PORTABLE DNA DETECTION SYSTEM BASED ON ULTRAFAST SEGMENT-FLOW AND FLUORESCENCE DETECTION

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ABSTRACT

To detect the biological agents, the DNA detection is most usable due to the sensitivity and the selectivity. Although the key technology of DNA detection is real-time polymerase chain reaction (PCR), which uses exponential amplification of PCR and real-time detection of the amplified DNA fragments, the long thermal cycling time has been the problem to apply the rapid detection of pathogenic organisms. We developed a portable and rapid detection system based on ultrafast segment-flow PCR and fluorescent detection. Using the portable system, a model microorganism of biological warfare agent was successfully detected within 6.5 min with an enough high sensitivity.

KEYWORDS: Polymerase Chain Reaction, Portable System, Segment-flow, Fluorescence Detection

INTRODUCTION

A novel microfluidic system for PCR was developed and achieved ultimately fast and small-volume DNA amplification on a chip. In the decade, rapid gene detection has attracted interest from many researchers. In the excellent case, Kopp et al. has already reported flow-through PCR using a meandered microchannel contacted on three stainless blocks which were isothermally controlled at each reaction temperature for PCR [1]. This methodology for PCR amplification is very smart, because the temperature change of the heaters were unnecessary for PCR. In general, conventional PCR systems have used a heating/cooling unit having a large heat capacity encumbering rapid thermal cycling. On the other hand, the flow-through PCR device needs only to change the temperature of PCR sample solution, so that the thermal cycle could be carried out rapidly. However, the flowing PCR solution in the microchannel often suffered trouble by babble formation on denaturing process at about 95˚C. To prevent babble formation, we have tried to pressurize the solution in the microchannel against the vapor pressure at about 95˚C [2]. For example, the microchannes was previously filled with viscous oil, which was laborious pretreatment and reduceed the flow rate.

In the present research, we improved the flow-through PCR to accelerate the thermal cycling time and simplify the operation. The volume of PCR solution was decreased and transported in the meandered microchannel as a segment-flow [3]. The segmented PCR solution was moved over three temperature zones for denaturation, annealing, and extension reactions rapidly before the babble formation. Therefore, the DNA could be rapidly detected without any pretreatment.

EXPERIMENTAL

The microchannel device was made of polyolefin (PSP) film coated with pressure sensitive adhesives and cyclo-olefin polymer (COP) as shown in figure 1. The meandered microchannel was processed by micromachining. The enclosed structure of microchannel was fabricated simply by weighting the PSP film on the COP substrate. Furthermore, the real-time DNA detection systems were developed for the microfluidic device of segment-flow PCR. The heater units, syringe pumps, the fluorescence detector were integrated in a suitcase for portable use (figure 2). The PCR reagents and positive controls were purchased from Takara (Japan) as part of the Cycleave PCR Bacteria Screening Kit, which is optimized for real-time PCR of bacillus genes. The PCR solution was injected with a small volume, 1 to 5 μL, and flowed as segment-flow by an air syringe. The amplified DNA fragments was quantified with fluorescence detection at the end-point of the microchannel using a SELFOC μ-Fluorescence Detector (Nippon Sheet Glass, Japan).

RESULTS AND DISCUSSION

During segment-flow PCR, the difference of vapor pressures between the front and the end of segmented solution made a flow speed change, and the rhythm of flow speed was suitable for the elongation of extension reaction time. The flow rate could be risen ten-fold without babble formation compared with flow-through PCR using a same syringe pump. The miniaturized volume of PCR solution could decrease the resistance of flow. As a model agent of *Bacillus anthracis*, the spore solution of *Bacillus Subtilis* was used. Ultrasonic lysis of suspended spores is a promising alternative to conventional spore disruption techniques that include bead beating as the spore lysis gold standard. The spore solution was added in PCR mixture for bacillus detection after the ultrasonic treatment. The PCR sample was injected into the microfluidic device for 40-cycle PCR and then the amplified

fluorescence was monitored at the end-point of the microchannel. The fluorescence intensity clearly depended on the concentration of spores in the PCR solution. In general, the quantities of amplified DNA after enough efficient PCR achieves almost same level by the saturation of PCR products, which would indicate the same intensity of amplified fluorescence at the end-point of microchannel after real-time PCR. However, the amplified DNA could be quantified when the template DNA before PCR is not so high concentration. The fluorescent change of real-time PCR solution was observed by SELFOC fluorescence detector. As the results, target DNA could be obviously detected within only 6.5 minutes as shown in figure 3, and the detection limit was 10 genes/μL (figure 4).

