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Microfluidic system for DNA sequence detection

Kenichi Yamashita, Yoshiko Yamaguchi, Masaya Miyazaki, Hiroyuki Nakamura, Hazime Shimizu, Hideaki Maeda*

Micro-space Chemistry Laboratory, National Institute of Advanced Science and Technology, 807-1 Shuku-machi, Tosu, Saga 841-0052, Japan

Abstract

A microfluidic system offers superior controllability of fluids. This study developed a novel separation method for complexes formed in a microchannel. It presents an application for DNA sensor. This method is based on the molecular sieving effect, which is achieved by superior controllability of fluidics. Use of confocal fluorescence microscopy observation and computer simulation confirmed that the laminar secondary flow at the curing part of a microchannel enables this microfluidic separation. Also in this study, we applied this molecular sieving effect for sequence-selective DNA sensor. This measurement showed that the response of a complementary sequence target DNA was higher than that of a non-complementary sequence. Moreover, the response of a longer target was higher than a different-length complementary sequence target. This method does not require immobilization of probe or target DNA: solutions are merely injected into the microchannel and all reactions occur in the liquid phase. Such features might lower the experimental error and difference in data by operators.

Keywords: Microfluidic system; DNA sensor; SNP

1. Introduction

Rapid detection of specific sequence in genes is desired in a pathological examination site. [1] In addition, rapid analysis of single nucleotide polymorphisms (SNPs) in genes is important both for diagnosing the risk of common diseases such as hypertension, diabetes, and for determining optimum tailor-made medications [2,3]. Analyses for these purposes are carried out in medical examination sites or terminal medical facilities. Therefore, properties such as a high accuracy, low price, easy operation, and wide use are desired in contrast to a DNA microarray [1]. That is, large high throughput is not always needed. Recently, analytical methods that consider these properties have been reported. Currently, DNA analytical methods are carried out by one of two techniques that are based on enzymatic reaction [4,5] and DNA probe method [6,7]. The latter technique is especially useful for quick detection that has the above described properties. From this view point, new methods which use a groove binder [8] or intercalator [9–11] as a hybridization indicator have been reported actively. However, these methods can not satisfy requirements of high accuracy, low price, easy operation, and wide use simultaneously [12,13].

Microfluidic systems offer superior controllability of fluids [14]. This study developed a novel DNA analysis method that is suitable for terminal medical facilities. It uses a microchannel device for its high fluid controllability. The channel design of this microchannel and flow conditions, such as flow speed, are very important for fluid operation. The laminar flow in microchannel and its fluid operation enable the control of positioning and transfer of substances. Our breakthrough is high accuracy DNA detection through very simple handling.

2. Experimental

2.1. Microfluidic system

The microfluidic system chip used in this study was of two types prepared by mechanical methods as reported in a previous paper [15]. This chip is acryl-made and is 3 cm × 7 cm. The microchannel on this chip has cross-sectional plane whose size is 300 μm width, 200 μm depth, 2.0 mm diameter curve, and 24 cm length, or 1.5 mm diameter curve and 49 cm length.

2.2. Chemicals

All probe and target DNAs were obtained from Qiagen K.K. (Japan). The probe DNA was labeled by FITC at the 5′-end. Target DNAs 1–4 have complementary sequence to probe DNA, 5 and 6 have one base mutation sequence,

* Corresponding author. Tel.: +81-942-81-3676; fax: +81-942-81-3657.
E-mail address: maeda-h@aist.go.jp (H. Maeda).

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and 7 was prepared as a negative control. Sequences corresponding to probe DNA and target DNAs 1–6 were designed using part of a unique sequence of exon 4, on which the P72/R72 SNP resides, of cancer repression gene p53 [16]. Concentrations of these oligonucleotides were estimated from the molar absorptivities at 260 nm [17]: 171,800 $cm^{-1}\,M^{-1}$ for probe DNA; 98,300 $cm^{-1}\,M^{-1}$ for target 1; 144,600 $cm^{-1}\,M^{-1}$ for target 2; 186,000 $cm^{-1}\,M^{-1}$ for target 3; 648,600 $cm^{-1}\,M^{-1}$ for target 4; 101,800 $cm^{-1}\,M^{-1}$ for target 5; 189,500 $cm^{-1}\,M^{-1}$ for target 6; and 243,400 $cm^{-1}\,M^{-1}$ for target 7.

