

# SUB-MILLISECOND SEPARATION OF DNA AND MICRO-RNA BY NANOPILLAR ARRAY CHIPS

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## ABSTRACT

In this study, we fabricated a novel nanostructure that combined the pillar structures (nanopillar) and dammed structures (nanoslit) in nanometer-scale inside microchannels to realize ultra-fast separation. Nanopillar chips were fabricated by using electron-beam lithography, photolithography and plasma etching. We used the nanopillar chip to realize ultra-fast separation of T4 DNA (165.5 kbp) and microRNA (22 b). By decreasing height down to 100 nm, we succeeded in separating DNA and microRNA in sub-milliseconds.

## KEYWORDS

Nanostructures, Nanopillars, DNA separation, Electrophoresis.

## INTRODUCTION

DNA sequencer has been developed in recent years, and even faster DNA sequencer, such as nanopore sequencer, is under investigation, and becoming an increasingly competitive research field. For DNA sequencing by using the nanopore sequencer, the speed-up of biomolecules separation is critically required for a part of pretreatment steps. Researches, who work on separation technology, decided to use nanofabricated-structures to achieve their aims [1]. With the development of the nanofabricated technology, a number of researches on nanostructures have been reported. Researches have been performed separation of biomolecules by using nanofabricated structures as the separation medium instead of polymer and gel matrices. As one of the nanostructures, a nanopillar structure, which was made inside the channel on the quartz substrates, attracts attention of researchers [2]. In this research, nanopillar devices were fabricated, in which nanopillar were made inside microchannels on quartz substrates, and we used the nanopillar devices as the separation medium to separate the biomolecules. In this study, a novel nanopillar array structure were fabricated inside microchannels on quartz substrates to achieve ultra-fast separation and it was used as separation matrices to separate DNA molecules.

## THEORY

Separation of DNA molecules using the nanopillar array structure is based on trapping of DNA molecules at the entrance of this structure. While longer DNA molecules were trapped after physical collision with the nanopillar array structure, shorter DNA molecules passed through the nanopillar array structure without any collisions (Figure 1). Because of differences of physical behaviors, we can separate longer and shorter DNA molecules under applied electric fields.

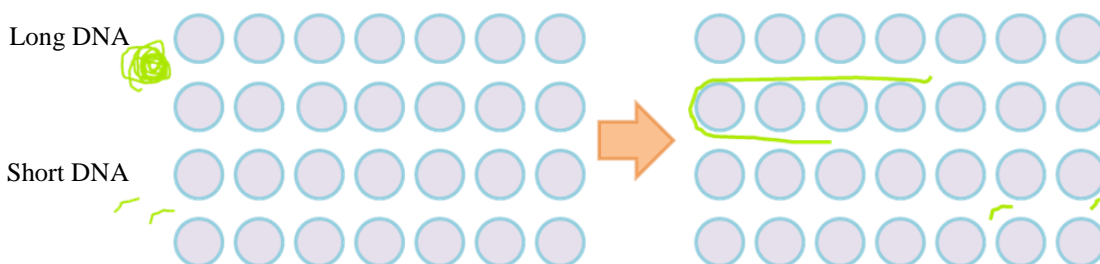


Figure 1. Principle of separation of DNA molecules under applied electric field.

## EXPERIMENT

In order to separate DNA molecules in less than milliseconds, we designed a novel nanopillar array structure, which is the combination of the nanopillar and nanoslit array structure. The novel chip was fabricated on quartz substrates by using electron-beam lithography, photolithography and plasma etching. The detailed fabrication of the novel nanopillar chip was shown in Figure 2. The nanopillar array structure had 318nm in diameter, 660 nm in spacing, and 100 nm in height (Figure 3), and the SEM image of the nanopillar array structure embedded in microchannel was also shown in Figure 3. The location of nanopillar structures were near to the cross of microchannel to realize ultra-fast separation. We used T4 DNA (165.5 kbp) DNA and microRNA (22 b) as a DNA

sample which were dyed with YOYO-1 fluorescent dye at a ratio of 1 dye molecule per every 10 base pairs and Alexa 488 fluorescent at the terminal of microRNA. DNA sample was introduced to nanopillar region by applying 7500 V/cm electric field. The measurements were performed with a fluorescent microscope system consisting of a Nikon Eclipse TE300 inverted microscope, 10×/1.40NA oil-immersion objective lens, and an high-speed camera.

