A DROPLET ON DEMAND MICROFLUIDIC DEVICE FOR DETECTING DNA SINGLE BASE SUBSTITUTION USING PNA PROBE

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ABSTRACT

Rapid detection of DNA single-base-substitution (SBS) using PNA (peptide nucleic acid) probe is realized in a droplet-handling microfluidic device. The device can conduct reaction process with 420-pL droplets and following electrophoresis process for analysis the reaction products. All liquid handlings were implemented by pneumatically manipulation through microcapillary vent on the device. As a demonstration, detecting of R553X mutation on CFTR (Cystic Fibrosis Transmembrane Regulator) gene was carried out within 1.5 min. By utilizing the droplet on demand format of the device, effect of the droplets configuration against DNA-PNA reaction was studied.

KEYWORDS: Droplet, Droplet on demand, PNA, DNA, Electrophoresis

INTRODUCTION

To realize rapid analysis with small volume samples and reagents by using microfluidic device, several methods for droplet handling have been proposed and developed [1]. Although there're still some issues to be discussed, the pneumatic handling of droplet is one of the promising ways because of the compatibility with conventional separation (e.g., electrophoresis, etc.) schemes. We previously reported a microfluidic device [2] for droplet-based reactions and following electrophoretic analysis using combination of hydrophobic microcapillary vent structures [3] with pneumatic manipulation. In the current work, we have improved the reliability of the device by minor changes of microchannel geometries then the present device can be conducted detecting of a SBS using PNA probe [4].

EXPERIMENTAL

The present device consists of a droplet-handling fluidic chip made of PDMS, a glass substrate equipped with Au/Cr electrode for electrophoresis, and a glass substrate which patterned ITO heater/sensor structure for temperature control for reaction and electrophoretic separation. Required positive/negative air pressures for series of liquid-handling operations are applied by a programmable air pressure control system (MyFlow; Arbiotec, Japan). The previous our report [2] shows the detail of the basic device design and liquid-handling operations schemes.

As a model of SBS, we selected mutation of R533X on CFTR gene. Two DNA oligomers of 15mer were used as ssDNA samples of wild type and mutant. Sequences are as follows: wild type (FITC-5'-AGG TCA ACG AGC AAG-3'), mutant (FITC-5'-AGG TCA ATG AGC AAG-3'). As probe, 15mer PNA oligomer which has complimentary sequence to the mutant DNA was used. The concentrations of DNA samples and PNA probe solutions are 10 μM and 40 μM. A 1% Hydroxyethyl cellulose (HEC) polymer solution containing 4 M urea [4] was used for both sieving matrix and salt bridge. Addition of urea as denaturerant was necessary to keep the melting temperature of the PNA/DNA hybrid within the controllable temperature range of the device.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

RESULTS AND DISCUSSION

Fig. 1 demonstrates the liquid-handling operations for reaction and electrophoretic separation. Fist, the two droplets of DNA (mutant) and PNA probe were metered and merged together (Fig. 1AB). The volume of droplet is about 420 pL. Then the droplets were merged with each polymer solutions for salt bridge and sieving matrix (Fig. 1CD). Finally, electrophoresis was conducted, and then the ssDNA and PNA/DNA hybrid were separated (Fig. 1E~H). All operations are successfully performed within 1.5 min.

Fig. 2 shows electropherogram of wild type and mutant DNA where the latter is a single base substitution in the target region hybridized with PNA probes, run at 42°C. By carrying out electrophoresis under this condition, it is possible to obtain the melting of PNA/DNA hybrid with a mismatch whereas those perfectly complementary are stable. From the difference of second peak height in Fig. 2, the SBS could be successfully detected by the presented device.

In this droplet-based reaction manner, we suppose that since PNA molecules have non-electric charge of its backbone [5], PNA molecules is remained at their droplet region as shown in Fig. 3. Hence, DNA-PNA binding reaction is advanced not only diffusion-based mixing but also during the DNA passing through the PNA droplet region by the electrophoresis (Fig. 3B). Therefore we also consider that the reaction efficiency of DNA-PNA binding is affected by the droplets configuration. To confirm the consideration, and, to get higher sensitivity and to reduce the time for detection of SBS, we demonstrated the DNA-PNA reaction under the four cases as shown in Fig. 4 by employing the droplet on demand format of the device. First, each case is successfully realized on the device as shown in Fig. 5A. Notes that diffusion-based mixing is not completed in case 3 and 4 (3 s). After incubation for in each case incubation time, electrophoresis is conducted. The resultant eletropherograms are shown in Fig. 5B. The first peaks are from ssDNA (not-reacted) and the second peaks indicated by arrows are from DNA/PNA hybrids as the reaction products. The each profile is normalized by their first peak height. To estimate the reaction efficiency in each case, we define a value of peak ratio that given by dividing second peak height by their first one as shown in Fig. 5C. By comparing case1 with case 2 (same incubation time but different droplets configuration), the peak ratio of case 1 that DNA droplet located behind of PNA droplet is better. Also, by comparing case 3 and case 4 (same time but amount