2.3. Apparatus

A syringe pump (KDS230; Kd Scientific, MA) controlled all injections of solutions into the microchannel. Laminar secondary flow velocity distribution of a cross-sectional plane was simulated by FLUENT (Fluent Asia Pacific Co., Ltd., Japan). The cross-sectional plane image and its fluorescence intensity profile were observed by confocal fluorescence microscopy (C1; Nikon Inc., Japan). Fluorescence intensity in sequence-selective DNA sensing was measured using fluorescence microscopy with a fluorometer (C7473; Hamamatsu Photonics K.K., Japan), Ar-gas laser (Stabilite 2017; Spectra-physics, CA), and a longpass filter (OG515; Edmund Industrial Optics Co. Ltd., NJ).

2.4. Sequence-selective DNA sensing

Probe and target DNA solutions were charged into the microchannel by syringe pumping simultaneously. Then fluorescence intensity of target solution side and probe solution sides were measured near the outlet of the straight part of the microchannel using above described fluorescence microscopy. Detection was evaluated with a ΔF value (the ratio of fluorescence intensity (b/a) of target solution side (b) and probe solution side (a) of microchannel) when irradiated with an excitation laser.

3. Results and discussions

Fig. 1 shows an outline of our method. The operation of our method is very simple: one simply injects probe and target DNA solutions into the microchannel simultaneously. Then this probe DNA solution must be injected into the inlet that goes on to the inner side at the first curve of microchannel. The microchannel design will be optimized for respective separation purposes. These two solutions form multiple laminar flow in microchannels. That is, a "differential medium laminar interface" will be formed in cases of the two soluble solutions. In a straight microchannel, mixing of two solutions depends simply on diffusion. If probe DNA has a complementary sequence to the target, these DNAs will form double strands near the interface. Molecular weight increases by double strand formation; therefore, the

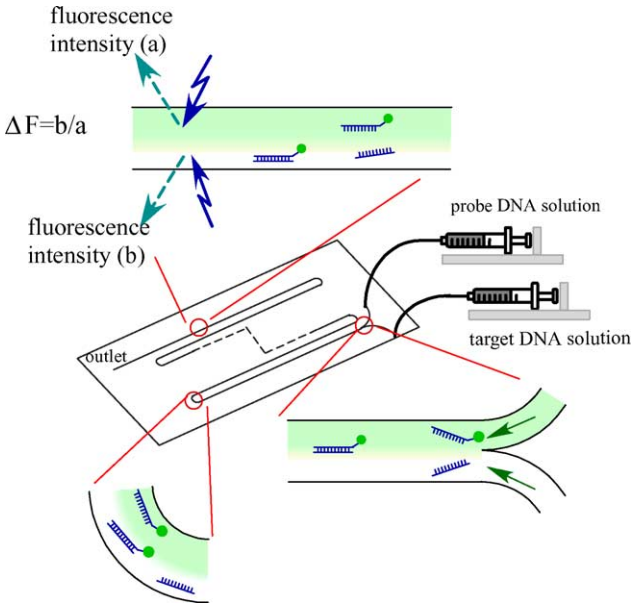

Fig. 1. Schematic principle and procedure of the gene diagnosis using the microfluidic system.

molecule localizes near the interface. In short, if the probe DNA that transformed to the interface by Brownian motion forms a double strand with target DNA, this double stranded DNA stays near the interface, otherwise it returns or goes forward. However, in cases of going forward, these forward DNAs will be canceled out by the laminar secondary flow at the next curve. Moreover, at the position through curves of the number of odd times ahead, this interface is transformed and expanded. Thereby, the formed double stranded DNA will increase further. At the curving part of the microchannel, internal force of the fluid produces a laminar secondary flow within the channel. Such laminar secondary flow at the turn disrupts the interface and moves double stranded DNA molecules to the outer side. When solutions were charged as

Fig. 2. Structures of probe and target DNAs 1–7 used in this study.

