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Integrated DNA Purification and Detection Device for Diagnosis of Infection Diseases

Shingi Hashioka[1], Ryo Ogawa[1], Hiroki Ogawa[2], and Yasuhiro Horiike[1]

[1]National Institute for Materials Science, Nanotech-driven Materials Research for Information Technology
1-1, Namiki, Tsukuba, Ibaraki 305-0047, Japan
Phone: +81-29-851-4652 E-mail: HASHIOKA.Shingi@nims.go.jp
[2]Adbic Incorporation
2-1-6 Sengen, Tsukuba, Ibaraki 305-0047, Japan

1. Introduction

This paper reports the development of a diagnosis chip of infection diseases originated from viruses. To realize quick and safety detection of a specific virus, extraction of nucleic acid is a key technology. Especially, DNA purification and trapping are important. These processes should be done in continuous micro channels in a chip. We reported a new DNA stretching method by using simple micro channel structure equipped with nano gap array and then detection of target DNA by hybridization of probe DNA [1]. In addition to the nano gap array device, we have attempted to contain DNA purification tool consisting of alumina (Al_2O_3) coated micro pillars and a hydrogel valve, whose functions is changed by pH of washing solutions.

2. Experiments and Results

Figure 1 shows a model of the DNA purification and detection chip to be developed, in which DNA is extracted from lyses of viruses in plasma separated from whole blood and washed. After washing, DNA is trapped and detected in nano gap array.

The purification device shown in Fig. 2 (a) was studied. Fig. 2 (b) shows Si micro pillars (60 μm height, 10 μm diameter, 10 μm pitch), which was fabricated by a Si deep etching. To purify DNA extracted from virus with solution such as Chaotropic salt, DNA has to be immobilized to the pillars for flow of a washing solution. To immobilize DNA with a negative charge to the pillars, coating of Al_2O_3 on the Si pillar surface was investigated. Al_2O_3 was coated by the atomic layer deposition (ALD). Deposition rate of the about 0.1 nm was obtained by one cycle which consists of exposure of H_2O_2 at $1x10^{-4}$ Torr and 1 sec. and after interval of 2 sec., exposure of $Al(CH_3)_3$ at $1x10^{-4}$ Torr and 1 sec.

Figure 3 shows pH dependence of the zeta potential of Al_2O_3 surface where thickness was 40 nm by repeating 400 cycles. The zeta potential was measured using ELS-6000 (Ohtsuka electronics). The zeta potentials vary from positive values at acidic solution to negative values at alkaline solution via zero zeta potential around pH=6.

Next, a glass layer was anodic-bonded on the Si substrate containing Al_2O_3-coated micro pillars. T4 DNA (166 kbps, length; about 50 μm) stained by YOYO-1 and was diluted by TBE buffer with various pH. These solutions were then introduced into the channel using centrifugal force. Figure 4 shows variations of the fluorescence images before and after introduction of the solution. When DNA solution with pH=4.7 was introduced, much DNA was remained even after removal, whereas most of DNA with pH=10.1 was removed after rotation.

Figure 5 shows pH dependence of the fluorescent intensity for the experiment performed in Fig. 4. The great decrease in fluorescent intensity from acid to alkaline condition demonstrates satisfactory control of immobilization and extraction of DNA. Since the purification tool needs a valve, which controls the flow of the washing solution, we also have developed a pH sensitive hydro gel valve reported by D. J. Beebe et al [2] as shown in Fig. 2(c). The combination of the Al_2O_3 coated pillars and hydro gel valve will perform the purification procedure, which DNA immobilized at acid condition is washed by closing the valve and the purified DNA is transferred to the nano gap array as changing the pH from neutral to alkaline conditions.

Figure 6 shows microscopic images of a DNA detection device, nano gap array, which is an analysis stage for our final goal. The 816 triangle shaped channels in an area of 85 x 240 μm^2, in which depth, maximum and minimum widths were 140 nm, 5 μm and 50 nm, respectively, were fabricated on a quartz plate by an EB lithography and reactive ion etching processes. T4 DNA was introduced into the channel by electrophoresis using applied voltages of 50-100 V. Fig. 6(c) shows a fluorescence image of a number of DNA trapped with stretching in nano gap array. A length of these DNA fixed with stretching exceeds 20 μm in the channel. Subsequently, probe DNA was introduced from the reservoir into the nano gap array to hybridize the trapped DNA. A sequence of probe DNA is designed from parts of a sequence of Bacteriophage T4 gene. Fig. 6 (d) shows a fluorescent image, when probe DNA is introduced after trapping of T4 DNA. Intensive luminescence was seen in the channels.

3. Conclusions

The fundamental experiment for novel one-chip DNA purification technique by using the micro pillars and the hydro gel valve was performed. Additionally, Simple and quick purification and detection of target DNA by hybridization in nano gap channel array was demonstrated. Those chip systems will allow us to analyze DNA from the virus lysis quickly, easily and safety in the near future.

Acknowledgements

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References

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[2] D. J. Bebee, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss and B-H. Jo, Nature (2000) 588.

