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A miniaturized quantitative polymerase chain reaction system for DNA amplification and detection

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ABSTRACT

In this study, a new polymerase chain reaction (PCR) system incorporated with a fluorescence detection module and a micro PCR chip has been demonstrated to allow for on-line detection of infectious diseases. A new flow-through PCR chip was developed to reduce cooling and heating times for a PCR process. It is comprised of two micro modules for thermal and microfluidic control. The microfluidic control module in this chip adopts three serpentine-shape micropumps to rapidly transport DNA samples and PCR reagents through three reaction chambers. Using this approach, one can randomly adjust cycle numbers and reaction times of the DNA samples for each reaction, thus optimizing a PCR process. The micro thermal control module consists of three individual array-type heaters and temperature sensors to modulate three specific temperatures for three thermal steps of a PCR process. The optical detection system is then used for detection and quantification of the amplified products. The amplification and detection of detection genes associated with two viruses, specifically hepatitis B virus (HBV) and hepatitis C virus (HCV), has been performed to demonstrate the capabilities of the developed system. The miniaturized PCR system has been successfully used for DNA amplification and quantification and is a promising new tool for molecular diagnosis.

1. Introduction

In recent years, microfluidics has been an enabling technology, especially for biomedical and chemical analysis applications. It is envisioned that microfluidic systems made of micro-electro-mechanical-systems (MEMS) technologies can perform critical functions, including sample preparation, mixing, reaction, transportation, separation and detection, on a single chip in an automated fashion. The advantages of the microfluidic systems include less consumption of samples and reagents, faster analysis and higher sensitivity. Among promising biomedical applications, miniaturized polymerase chain reaction (PCR) systems using MEMS technology has attracted considerable interest recently [1–2]. PCR provides a well-developed method for nucleic acid amplification for genetic identification, diagnosis and many other applications. Based on the proper selection of specific primers, PCR can be used to perform fast nucleic acid amplification *in vitro*, resulting in the production of a large quantity of a target nucleic acid replicates. However, it is time-consuming to detect PCR products on a slab gel. Furthermore, precise quantification of the PCR products is challenging. Therefore, an improved method, usually referred to as quantitative PCR (Q-PCR), has been developed to amplify and simultaneously quantify a targeted DNA sequence [3,4]. Fluorescence-based optical detection measuring the intensity of the fluorescence signal resulting from the interaction between the fluorescent dyes/probes and an increasing amount of dsDNAs is commonly used for the Q-PCR detection. This method provides a high sensitivity and can quantify the PCR products in real-time without performing slab gel or capillary electrophoresis. However, the Q-PCR system is usually bulky and costly due to its complicated optical and thermal control components.

Recently, micro PCR chips have been extensively investigated and have shown their potential to replace their large-scale counterparts. Typically, micro PCR chips can be classified into two major categories, namely, micro stationary PCR chips [5–8] and

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Nomenclature

Al	aluminum
Au	gold
CCD	charge-coupled-device
DNA	deoxyribonucleic acid
EMV	electromagnetic valve
HBV	hepatitis B virus
HCV	hepatitis C virus
IR	infrared
LED	light emitting diode
MEMS	micro-electro-mechanical-systems
Mg^{2+}	magnesium ion
PCR	polymerase chain reaction
PDMS	polydimethylsiloxane
Pt	platinum
Q-PCR	quantitative PCR
S-shape	serpentine-shape
Ti	titanium
UV	ultra-violet
a.u.	arbitrary unit
bp	base pairs
ddH_2O	double distilled water
dsDNA	double-stranded DNA
C_t	threshold cycle
E_{ff}	amplification efficiency
R^2	coefficient of determination

continuous-flow PCR chips [9–16]. By modulating the thermal cycling (including number of cycles and reaction temperatures) within a reaction chamber, DNA amplification can be performed in the micro stationary PCR chips, similar to the manner in which conventional PCR instruments are operated. Due to the relatively small dimensions of the micro stationary PCR chip, it can perform thermal cycling faster as compared with its conventional large-scale counterparts. However, as the DNA templates and PCR reagents are stationary inside the reaction chamber, heating and cooling cycling of the DNA sample is still inevitable, thus increasing the entire reaction time. Besides, temperature uniformity inside the reaction area is crucial. Low duplication efficiency of the DNA may be caused by an appreciable non-uniformity in the temperature field.

