The International Symposium on Micro-Mechanical Engineering
December 1-3, 2003

B22-036

AN ARTIFICIAL GEL STRUCTURE FABRICATED BY SILICON MICROMACHINING

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Abstract

We have successfully fabricated an artificial gel for sieving DNA with length by silicon micromachining. The artificial gel has a potential to optimize device design for DNA sizing analysis. A novel fabrication technique for pillar structures with sub-micron spaces has been established, that requires only conventional lithography and electrochemical etching of silicon. Less than 100nm spaces have been achieved. For evaluation, DNA molecules were injected into pillar structure by applying DC voltage. We used 700bp (base pair), 5000bp and 48500bp DNA for observation by a fluorescent microscope. Compared 700bp with 5000bp, the difference of motion speed is clear in pillar structures with 400nm, whereas difference is less clear between 5000bp and 48500bp. Main cause of separation is the duration time for DNA to deform the shape when DNA molecule gets into narrow spaces. Deformation of the DNA molecule during passing through 300nm spaces is verified by fluorescent imaging.

Keywords: *Silicon micromachining, Electrochemical etching, Artificial gel, DNA separation*

INTRODUCTION

Recently, DNA analysis is getting into the limelight and move on the single nucleotide polymorphism (SNP) analysis. However, it is not the time to utilize DNA analysis for human disease diagnosis regard to the throughput and cost.

Several groups have reported DNA sieving devices using an artificial gel fabricated by micromachining technique [1-5]. When the DNA fragments go through a small space of sieving material, usually gel or polymer, the interactive force between the sieving materials or deformation of DNA by obstacle occurs then DNA fragment has dependence of migration speed on the DNA fragment's length. Therefore, the duration of the time for DNA to reach the outlet is different with the length. The Artificial gel has potential to optimize design for DNA sizing analysis. However, mechanism of separation in the artificial gel is not yet clear, so that design optimization has not been achieved.

In this paper, we have presented novel fabrication technique for pillar structures with sub-micron spaces that requires only conventional lithography and electrochemical etching of silicon. We have successfully demonstrated that motion speed of DNA molecules differs with size of DNA in the pillar structure with sub-hundred-nanometer space, and discuss DNA behavior in the pillar structures with sub micron spaces and separation mechanism by fluorescent imaging and analysis.

FABRICATION PROCESS

Figure 1 shows the schematic view of the DNA sieving device that we have proposed. The silicon dioxide (SiO_2) micro pillars in a micro-channel, which function as sieving matrix, were fabricated on silicon substrate. An inlet and outlet to apply voltage were fabricated at both ends of channel. In this device, the key is the fabrication of some hundred-nanometer space. To form such a small structure, electron beam lithography is often used. Contrarily, novel technique based on electrochemical etching and thermal oxidation will be presented as follow.

The process sequence for device fabrication is given in Fig. 2. First, a silicon nitride layer on n-type silicon was patterned, then silicon was etched with alkaline solution to form initial pits for initiation of macropores

(b). Macropores were fabricated by electrochemical etching in hydrofluoric acid (c). In the electrochemical etching, a backside of the sample is illuminated to generate electric holes, which is necessary to etch silicon. Electric holes are concentrated around the initial pits by an electric field. Therefore, silicon etching is defined at initial pits that make it possible to form macropores with high aspect ratio [5]. Then, the first thermal oxidation step was applied. Silicon dioxide layer was formed along the macropores. In this thermal oxidation step, silicon turns into silicon dioxide. Therefore space between each oxide layer is depending on thermal oxidation thickness. The accuracy of SiO_2 thickness can be less than 100Å, so that precise control of space is possible just by controlling thermal oxidation time. The surface of SiN layer was removed by mechanical polishing (d). The mechanical polishing also has a function to makes surface flat, since an internal stress of SiO_2 induces the wafer warp. Less than 0.2 um flatness was achieved by mechanical polishing. The second thermal oxidation was applied and oxide layer was patterned to form a micro channel (e). To pattern the surface silicon dioxide layer, ion beam etching was applied. Sample is tilted against ion beam flux that makes it possible to remove only surface SiO_2 layer not SiO_2 along macropores. Tetramethly hydroxide (TMAH) was used as alkaline etching. TMAH removes only silicon not SiO_2. Therefore, SiO_2 micropillars were formed in the microchannel (f). The third thermal oxidation step was applied to form the electric isolation and surface hydrophilic (g). Finally grass wafer was covered on the fabricated sample. The bonding was done by anodic bonding, which is suitable to seal the liquid in the micro channel (h). Prior to the bonding, the throughholes are fabricated in the glass substrate by sand-blustering to form the inlet and outlet. Bird's-eye-view of the fabricated sample is given in Fig. 3(a). Top view of micropillars is given in Fig. 3(b).