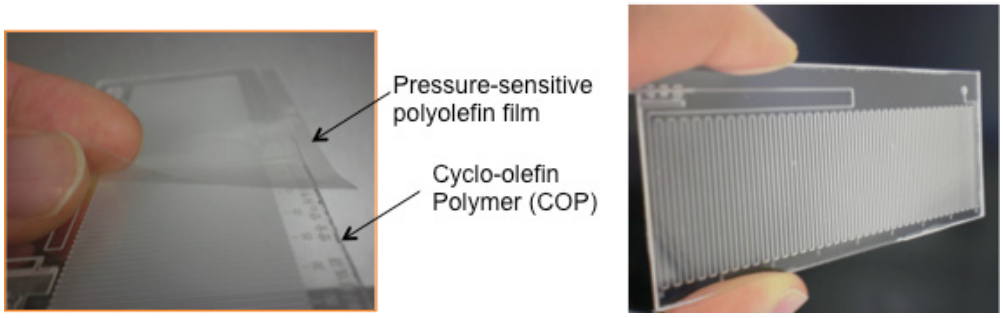

Figure 1: The microfluidic device for segment-flow PCR consists of COP and thin film.

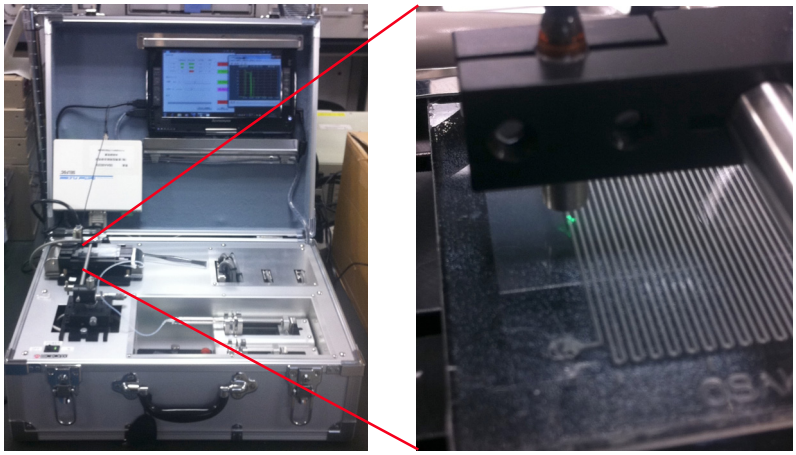

Figure 2: Portable DNA detection system based on segment-flow PCR (left) and fluorescence detector (right).

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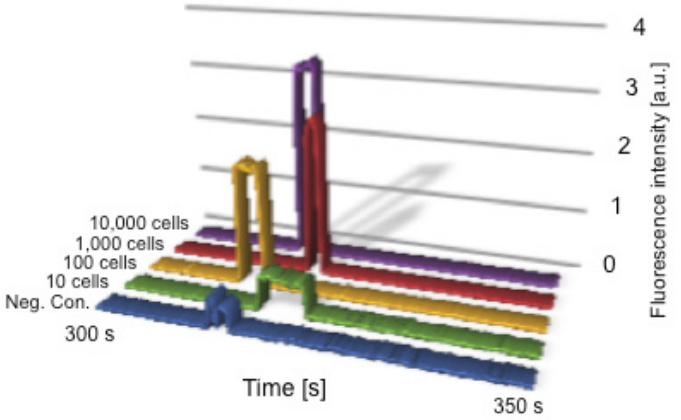

Figure 3: The end-point detection of fluorescence on the microfluidic device for segment-flow PCR.

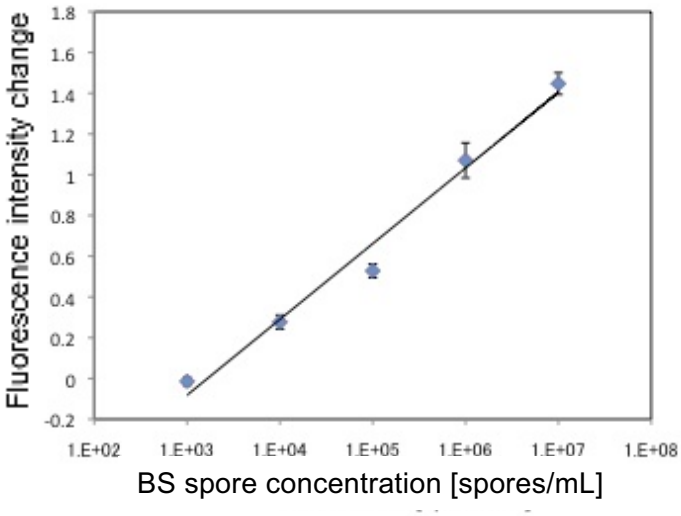

Figure 4: The quantification of anthrax genes using the microfluidic device for segment-flow PCR.

CONCLISION

As a segment-flow PCR, ultra-rapid DNA detection was accomplished with a minimal amount of PCR reagent. Using the developed portable DNA detection system, a model pathogen microorganism could be quantified within 6.5 min. The quantification of the microorganisms could be obtained good linearity with the enough high sensitivity.

ACKNOWLEDGEMENTS

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