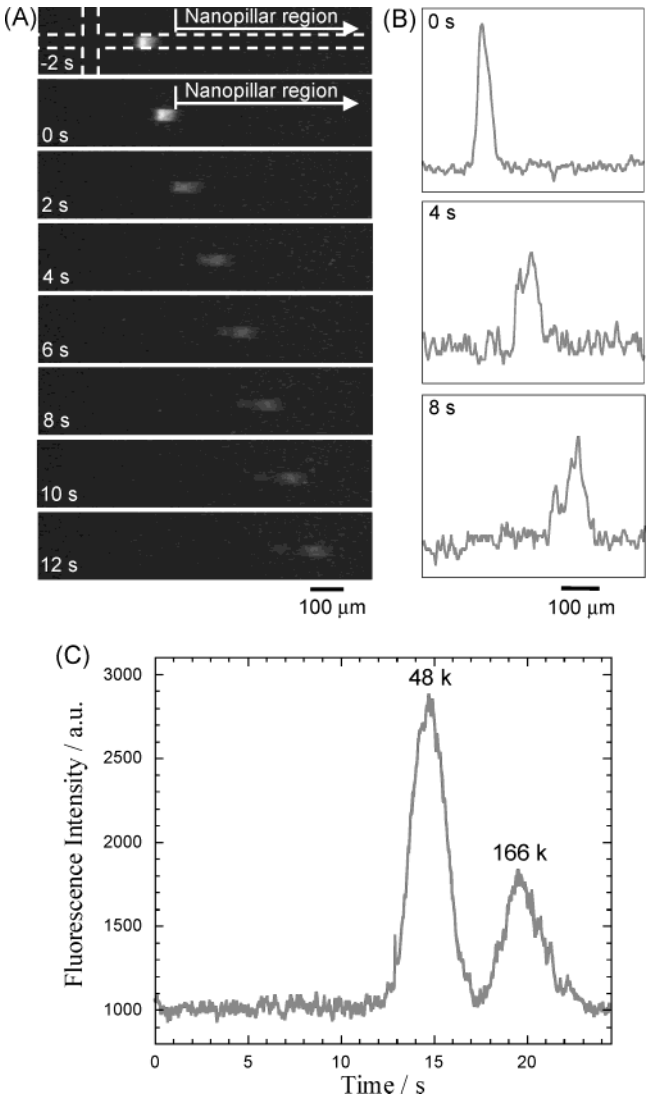

Figure 6. (A) Consecutive fluorescence images of λ DNA and T4 DNA separation at 5 kV after injection into a nanopillar region. White dotted lines show an external view of the microchannel. At 0 s, sample plug began to inject into the nanopillar region. Each separated band was confirmed as each DNA—right and left bands were λ DNA and T4 DNA, respectively—by observation using a 40-power objective lens. (B) Fluorescence intensity profiles along a migration direction. Each profile corresponds to the left fluorescence images shown in (A). (C) Electropherogram of λ DNA (48 kbp) and T4 DNA (166 kbp) separation at the first detection point (380 μm below from the nanopillar entrance). Conditions: In sample loading phase, potential at E_2 well was 300 V, and E_1, E_3, and E_4 wells were grounded. In separation phase, potentials at E_2, E_3, and E_4 wells were 500, 5000, and 500 V, respectively, and the E_1 well was grounded. The DNA migration velocity was rather slow compared with the applied field (~5.0 kV) and the foregoing experiments. In this run, bubbles were observed in some nanopillar-free regions and they might cause a conduction failure linked to the anomalous migration velocity.

migration distance was sufficient to separate both DNAs. The DNA bands are surely detected as separated bands at the first detection point as shown in Figure 6C.

Figure 7A shows the electropherograms of the λ DNA and T4 DNA separation at each detection point. The resolution was 0.89 and 1.82 at the first and third detection points, respectively. Three bands could be resolved in the 48−166-kbp range with 1450-μm migration distance under the condition used here. This result indicates that even such long DNA molecules can be separated in a pseudo-gellike structure. Moreover, to our knowledge, a standard agarose gel electrophoresis under a dc field loses its resolving power for DNA molecules longer than ~40 kbp[3,15] and other techniques, such as electrophoresis with pulsed field,[11,12,18] entropy trapping,[3,4] or entropy recoiling separation,[5] are required to defeat this limitation. In contrast to this λ DNA and T4 DNA separation, the mixture of 200 and 1000 bp was never separated on the same nanopillar chip with a short migration distance as shown in Figure 7B. To achieve separation of such small DNA fragments by nanopillars, much smaller nanopillars with smaller spacing are required. However, on a quartz substrate, a fabrication of smaller nanopillars (~100 nm) is difficult. At present, the nanopillar chip shows a low resolving power for small DNA molecules.

