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# One-Chip Integration of the Rapid Diagnosis Infectious Disease Chip Based on New Phenomena of DNA Trap and Denature in Nano-Gaps

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## 1. Introduction

Infectious diseases such as HIV, viral hepatitis, bird influenza, etc. pose increasingly grave menace to humankind. Virus-caused infectious diseases can be detected by hybridization of the probe DNA with single strand (ss) DNA denatured from double strand (ds) DNA of virus particles in blood. Trap and then extension of ssDNA facilitates this hybridization process. Some studies reported DNA trapping using nano-micro-channels [1, 2]. However, they need complicated systems for detection in a chip. To aim at a one-chip which enables us to diagnose infectious diseases, we have studied the functions consisting of a virus lysis, a DNA purification, a DNA trapping and subsequent detection [3]. Such detection is advantageous in avoiding the risk of infection of medical staff. The paper reports the one-chip integration of those devices.

## 2. Experiments and Results

Figure 1 shows a schematic structure of a chip fabricated. A purification chip of dsDNA extracted from lysis of viruses is stacked with a detection chip via a through-hole. Si and quartz substrates were bonded by press with 0.35 MPa with heating to 65 °C after soaked in the H<sub>2</sub>SiF<sub>6</sub> solution. Glass cover was anodic bonded. Figure 2 shows the pre-treatment device chip. To wash dsDNA, dsDNA is immobilized on the Si micro-pillars fabricated by Bosch etching, allowing a cleaning solution flow. Alumina is coated on the micro-pillars by an ALD method. Zeta potential of alumina varies from positive in acid to negative values in alkali. Negatively charged dsDNA is immobilized on the alumina-coated-micro-pillars during purification in acid solution and released during elution in alkaline solution. Two types of pH-sensitive hydrogel valves [4] were applied to the washing process. A positive-type-valve (posi-valve) is closed in acid by hydrogel-expansion and opened in alkali by shrinkage. A negative-type-valve (nega-valve) is opened in acid and closed in alkali. Washed dsDNA is transported to the nano-gap-array for the detection.

Pre-treatment procedures were performed as shown in Fig. 3. First, T4 dsDNA (166 kbp) was introduced into the chip with solution of pH=4.7. The chip was then rotated. Figure 3(a) shows the valves and micro-pillars after the introduction of dsDNA. That dsDNA accumulated on the micro-pillars by closing the posi-valve and opening the nega-valve. Next, NaOH (0.02 M) was introduced into the channel. As shown in Fig. 3(b), dsDNA immobilized on the

micro-pillars passed through the posi-valve. In the meantime the nega-valve blocked dsDNA as shown in Fig. 3(c).

Finally, washed dsDNA was introduced into the nano-gap-array by electrophoresis. Figure 4 shows the nano-gap-array, which has 816 triangular channels with minimum width of 50 nm. As shown in Fig. 5(a), DNA dyed by YOYO-1 was trapped in the nano-gap-array and stretched up to a length of 20 μm. We found a marvelous phenomenon that dsDNA denature to ssDNA. Indeed, a probe ssDNA was introduced to the trapping area, thereby hybridizing the trapped ssDNA as shown in Fig. 5(b). This demonstrates just capture of viral DNA. We presumed that this denature was caused by joule heating due to ion current during trapping in nano-gaps. To confirm, we investigated the fluorescence as a function of trapping time as applying voltage of 50V. As shown in Fig. 6(a), the fluorescence started to go out around 23 min. and the dark area expanded. As shown in Fig. 6(b), the initiation time for darkening diminished as applied voltage increased. Measured electric current during the DNA trapping was about 0.03 μA at applied voltage of 50V. It is calculated that DNA solution in micro- and nano-channels is heated by about 90 μJ for 1 min. The heating was assumed to elevate temperature of the electrolytic solution up to 100 °C, which was enough temperature to denature dsDNA.

## 3. Conclusions

A new DNA diagnostic chip in which integrates procedures from the virus lysis to the detection has been developed based on a finding of denature of dsDNA trapped in the nano-gap.

## Acknowledgements

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## References

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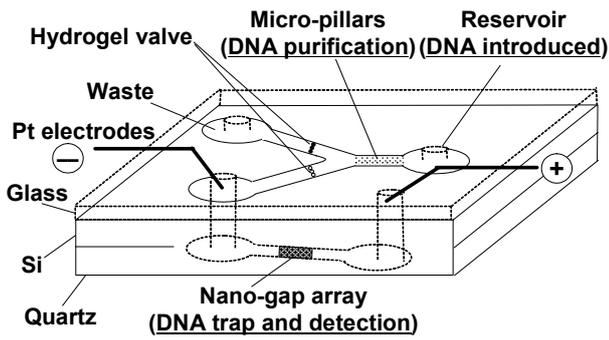


Figure 1: A schematic structure of a chip fabricated.