The black squares are the macropores formed by electrochemical etching. The black areas around the white circle, that are silicon dioxide, represents the space of each pillar that acts as the DNA sieving matrix. The reason that circle shaped SiO_2 pillars are formed around the square macropores is the compressive stress concentrated at the corner of the pore that reduces the oxidation rate.

The less than 100nm space has achieved by optimizing thermal oxidation, as shown in Fig.4.

EVALUATION OF THE FABRICATED DEVICE

To evaluate the fabricated device, DNA molecules were put into one reservoir and injected into the pillar structure by applying DC voltage between 30mm-long channel. Before the injection of the DNA, the channel was filled with a buffer solution, specifically 1×Tris borate EDTA (TBE). Surface of the channel is so hydrophilic that the buffer solution can be filled in the pillar structures only by putting solution into an either reservoir. We used 700bp (base pair), 5000bp and 48500bp DNA which were stained with a fluorescent reagent (Yo-PRO-1) prior to injection.

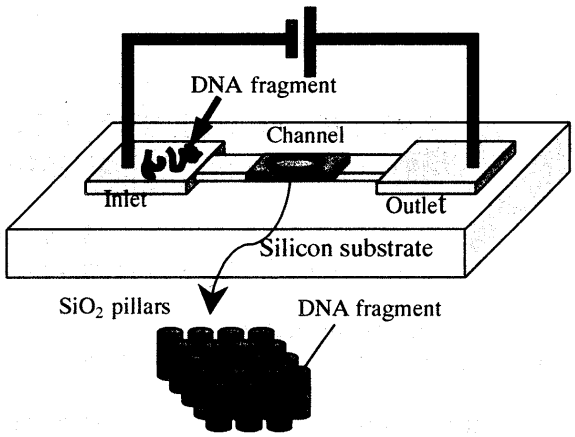


Fig.1 Schematic view of DNA sieving device

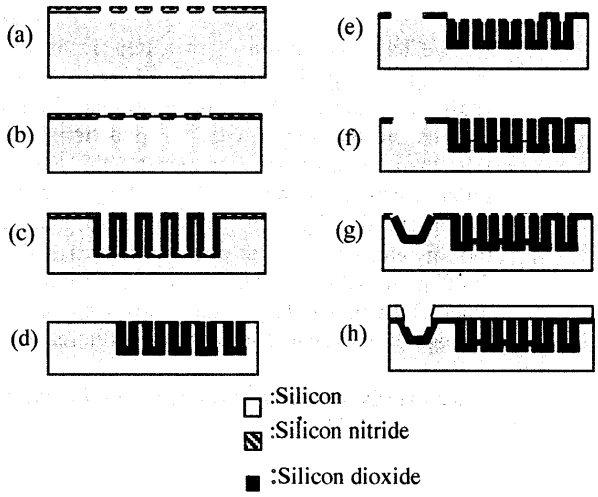


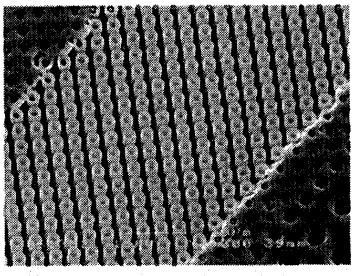
Fig. 2 process sequence of DNA sieving device

The SiO_2 surface has negative zeta-potential in the buffered solution, which causes electro-osmotic flow in the microchannel. The direction of electro-osmotic flow is opposite to that of electrophoresis flow, which leads to generate complicated flow in the microchannel. To suspend an electro-osmotic flow, POP-6(1%, Applied Biosystems) is added to the buffered solution.