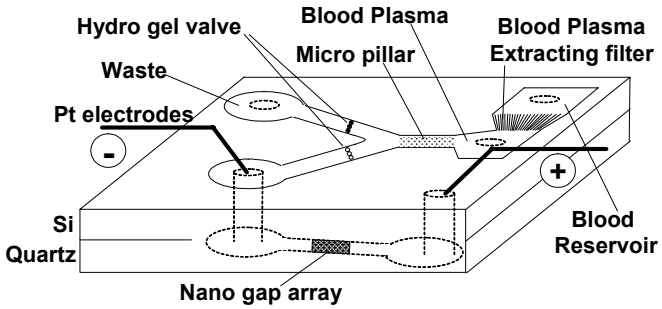

Fig. 1 A model of the DNA purification and detection device to be developed.

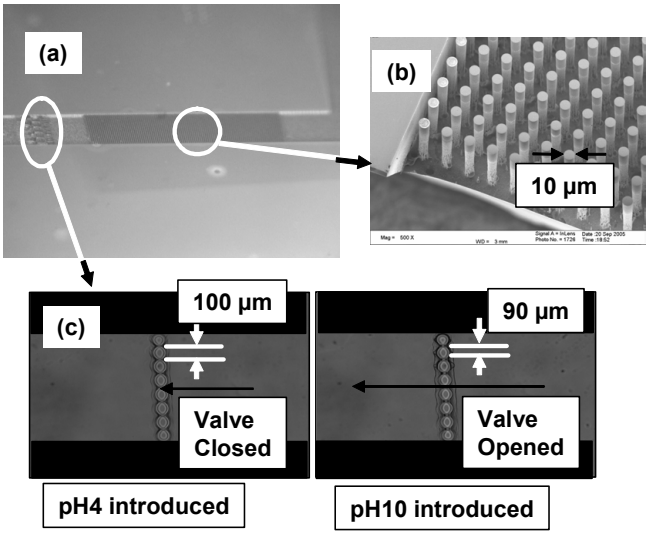

Fig. 2 Microscopic images of DNA purification device by using micro pillars and hydro gel valve.

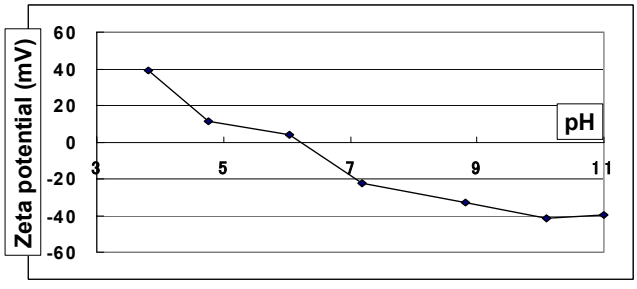

Fig. 3 pH dependence of the zeta potential of Al_2O_3.

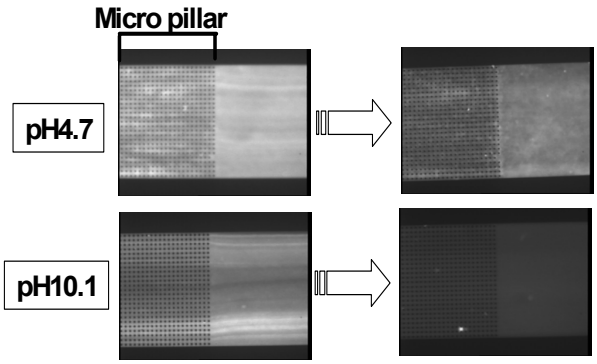

Fig. 4 Variation of the fluorescence images before and after introduction of DNA solution.

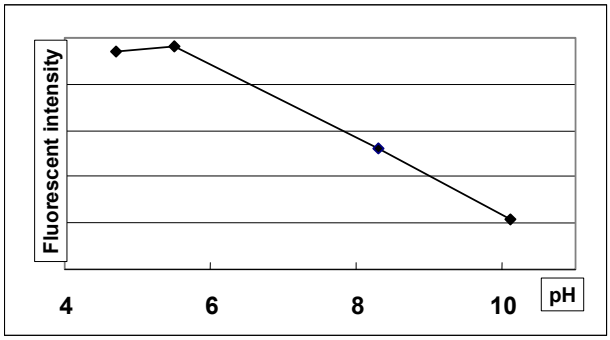

Fig. 5 pH dependence of the fluorescence intensity in micro pillars.

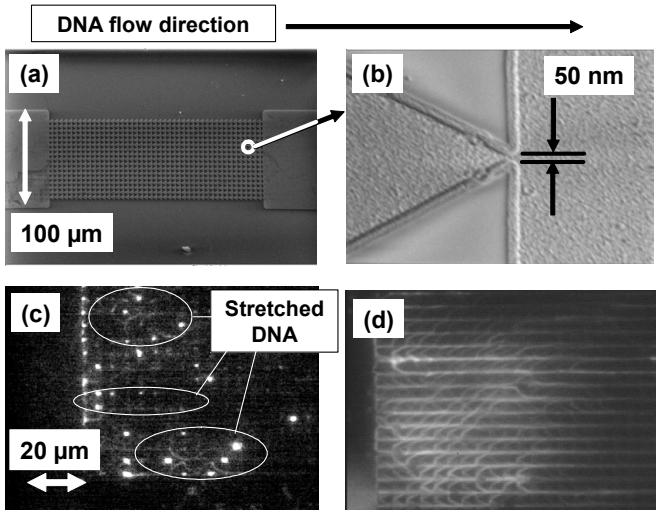

Fig. 6 Microscopic images of DNA detection device by using nano gap array.