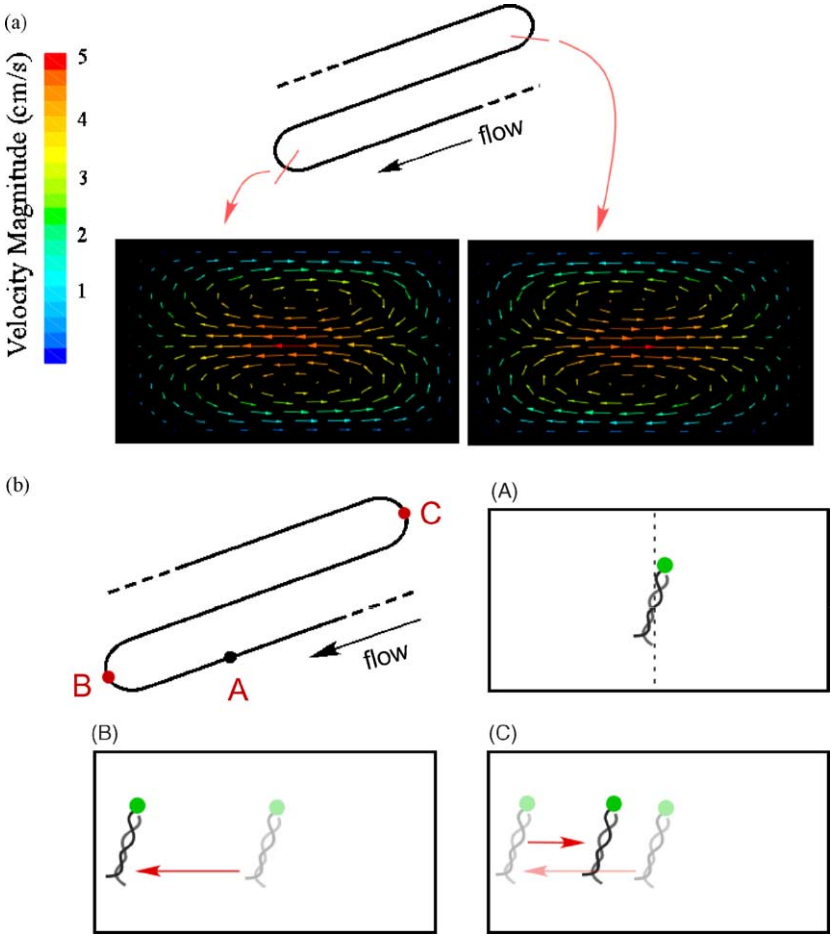

Fig. 3. (a) Laminar secondary flow velocity distribution of a cross-sectional plane at the curve of microchannel simulated by FLUENT in the same condition of Figs. 4 and 5. (b) Schematic separation principle of double stranded DNA, which is formed at the "differential medium laminar interface" of microchannel.