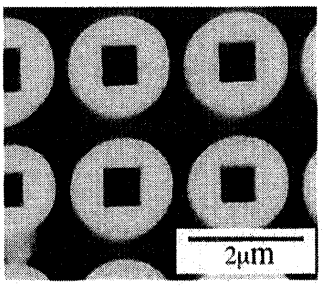
DNA molecules were observed by a fluorescence microscope with a silicon intensified target camera that enables to observe single DNA molecule.

Electric voltage generates a driving force for DNA molecules to migrate between micropillars. SiO_2 is formed on the surface of microchannel for electric isolation. However, more than 200V cause an electrical breakdown of the SiO_2 layer that lead to bubble formation on each reservoir because of electrochemical decomposition. In this experiment, lower than 200V was applied.

Fig.5 shows the dependence of motion speed on DNA length in 300nm-space device. DNA motion speed is linear dependent on applied voltage. Compared 700bp



(a) Bird's-eye view of fabricated sample.



(b) Top view of fabricated sample

Fig. 3 SEM photograph of fabricated device

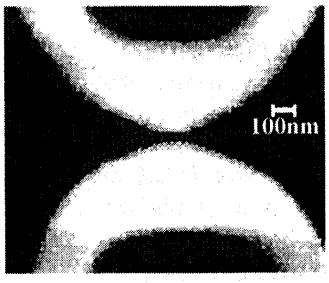


Fig.4 top view of micropillars with less than 100nm space

with 5000bp, the difference is clear, whereas the difference is less clear between 5000bp and 48500bp. If the separation were mainly caused by friction between DNA and pillars at narrow space, the difference of motion speed between 5000bp and 48500bp would be clearer. End to end distance of 1000bp DNA with fluorescent reagent is about 170nm, and that of 500bp DNA is about 90nm[5]. Therefore, end to end distance of 700bp DNA is expected to be less than 300nm. Those of 5000bp and 48500bp are expected to be more than 300nm, according to the worm-like chain model. 700bp DNA can pass through 300nm spaces without deformation of DNA shape, while, in case of 5000bp and 48500bp, deformation from random-coil to stretch is necessary. It is considered that the main cause of the separation is the duration time that is required for the deformation when DNA molecule gets into narrow spaces. Fig.6 shows motion of single DNA molecule of 48500bp in the pillar structures with 300nm space. Deformation of the DNA molecule with 48500bp during passing through 400nm spaces is verified as shown in Fig.6. The DNA molecule is coiled in wide space in Fig 6(a). To pass the narrow space, DNA molecule is deformed, as shown in Fig 6. (b).

The motion speed analysis is applied to investigate variation of the motion speed when DNA molecules pass through the pillar structures. Figure 7 shows the variation of the motion speed during regard to 5000bp and 48500bp. In 48500bp and 5000bp, motion speed is periodically changing in accordance with the pillar structure. The motion speed becomes fast in narrower space, and maximum speed of 5000bp is almost same as 48500bp. This implies that friction force between the pillars and DNA molecules is not dependent on DNA length. In 700bp, motion analysis for each single molecule is not possible because of too small molecules. However, average motion speed of 700bp is around 35 μ m/sec that is as same as maximum speed of 5000bp and 48500bp. From these results, duration time for DNA molecule to deform the shape from wider space to

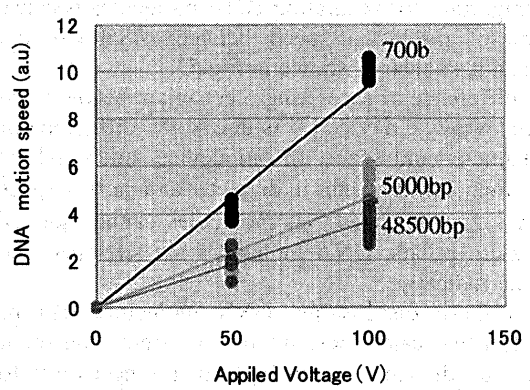


Figure 5 Dependence of averaged motion speed on various DNA chain length. Pillar space is 300nm. on DNA size. Space of pillars is 300nm.

narrower space leads to separate DNA by 700bps and 5000bp, 48500bp.