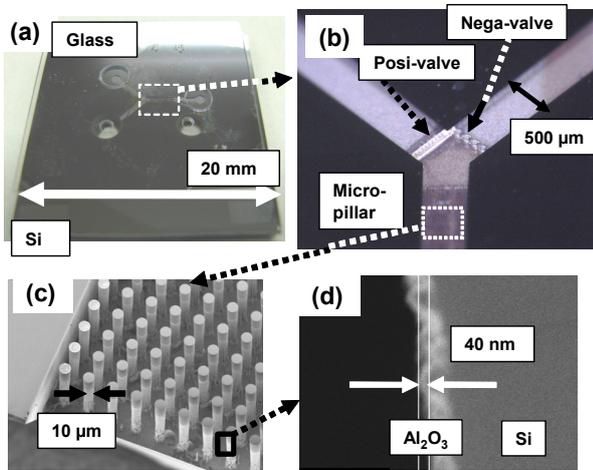


Figure 2: (a) A photograph of DNA pre-treatment device side of the chip (b) Microscopic photograph of the pre-treatment device (c) SEM image of deeply etched Si micro-pillars (d) Magnified image on sidewall of pillar coated with alumina.

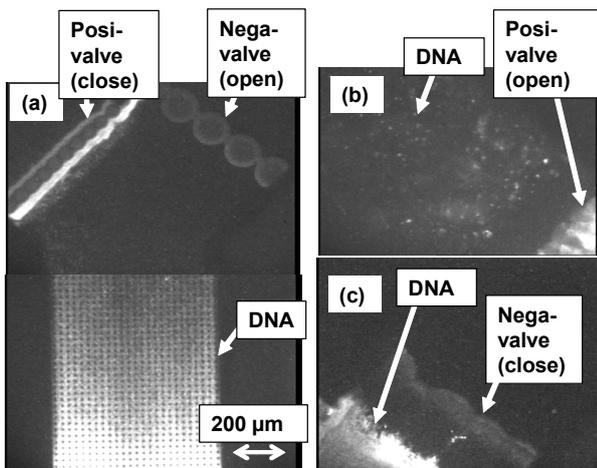


Figure 3: (a) Valves and pillars after introducing DNA in acid. (b) Fluorescence image of DNA which was eluted by alkali solution and passed through the posi-valve. (c) DNA blocked by nega-valve.

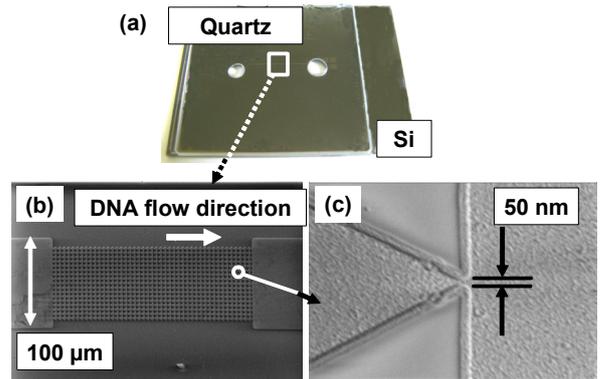


Figure 4: (a) A photograph of nano-gap-array side of the chip (b-c) SEM images of nano-gap-array.

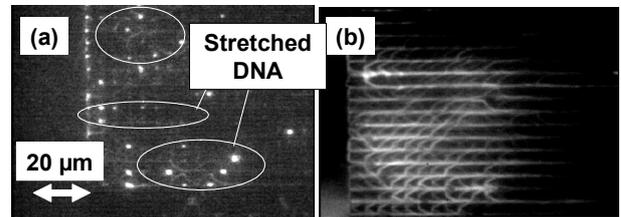


Figure 5: (a) Number of T4DNA trapped by stretching up to a length of 20 μm in nano-gap-array. (b) Introduced probe DNA hybridizing the trapped DNA.

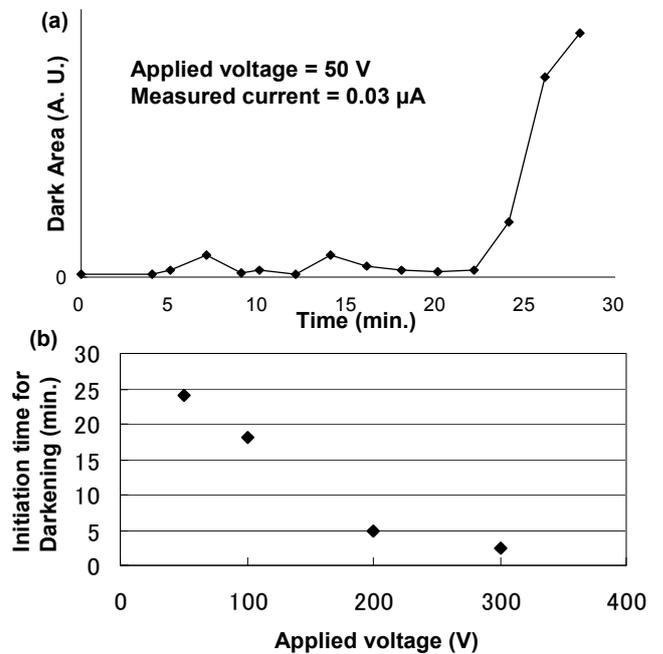


Figure 6: (a) Expansion of Dark Area in the fluorescence image after DNA trap. (b) Initiation time for darkening of DNA against applied voltage.