To understand these different results between a gel and a nanopillar system qualitatively, we performed a single DNA molecule observation in the nanopillar region. When a long DNA molecule is driven through a gel, the DNA chain frequently loops

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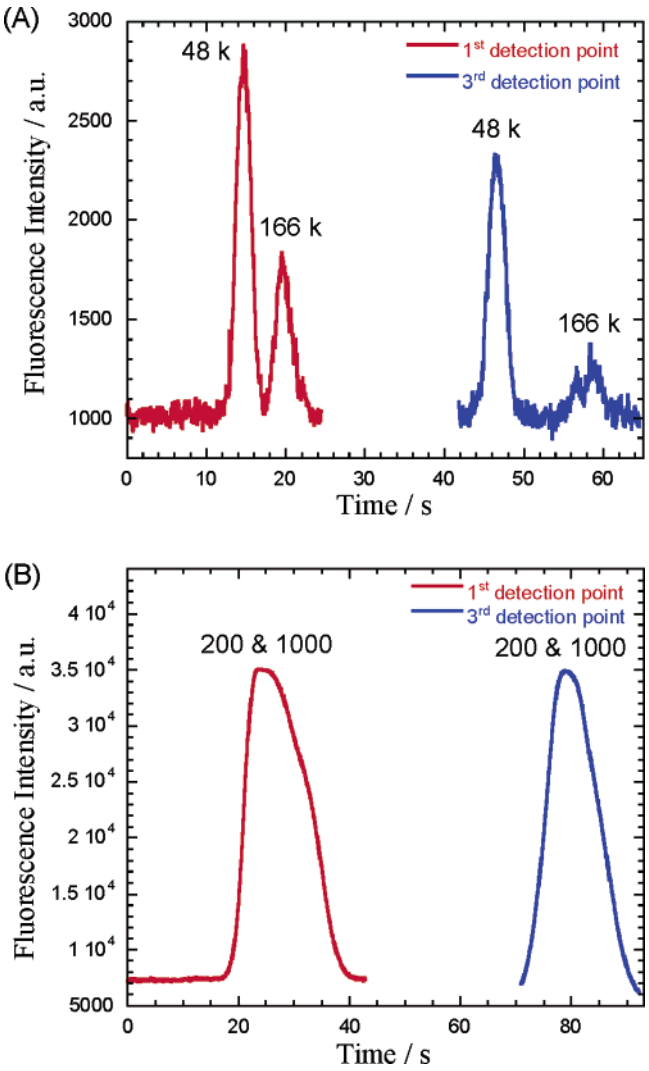

Figure 7. (A) Electropherograms of λ DNA and T4 DNA separation on the nanopillar chip at each detection point. The separation conditions are identical to these in Figure 6. (B) The mixture of 200- and 1000-bp DNA was not separated on the nanopillar chip with a short migration distance, which accomplished the separation of λ DNA and T4 DNA. The separation conditions were as follows: In sample loading phase, potential at E_2 well was 100 V, and E_1, E_3, and E_4 wells were grounded. In separation phase, potentials at E_2, E_3, and E_4 wells were 100, 110, and 100 V, respectively, and the E_1 well was grounded.

around gel fibers, extends to the U-shape in the field, and relaxes into a more compact state.[19,20] These dynamics during gel electrophoresis were also observed in the nanopillar region as shown in Figure 8A and B. In addition, a high electric field (>35 V/cm) generated a frequent trapping by a nanopillar with formation of an extended U-shape and prevented λ DNA and T4 DNA molecules from a continuous migration. This fact is also consistent with macroscopic observation during an agarose gel electrophoresis.[21]

A scrutiny of the DNA electrophoretic behavior revealed that λ DNA spent most of the time with a compact conformation and occasionally formed a U-shape conformation. Panels A and B of Figure 8 show consecutive fluorescence images of a single λ DNA and T4 DNA electrophoretic behavior in a nanopillar channel. Figure 8C shows time sequences of R_l, which is the radii along the horizontal axis of λ DNA and T4 DNA exhibited in Figure 8A and B. Though the radii along the vertical axis of both DNAs keep a constant value, a periodic change of R_l indicates a continuous conformational change as mentioned above due to a collision with nanopillars. We can see this periodic conformational change in T4 DNA migration during electrophoresis. On the other hand, λ DNA showed this periodic change during few seconds and then migrated while retaining a compact state. We interpreted these two distinct behaviors in the nanopillar region from the standpoint of a gyration radius given by