DISCUSSIONS

Several DNA separation mechanisms in polymer or gel matrix have been presented [6]. In reptation model, DNA molecules behave like a snake entangling with gel matrix. Viovy et al[5] showed that sub-micrometer diameter pillars with more than 5 μm space also has ability to separate the DNA by chain length. In their device, DNA is hooked and stretched around micro pillars, and then detachment time from the micro pillars is depending on DNA chain size.

On the other hands, in our device, the deformation of DNA is mainly lead to separate DNA by length in this device. The friction between pillars and DNA molecules

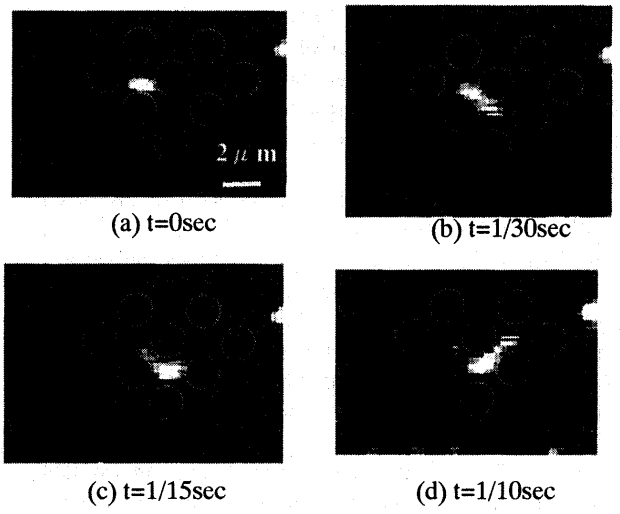


Figure 6 Video images showing the motion of single DNA molecule with 48500bp in pillar structures. Dotted circles represent pillar structures.

are not important for separating DNA molecules because difference of migration speed between 48500bp and 5000bp is not so clear as shown in Fig.5.

The difference of separating mechanism between our device and Viovy's device [3] is due to the difference of pillars diameter and space. In our device, micro pillars has more than 1 micrometer in diameter and narrow space less than 1 μm, so that DNA molecules cannot hooked around micro pillars. DNA molecules are stretched and recoiled periodically.

From these discussions, DNA molecules can be separated in two categories, smaller or larger than pillar space in our device. Various spaces are necessary to separate DNA with various chain lengths.

CONCLUSIONS

We have successfully fabricated artificial gel for sieving DNA with chain length by silicon micromachining. The novel fabrication technique for pillar structures with sub-micron spaces has been established, that requires only conventional lithography and electrochemical etching of silicon. Less than 100nm spaces have been achieved. Compared 700bp with 5000bp, the difference of motion speed is clear in pillar structures with 300nm, whereas difference is less clear between 5000bp and 48500bp. Main cause of separation is the duration time for DNA to deform the shape when DNA molecule gets into narrow spaces. Deformation of the DNA molecule during passing through 300nm spaces is verified by fluorescent imaging.

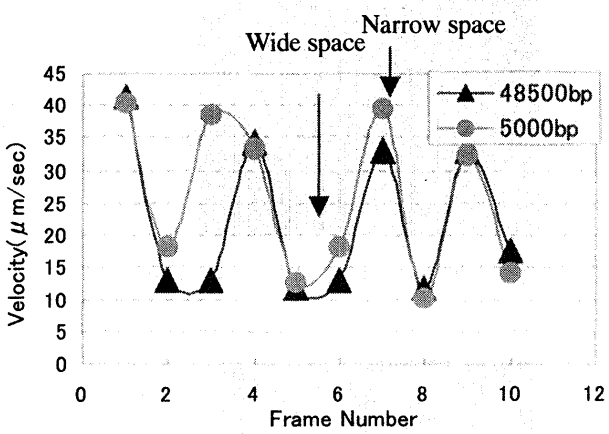


Fig.7 Motion speed analysis of each DNA passing micropillars. Each frame corresponds to 1/30sec.

ACKNOWLEDGMENTS

This work was partially supported by Millennium Project of the Ministry of Education, Culture, Sports, Science and Technology of Japan (12408).

The authors would like to appreciate Porf. Masao Wasizu and Mr. Osamu Kurosawa for fruitful advices.

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