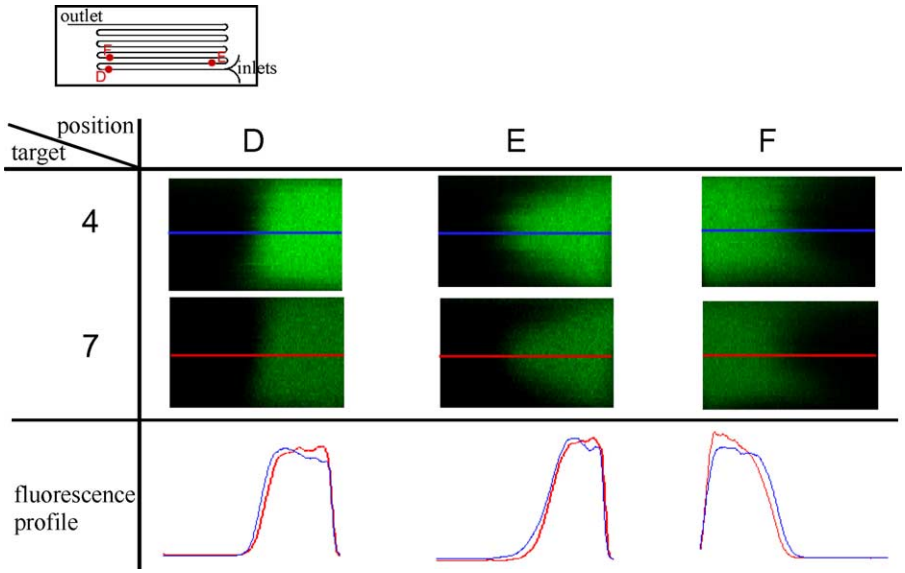

Fig. 4. Cross-sectional plane images and their fluorescence intensity profiles of fluid in a microchannel at positions D–F by confocal fluorescence microscopy observation. Blue and red lines are for targets 4 and 7, respectively. Probe and target DNA solutions are 0.50 pM in 5 mM phosphate buffer (pH 7.0) and 50 mM NaCl. All measurements were carried out at 25 °C; an Ar-gas laser (488 nm) was the excitation light source. Total flow speed is 80 μl min^{-1} (2.2 cm s^{-1}).

shown in Fig. 1, we have only to measure fluorescence intensity at the target DNA solution side of the microchannel.

Structures of probe and target DNAs 1–7 are shown in Fig. 2. The probe DNA was labeled by FITC at 5′-end. Target DNAs 1–4 have a complementary sequence to the probe DNA, 5–6 have one base mismatch against the probe sequence, and 7 was prepared as a negative control. In addition, instead of target DNA solution, the case of buffer solution was also measured as a blank.

Fig. 3a shows a velocity distribution of laminar secondary flow at the microchannel curve. This velocity distribution was simulated in the same condition of Fig. 5. As shown in Fig. 3b, double stranded DNA that was formed at the interface will be transformed to the outer side of microchannel at the first curve. At the next curve, the transfer distance is smaller than that of the first curve because this DNA should be positioned at inner side. This difference is a net transfer distance of a round trip. This DNA will get sufficient transfer distance and quantity in passing through several curves. In contrast, for a non-complementary sequence, this transportation and separation does not occur because double stranded DNA does not be form and accumulate at the interface. In addition, because of the influence of mixing by laminar secondary flow in cases of very rapid flow and diffusion in case of very slow flow, these flow conditions will not be applicable to our method.

Fig. 4 shows the cross-sectional plane image and the fluorescence intensity profile of fluid in a microchannel at any positions D–F by confocal fluorescence microscopy observation. At position D, the fluorescence intensity peak of target 4 shifts toward the interface in contrast to target 7. At position E, such movement is more conspicuous: the second intensity peak appears. At position F, in comparison to target 7, the fluorescence intensity of target 4 becomes broader and shifts to target solution side of microchannel. These results support the schematic separation principle, as shown in Fig. 3b.

Detection results at all positions are shown in Fig. 5a. At positions I and K that are past curves of an even number of times, only the response of fully complementary sequence target 3 is large in comparison with target 7 and blank solutions. In contrast, at positions H and J, i.e., through curves of the number of odd times, three responses are almost identical. That is, the detection position must be set-up through an even number of curves in our method. This is the transformation of the interface that is caused by laminar secondary flow at the microchannel curve. The interface between two solutions is transformed at the first curve, then restored at the next curve. Proper detection can not be accomplished in the state of the interface transformed because two solutions appear to overlap.

Fig. 5b and c shows detection results for all target DNAs at two different temperatures and solution conditions at position F. The coefficient of variation of these analyses was 5%. At higher salt concentrations and lower temperature conditions as in Fig. 5b, the ΔF values of targets 1–6, which is

