ULTRA-FAST AND HIGHLY-EFFICIENT FLOW-THROUGH PCR MICROFLUIDICS USING VAPOUR PRESSURE AND ITS APPLICATION TO RAPID FIELD DETECTION

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ABSTRACT

Novel flow-through PCR microfluidics system using vapour pressure of destabilizing-flow source was developed and achieved ultimately-rapid and small-volume DNA amplification on a chip. The yield of PCR amplification compared to the thermal cycler was investigated against the cycling time, and resulted in about 80\% for 4 minutes. The chip could be discriminated the addition of sterilized anthrax to sample solution or not. The integrated chip device was fabricated for performing all the steps of mixing, amplification and detection on single chip only a pumping operation.

KEYWORDS: Polymerase Chain Reaction, DNA, Flow-Through, Anthrax

INTRODUCTION

The need for counter-terrorism measures has become even more important in the face of an increasing number of global terroristic threats. Although several new methods have been developed to identify biological threats, more sophisticated devices are still needed. In Japan, research and development projects have been ongoing toward this goal since 2007 with support from Japan's Ministry of Education, Culture, Sports, Science and Technology. The ideal detectors for biological agents should be fast, robust and sensitive and should be able to rapidly detect a biological agent in real time and in the field. A flow-through PCR microfluidics device holds the strongest promise for this application due to rapid DNA amplification and facile introduction of Micro-total analysis system (μ-TAS) by incorporating many functionalities [1]. The typical device is composed of three temperature zones (denaturation, extension, annealing) and a long micro-flow channel, which carries the PCR reagents to each of the temperature zones. Since the heat transfer coefficient is independent of the heating and cooling ramping rates, the flow-through PCR system can perform DNA amplifications faster than other types of PCR instruments. However, one of the biggest drawbacks of using microfluidics for PCR analysis is the need to circumvent the destabilization of flow caused when bubbles are generated in the denaturation zone as the temperature approaches the boiling point of water.

Normally, to introduce a highly viscous liquid to the microchannel is effective in bubble prevention because of the viscous liquid increases the internal pressure of the microchannel [2]. However, using the viscous liquid necessitates a complex operation and long amplification.

In our study, the vapour pressure of the destabilizing flow source was contra applied as the driving force, and ultimately rapid and successful amplification was accomplished with a minimal amount of PCR reagents required for detection. This method utilized only pumping force and heat to create a simple device with practical applications. Furthermore, the flowing rhythm created by vapour pressure minimized residual PCR products, leading to highly efficient amplification. Thus, the present device has achieved the breakthrough in several of the limitations of previous flow-through PCR devices.

The chip device was made of pressure-sensitive polyolefin (PSP) film and cyclo-olefin polymer (COP) substrate, which was processed with a micro-Numerical Control machine to produce a microchannel. The enclosed structure of the microchannel was fabricated solely by weighting PSP film on COP, resulting in a practical application that excels when compared with other devices [3].

For automatic-mixing on a single chip, pillar structures were constructed in a reaction chamber. Droplets of reagents were captured among these pillars by surface tension. When the sample solution was loaded into the chamber, the reagents were mixed and then flowed to the opposite side of microchannel.

As a real test sample, sterilized anthrax was employed in this study. Detection of two toxic plasmid genes was attempted. Air dust was collected in a public space with assistance from the security company.

THEORY

The combination of using vapour pressure and the serpentine design of the microchannel on the chip creates a flowing rhythm, which plays a critical role in PCR amplification. As the droplet flows from the denaturation to the annealing zone, the flow rate increases, while the flow rate decreases during flow in the opposite direction (Figure 1). Although the vapour pressure accelerates the droplet movement toward the annealing zone, movement in the opposite direction inevitably decreases. In addition, only a small quantity of fluid is used, such that the sample flows through the denaturation area of the chip before bubbles can be generated in the PCR reagents.

In general, the PCR process from denaturation to annealing requires moving the fluid as quickly as possible. This is because the denatured single-stranded DNA is very likely to form a double-stranded bond with the template strand or the complementary fragments in this process, resulting in the reduction of DNA amplification efficiency. On the other hand,
our method using vapor pressure not only has the fast speed required for the PCR process toward the annealing zone, but also allows for the slow speed in another PCR process from annealing to denaturation. The time-ratio recommended for PCR is 1 : 3 : 2 for denaturation : extension : annealing. This slow speed is very convenient for PCR, and it makes flow-through PCR more simple and automatic.