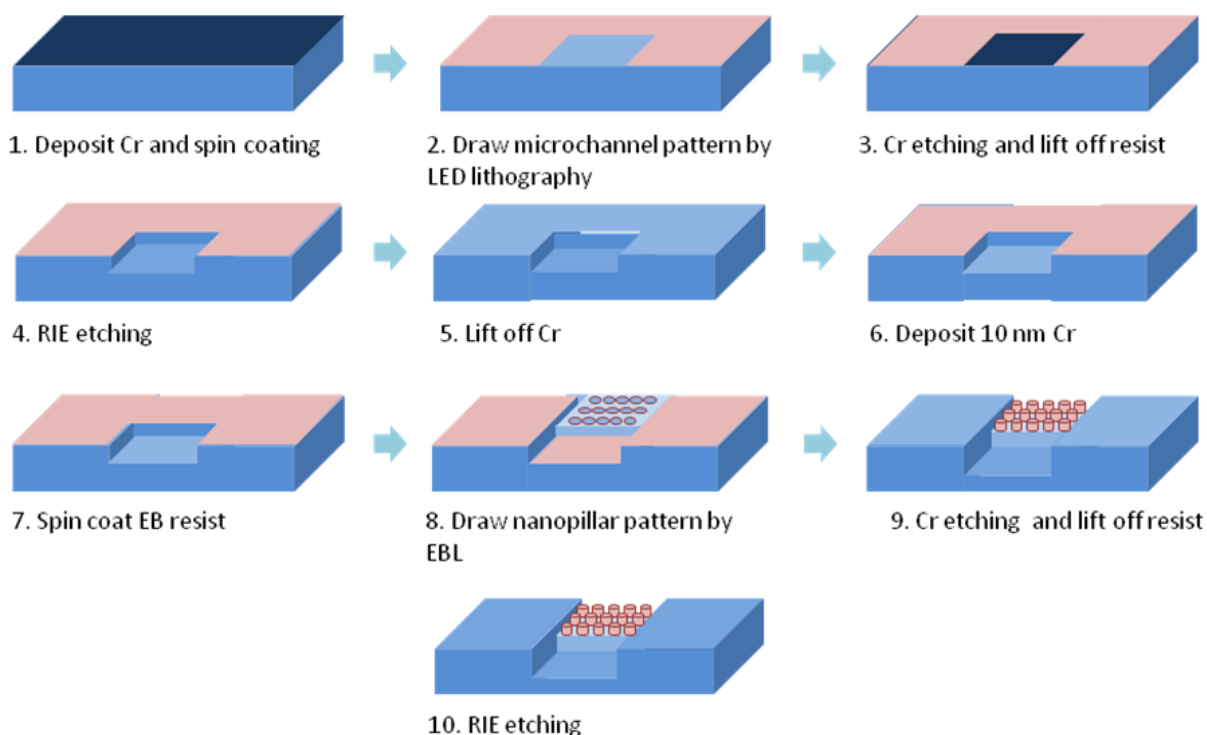


Figure 2. Fabrication of the novel nanostructure that combined the pillar structures (nanopillar) and dammed structures (nanoslit) in nanometer-scale inside microchannels.