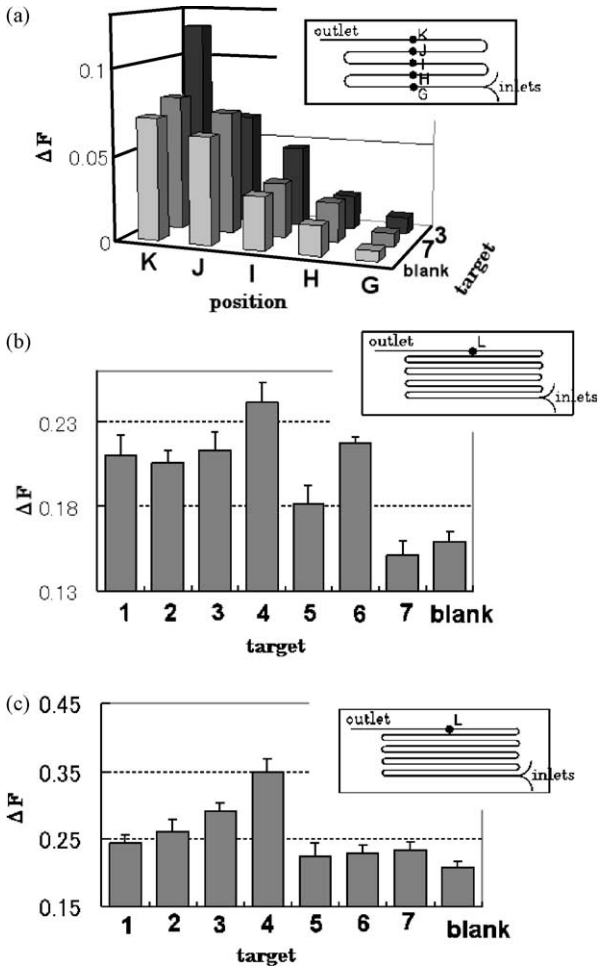

Fig. 5. Detection results of all positions (a) and all targets (b) and (c). ΔF (=fluorescence intensity b/a) value is the ratio of fluorescence intensity of target solution side (b) and the probe solution side (a) of microchannel when excitation laser is irradiated. ΔF values are shown as averages ($n = 10$). Error bars show standard deviation. (a) This microchannel has a 2 mm diameter curve and 24 cm length. Total flow speed was 40 μl min^{-1} (1.1 cm s^{-1}), and carried out at 23 °C. Probe and target DNA solutions are 0.50 pM in 5 mM phosphate buffer (pH 7.0) and 50 mM NaCl. (b), (c) This microchannel has 1.5 mm diameter curve and 49 cm length. Total flow speed was 80 μl min^{-1} (2.2 cm s^{-1}), and carried out at (b) 25 °C or (c) 35 °C. Probe and target DNA solutions are 0.50 pM in 5 mM phosphate buffer (pH 7.0) and (b) 50 mM, or (c) 5 mM NaCl.

complementary, or one base mismatch sequence are larger than that of target 7 and blank. In contrast, at lower salt concentration and higher temperature condition such as those in Fig. 5c, ΔF values of short or one base mismatch sequences are almost equal to those of target 7 and blank. Fig. 5c shows that, in cases of targets 1–4, whose sequences are complementary to the probe DNA, responses grow larger as target sequences lengthen. Especially, in case of the longer sequence target such as 3 and 4, its response is sufficiently large in comparison with the target 7 or blank solution as a negative control. These results support the principle shown in Fig. 1. On the other hand, responses of targets 5 and 6, which have one base mismatch against the probe sequence, are

almost identical to the negative control responses. That is, our method can detect one base mutation accurately. These results are explained by the difference of thermal stability of DNA duplexes in different salt concentrations. Calculated melting temperatures (T_m) [17–19] of the prove DNA and target 3 duplex are 51 °C ($[Na^+] = 50$ mM as in Fig. 5b) and 34 °C ($[Na^+] = 5$ mM as in Fig. 5c). T_m of prove DNA and target 6 duplexes, which contain one base mutation, are low about 5 °C [20].

4. Conclusion

We developed a novel separation method and a genetic analysis method using microfluidics. This method enables separation of complexes that are formed at the "differential medium laminar interface" of a microchannel by simple use of syringe pumping. That is, this separation is accomplished by molecular sieving effect microfluidics. Simple handling of our method enables minimum experimental error and difference in data by operators. This method requires no immobilization procedure. Therefore, no errors occur by scattering of immobilization quality and quantity. All reactions occur in the liquid phase. Moreover, our method can be applied not only to DNA, but also to complexes formed by specific binding. These features might be suitable for on-site use or analysis methods for biology, such as proteomics.

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