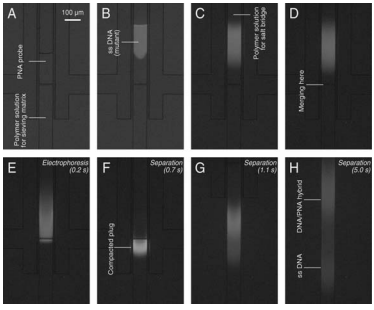

Figure 1. Droplet-based reaction and electrophoresis operations. (A, B) droplet generation and merging. Merging with salt bridge (C) and sieving matrix (D). (E~H) Following electrophoretic separation.

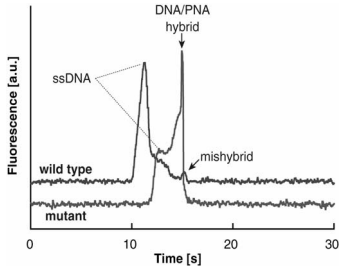

Figure 2. Electropherogram of wild type and mutant DNA reacted with PNA probe. Electrophoresis run at 42°C, Electric field is 168.3 V/cm. Detection at 5 mm. Incubation time is 30 s.

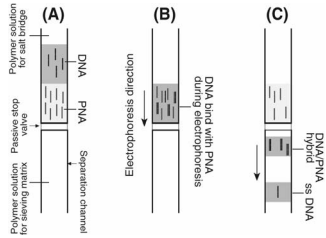

Figure 3. Schematic illustration of DNA-PNA biding process on device.

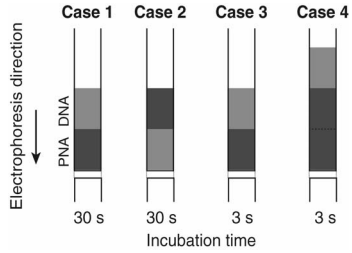

Figure 4. Schematic illustration of reaction condition of each four case.

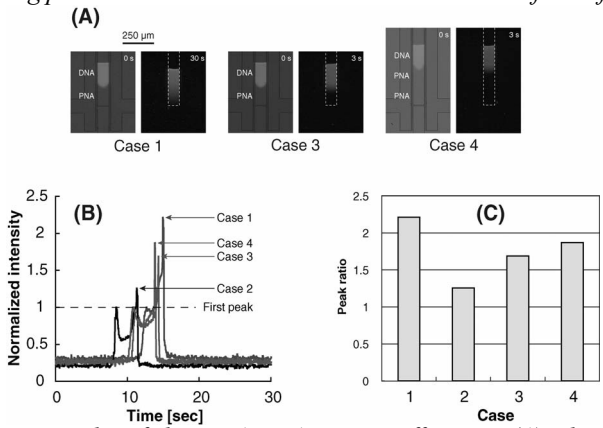

Figure 5. Results of the DNA-PNAreaction efficiency. (A) Photographs of each case after merging (left) and after incubation (right). Case 2 is not shown. (B) Electropherograms of each case. Running condition of electrophoresis is same as Fig. 2. (C) Results of peak ratio. Value of the peak ratio is given by dividing second peak height (reaction product) by first one (not-reacted).

of PNA droplet is twice), the case 4 (two droplets) is better than case 3 (one droplet). From the results, the validity of our expectation was confirmed.

We demonstrated a rapid detection of SBS using PNA probe by the droplet-handling microfluidic device. From the study for droplets configuration against the reaction, it was revealed the efficiency of DNA-PNA binding reaction is enhanced by the droplets configuration before the electrophoresis process. The presented results show the feasibility of further reducing the time and getting higher sensitivity for the SBS detection with PNA probe by the utilizing of PNA molecules nature and droplet on demand format of the present device.

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