MICRO Q-PCR CHIP ON A MINIATURIZED DETECTION SYSTEM FOR DNA DETECTION AND QUANTIFICATION

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ABSTRACT

In this study, a miniature quantitative real-time polymerase chain reaction (Q-PCR) detection system was demonstrated to allow for the detection of infectious diseases. A new flow-through PCR chips with three serpentine-shape (S-shape) pneumatic micropumps were designed to perform real-time PCR in an automatic format. The new design of array-type microheaters was adopted to improve the thermal uniformity. The fluorescence-based detection method was employed. With the integration of the fluorescence detection system, quantitative detection of hepatitis B virus (HBV) can be achieved.

KEYWORDS: Q-PCR, Microfluidics, MEMS, Micropumps

INTRODUCTION

To reduce the extra cooling and heating time for a PCR process, flow-through PCR microchips have been used. However, the entire reaction time for a PCR process is still determined by sample transport [1]. To tackle these technical challenges, the present study simplifies the control task by adopting S-shape pneumatic micropumps to precisely drive the sample flow [2]. In addition, a new design of array-type microheaters [3] and open reservoirs were adopted to improve the thermal uniformity in the PCR chambers and to facilitate the operation for temperature calibration.

Q-PCR systems based on PCR with fluorescence-based detection have been intensively used to amplify and simultaneously quantify a targeted DNA molecule. Therefore, a miniature Q-PCR detection system integrated with the flow-through PCR microchip was demonstrated in this study to allow for the detection of infectious diseases.

EXPERIMENTAL

A schematic diagram and a photograph of the miniaturized Q-PCR system were shown in Figs. 1(a). The fluorescence detection system was employed for detection of fluorescent signals [4]. A full wavelength fluorescence detection ranging from 200 to 1100 nm can be achieved with this approach. As shown in Fig. 1(b), the micromachined flow-through PCR chip comprised two micro modules for thermal and microfluidic control. The microfluidic control module, consisting of three S-shape micropumps, was used to store and rapidly transport the DNA samples through the three heating sections. Fig. 1(c) shows the membrane activation of the S-shape micropump controlled by one electromagnetic valve (EMV). In the micro thermal control module, three individual array-type
heating and temperature-sensing sections as shown in Fig. 1(d) were integrated to modu-
late the specific temperature field for three thermal steps of a PCR process.

The miniature Q-PCR system was then used for detection of HBV with a detection
gene (350-bp) by using SYBR Green fluorescent dye. The three S-shape micropumps
were controlled individually to adjust the cycle numbers and detention times of the sam-
ple in the three temperature control zones, where the PCR thermal cycles were performed.
The PCR sample was excited by a 470 nm blue LED and detected at 530 nm.

![Figure 1. Schematic diagram and photograph of (a) the miniaturized Q-PCR system [4] and (b) the flow-through PCR chip, including three reaction reservoirs and three S-shape micropumps. (c) Membrane activation of the S-shape micropump. (d) Schematic diagram and SEM photograph of the array-type microheater [3].](image)

RESULTS AND DISCUSSION

As shown in Fig. 2(a), the pumping rate of the S-shape pump was determined by the
driving pressure of the compressed air and the driving frequency of the EMV. Array-type
microheaters can be used to provide a uniform temperature distribution inside the reac-
tion chambers as shown in Fig. 2(b).

As shown in Fig. 3(a), biosamples with a concentration ranging from $10^4$ and $10^8$
copies/ml can be successfully detected using this system. By repetitive determinations of
known concentrations of the DNA template, the threshold cycles ($C_t$) were can be deter-
mined and shown in Fig. 3(b). Slab-gel electropherograms in Fig. 3(c) also proved that
the detection gene for HBV could be successfully amplified by using the new flow-
through PCR chip.

![Figure 2. (a) The relationship between the pumping rate and the driving frequency of the EMV. (b) Infrared image of the PCR chip and temperature distribution.](image)
CONCLUSIONS

In this study, a miniature Q-PCR system was developed to allow for the detection of infectious diseases. The new micromachined flow-through PCR chips were designed to perform real-time PCR in an automatic format. With the integration of a fluorescence detection system, quantitative detection of HBV can be achieved. The development of the miniature Q-PCR system may provide a useful platform for molecular diagnosis.

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REFERENCES