$$R_g = \frac{1}{\sqrt{6}}\sqrt{bL} \qquad (1)$$

where L is the contour length.[23] From eq 1, R_g of λ DNA and T4 DNA are estimated as 520 and 970 nm, respectively. The 500-nm spacing allows λ DNA to migrate through the nanopillar region with a rather spherical conformation, whereas it forces T4 DNA to deform. Even if λ DNA comes into collision with a nanopillar, such a wide nanopillar equivalent to the gyration radius is unfavorable to loop around. These different migration behaviors in the nanopillar region also remind us that the DNA electrophoretic behaviors are classified into different electrophoretic regimes: Ogston sieving, reptation without orientation, and reptation with orientation.[24] Unfortunately, these models are not sufficient to quantitatively describe DNA dynamics during electrophoresis. But qualitatively, these models can account for the fact that λ DNA and T4 DNA belong to different regimes. The motion of λ DNA might appear us as Ogston or reptation without orientation regime rather than reptation with orientation regime. In contrast, T4 DNA, which shows the cyclic motion, seems to obey reptation with orientation regime that produces no separation under a dc field. These different attributes make it possible to separate long DNA, which usually leads to no separation or band broadening in agarose gel, even under a dc electric field. This concept, which is from the standpoint of a gyration radius, also gives a reasonable explanation why a 300-nm-wide and spacing nanopillar chip could not separate λ DNA and T4 DNA.[14]

These results suggest that even a long DNA (~40 kbp), which could not separate using conventional gel electrophoresis, can separate within a few seconds by adjusting the nanopillar diameter and spacing based on the gyration radii of target DNA molecules. One of the great advantages of nanopillar chips is their flexibility of design, adjusting target molecules and separation modes. Various sizes of nanopillar diameter and spacing can be fabricated in the same microchannel at will toward a wide range DNA separation with a high efficiency. The optimal nanopillar design has great potential to defeat the separation limit attributable to

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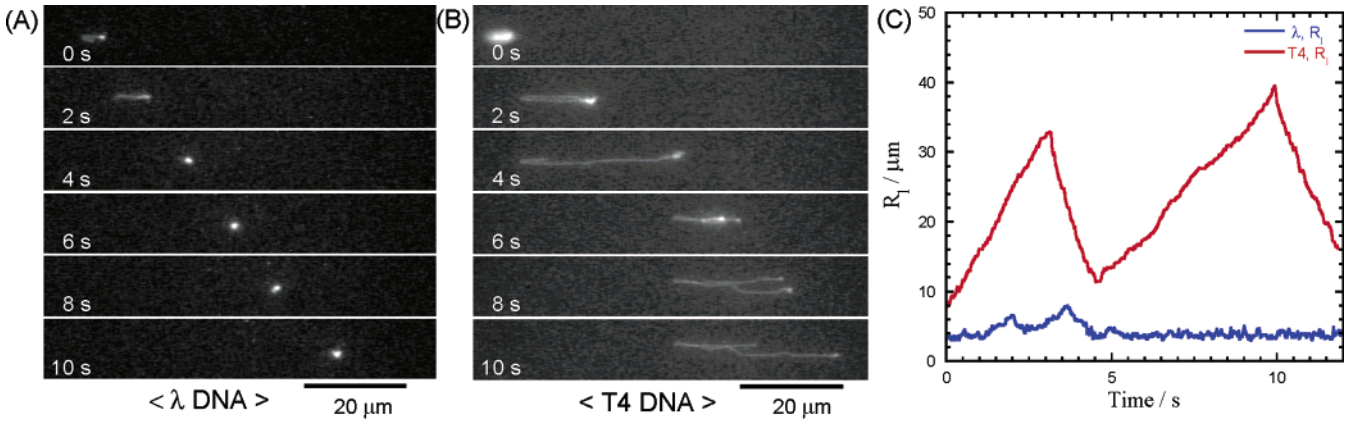

Figure 8. Fluorescence images of (A) a λ DNA and (B) a T4 DNA migrating in a nanopillar region at 7 V/cm. (C) Time sequences of R_I, which are the radii along the horizontal axis, of λ DNA and T4 DNA during electrophoresis. Throughout a long-time observation over 10 s, T4 DNA appeared as a U-shape formation with considerable frequency comparing with λ DNA. During the electrophoresis, λ DNA spent most of the time as a rather spherical conformation.

conventional sieving matrixes and expect to contribute an advance of μTAS.

CONCLUSIONS

We have shown the nanofabrication technique for constructing a nanopillar structure as a DNA sieving matrix on a quartz plate. The nanopillar channels exerted their molecular sieving ability as well as gels or polymers, and moreover, the optimal design based on the gyration radius of DNA molecules can fractionate long DNA molecules even under a dc electric field. Since our nanopillar chip reported here does not need to fill DNA sieving matrixes into a microchannel, it can be applied for an integrated bioanalysis system, which realizes whole laboratory procedures such as DNA extraction from cells, purification, PCR, separation, detection, and collection on one chip.

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SUPPORTING INFORMATION AVAILABLE

Video clip of separation of λ-DNA digested by *Hind*III (.avi format). This material is available free of charge via the Internet at http://pubs.acs.org.

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