Instead of moderating three reaction temperatures in a single reaction chamber, continuous-flow (also referred to as flow-through) PCR devices can perform the PCR procedure by driving the mixture of DNA templates and PCR reagents to flow continuously through a capillary or a microchannel, which have three functionally integrated but thermally isolated reaction zones. The three thermally isolated reaction zones can be achieved by using three sets of oil-baths and thermostats [9] or three copper blocks [10]. With this approach, the time required for the heating and cooling of the samples can be greatly reduced. The layouts of a serpentine channel [13] or a capillary tube [9] were commonly adopted to perform the PCR procedure. However, the number of thermal cycles and the duration of the annealing, extension and denaturation processes are determined by the individual layouts of the serpentine channels or the capillary tubes. Thus these chips can only perform three thermal steps with a constant time ratio and are usually relatively large when compared with the stationary PCR chips. This limitation was initially addressed by a circular arrangement of three temperature zones in a flow-through PCR chip [11,17–20]. Since the dimensions of the three heating regions and channels are fixed, it is still difficult to optimize the PCR processes by adjusting the time ratios of the three temperature process. To arbitrarily adjust the timing ratios and number of cycles, a flow-through PCR chip with close reaction chambers using multiple-membrane activation was reported by our group [21]. Furthermore, large-scale syringe pumps or external electric power supplies are usually inevitable precise fluid control of hydrodynamically driven [9–10,13,17,22] or electrokinetically driven [15] flow-through PCR chips. Alternatively, miniature components used for transport including magnetohydrodynamically driven pumps [12,18–20], thermally driven pumps [23], or pneumatic pumps [11,21] have been reported to continuously drive samples to flow-through the microchannel.

For most miniaturized Q-PCR detection systems with stationary [24–27] or flow-through [22,27] chips, bench-top light sources including bulky lasers and mercury lamps were commonly adopted to excite the labeling dyes or probes. In addition, microscope-based charge-coupled-device (CCD) cameras or other optical sensing instruments were used to accomplish the fluorescence detections. Compared to these large systems with high power requirements, compact excitation sources (light emitting diodes, LED) and optical sensors (photodiodes and photomultipler tubes) have been used to perform the fluorescence detection. For instance, a fluorescence detection system was incorporated with a capillary PCR system for on-line detection of PCR products [28]. However, it still used a glass capillary and required an external heater for performing PCR process. Recently, miniaturized systems with both integrated fluorescence detection and on-chip heaters/sensors have been reported [29–30]. Even though these micro systems can perform nucleic acid amplification and detection on a single chip, it still requires relatively complicated process to integrate these devices.

To tackle these technical challenges mentioned above, the present study therefore reported a new micro PCR system. A pneumatic micropump [31] was adopted to precisely drive the sample flowing through three reaction chambers. A new design of array-type microheaters [32] and open reservoirs were adopted to improve the thermal uniformity in the PCR chambers and to facilitate the operation for temperature calibration, respectively. The flow-through PCR chip was then incorporated with a fluorescence detection module for DNA amplification and quantification.

2. Materials and methods

2.1. Chip design

The micromachined flow-through PCR chip is comprised of two micro modules for thermal and microfluidic control as shown in Fig. 1(a). It was made of two polydimethylsiloxane (PDMS) layers and a glass substrate. The top PDMS layer is for serpentine-shape (S-shape) pneumatic microchannels and reaction chambers. The bottom PDMS layer is for liquid microchannels. The glass substrate is then used for depositing microheaters and micro temperature-sensing resistors. In the micro thermal control module, three series of array-type heating and temperature-sensing resistors are individually integrated to modulate the three specific reaction temperatures for the denaturation, annealing, and extension steps of a PCR procedure. A new design of array-type microheaters with dimensions of 6 mm × 6 mm, as shown in Fig. 1(b), is adopted in this study to enhance the thermal uniformity in the reaction region of the PCR chip. With this approach, a constant voltage source can be used to generate the same current passing through equivalent resistances for each heating element, thus providing a uniform temperature distribution. To ensure a precise thermal control process, platinum (Pt), with its linear temperature coefficient of resistance, is used to fabricate the heating and temperature-sensing resistors using the same process in order to simplify the entire fabrication procedure.

The microfluidic control module is comprised of three micropumps and three reaction chambers. A pneumatic micropump capable of generating higher pumping rates is adopted in this