**EXPERIMENTAL**

The flow-through PCR microfluidic device could be packed the reacting solution and other materials into the chip device contamination-free before enclosing the microchannel. So, the integrated chip device was fabricated to perform all the mixing, amplification and detection steps on a single chip using only a pumping operation (Figure 2). The pillar structures were constructed in a reaction chamber so that reacting solution and sample solution were mixed in only a pumping operation. Due to the electrochemical determination of amplified products, disposable electrochemical printed (DEP) chips was set in the chip device. Electrochemical determination by the DEP chip was based on the binding of target gene and mixed redox intercalator.

The heat-transfer aluminum blocks were fitted with a heat conductor and temperature sensor (Kyushu-Nissho Co., Fukuoka, Japan). The aluminum block for cooling was contacted with peltier cooling element (SPE-UC-100, SAKAGUCHI E.H Corporation). The PCR device was placed on three aluminum blocks, and the PCR mixture solution containing target genes was introduced through the inlet using a syringe pump. The aluminum block was configured such that the PCR solutions alternately passed through three different temperature zones. To determine the optimum temperature setting of the aluminum block, the thermal distribution of microfluidics was examined using an infrared imager (Ti10, Fluke Corporation, USA). The temperature measurements are very close to microfluidic temperature because of the transparency and thinness of the PSP film. Amplified PCR products were collected into microplate wells and detected by fluorescence intensity using a fluorescence microplate reader (Fluoroskan Ascent in Thermoscientific).

**RESULTS and DISCUSSION**

The sample was provided by the Osaka Institute of Environmental Sciences in Japan, our research collaborator. The product was detected using a Cycleave PCR Bacillus anthracis detection kit Ver 3.0 from TAKARA Bio Inc. The detection of two toxic plasmid genes, PA and CAP, was attempted following 40 PCR cycles.

Yields of PCR amplification compared to those with thermal cycler were investigated against cycling time (Figure 3). The values were normalized to the fluorescence of the product from the standard thermal cycler as a reference and plotted against the total cycling time. Significantly, flow time of 120 seconds showed a fluorescence of 15%, while the negative control showed less than 5%. The amplified product was also detected with sufficient sensitivity. To the best of our knowledge, 40-cycle PCR in 120 seconds were the fastest reported PCR assay to date. Fluorescence increased as the curve line being defined by sigmoid function, which is a typical curve of PCR amplification. At around 300 seconds of cycling time, the chip had about 80% of the highest in yields. One reason for not receiving 100% yield may be that the length of extension zone was not optimal.

Thus, vapor pressure based flow-through PCR succeeded in completing ultimately-rapid and efficient PCR more simply.

Fluorescence intensity by PCR was compared with the thermal cycler and the PCR chip (Figure 4). The detection limit in the PCR amplification for 5 minutes was 10 cells per microliter. For practical implementation of this PCR chip device, the identification of anthrax aerosol in atmosphere was constructed using a combination of detection equipment consisting of a particle counter, air sampler (Biocapture by mesosystems), and the developed flow-through PCR microfluidics device. If the air sampler runs for 2 minutes, 10 cells per microliter are concentrated in the buffer. As a
results, the chip was able to detect the addition or absence of required sensitive anthrax in the sample solution. Sensitivity in less than lethal concentrations was detected in a combination of detection equipment. The estimated time for detection was 8 minutes, significantly reduced as compared to rapid field detection.

CONCLUSIONS

The vapor pressure of the destabilizing flow source was used as the driving force in a PCR microfluidics system for the first time. The chip device was made of pressure-sensitive polyolefin (PSP) film and cyclo-olefin polymer (COP) substrate, and integrated with the DEP chip for the electrochemical determination of amplified products. When compared to a commercial thermal cycler, the PCR chip device obtained approximately 80% amplification efficiency with an assay time of about 5 minutes. At even faster assay times, a 15% increase in yield was accomplished in 120 seconds using 40 cycles. Sterilized anthrax was employed as a real test sample and the chip was able to detect the addition or absence of required sensitive anthrax in less than 10 minutes the process from the sample trapping to detection.

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REFERENCES


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