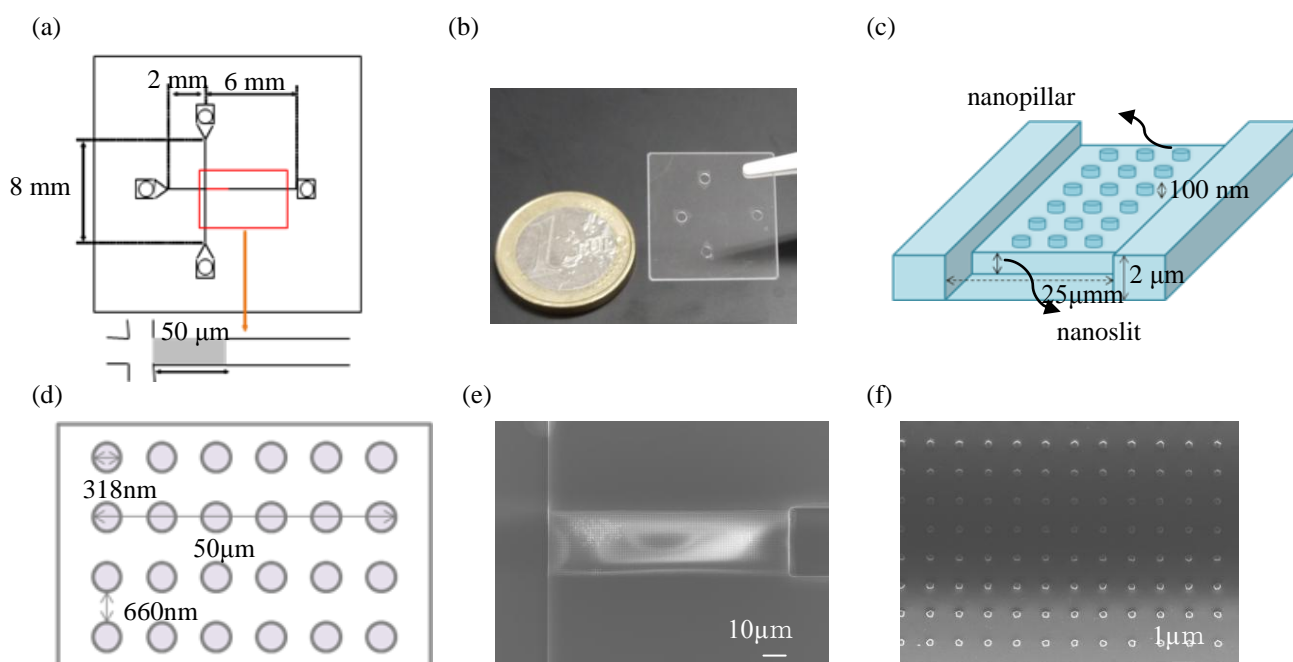


Figure 3. Novel nanopillar array structure fabricated on quartz substrates before sealing by coverslips. The nanopillar dimension was 318 nm in diameter, 660 nm in spacing and 100 nm in height. (a) Schematic and (b) An optical photograph of the nanopillar chip. (c) Illustration of the nanopillar array structure. (d) A top view of the nanopillar array structure. (e) SEM image of the novel nanopillar array structure. (f) Magnified SEM image in (e).

## RESULTS AND DISCUSSION

We successfully separated T4 DNA (165.5 kbp) and microRNA (22 b) in 1500  $\mu$ s by applying electric field of 7500 V/cm. Before the sample was introduced to nanopillar region, graph of fluorescence intensity against the distance was shown in Figure 4 (a), and after 1500  $\mu$ s, two peaks were revealed as shown in Figure 4 (b), in which T4 DNA and microRNA were left and right peak, respectively. Under this electric field, the mobility of microRNA was  $1.47 \times 10^{-4} \text{ cm}^2/(\text{V} \cdot \text{s})$  calculated by equation  $\mu=v/E$ , which was quite faster than the diffusion coefficient ( $D=1.0 \times 10^{-6} \text{ cm}^2/\text{s}$ ) of the same size DNA [3]. It was considering as the key of separating T4 (165.5 kbp) DNA and microRNA (22 b) in ultra-fast speed. So far, with the 100 nm height of nanopillar array structure, we made the separation speed drastically to 1500  $\mu$ s, and we would attain nanoseconds separation by considering nanopillar diameter, spacing between nanopillars and high electric field.

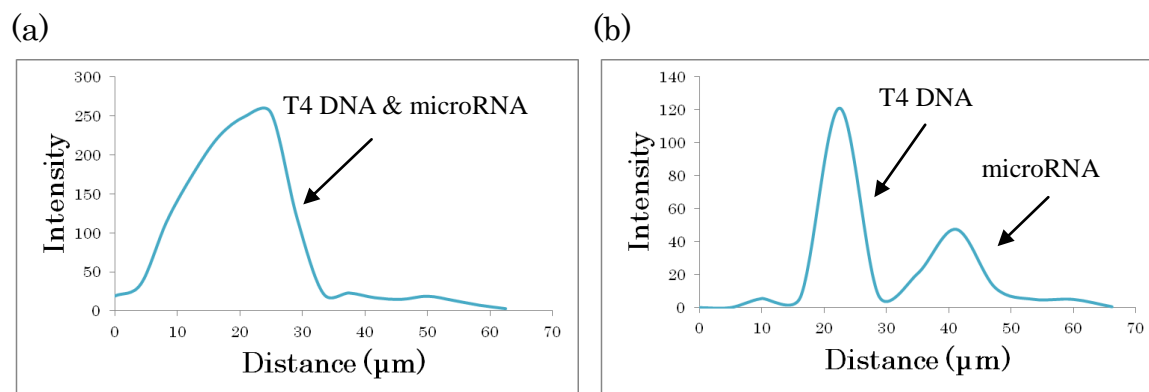


Figure 4. Results of separation of T4 DNA (165.5 kbp) and microRNA (22 b) by the nanopillar array structure with 100 nm height under applying electric field 7500 V/cm. (a) Result at the time of 0  $\mu$ s and (b) result at the time of 1500  $\mu$ s.

## CONCLUSIONS

In summary, we fabricated the novel nanostructure that combined the pillar structures (nanopillar) and dammed structures (nanoslit) in nanometer-scale inside microchannels to realize ultra-fast separation of DNA molecules. And we succeeded in using the height of 4  $\mu\text{m}$  nanopillar array structure to separate T4 DNA (165.5 kbp) and microRNA (22 b) in ultra-fast speed 1500  $\mu$ s.

## ACKNOWLEDGEMENTS

This research was partially supported by the Japan Society for the Promotion of Science (JSPS) through its "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)".

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