A Micromachined Low-power-consumption Portable PCR System

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Abstract

This paper presents a portable system for Polymerase Chain Reaction. The developed device incorporates a platinum thin film thermo-cycler fabricated using a simple and reliable Micro-Electro-Mechanical-System (MEMS) process and a battery-operated thermo-cycler controller. The portable system is comprised of two basic components, namely a PCR control module and a PCR chip module. The PCR chip module is fabricated from cheap and biocompatible soda-lime glass and PDMS materials, and contains a micro-heater and a micro-temperature sensor in a 10 μL PDMS micro chamber provided to load DNA samples. An accurate temperature is maintained within the PCR chamber by means of a predictive control system which operates in conjunction with a neural network. When the system is powered by a portable 9 V DC battery, the developed system is capable of increasing the temperature in the chamber at a rate of 20.5°C per second and decreasing the temperature at 7°C per second over a working range of 50°C to 95°C. The measured temperature shift at the target temperature is less than 0.2°C and the average power consumption of the portable system is less than 1.2 Watts. The capability of the developed system in performing the detection of infectious diseases is verified using the portable system to successfully detect the species-specific gene and the antimicrobial resistant determinant respectively in Salmonella. The development of the portable PCR system could be crucial for on-site diagnosis of infectious diseases.

Keywords: PCR chip, Thermo-cycler, Portable system, Diagnosis

Introduction

The continuous development of MEMS technology and micro-fabrication techniques over the last decade has facilitated many advances in the execution of chemical and biochemical reactions on a microchip. Of the various reactions, the polymerase chain reaction (PCR) is of crucial importance in the field of molecular biology applications. Since the PCR technique was first reported in 1986, it has been widely applied in a diverse range of fields, including molecular biology, medical diagnostics, food improvement, and environmental monitoring. The PCR thermocycle consists of three steps, namely denaturation, annealing, and DNA extension [1]. Briefly, the number of amplified DNA molecules is doubled during each PCR thermocycle. These three steps are generally performed at temperatures of 95°C, 40-72°C, and 72°C, respectively. After 30 cycles, the DNA segments are typically amplified by more than one billion and are therefore readily detectable using a conventional slab-gel electrophoresis technique.

An ever-growing number of MEMS devices are now finding use in various biomedical fields. The advantages of these micro-devices include their high-throughput, disposability, low cost, low power consumption, portability, and high efficiency. Several miniature devices have been designed specifically for the PCR-based analysis of DNA. Micro-PCR chips and thermocyclers [4] have generally been fabricated using silicon-based materials [2-5], glass substrates [7], or polymers [8,14]. Some of the reported devices utilize a hybrid chip fabricated using a combination of these materials [6,9-13,15]. It has been shown that the high thermal conductivity (157 Wm⁻¹K⁻¹) of silicon-based materials delivers a rapid temperature increase in the PCR chamber, while the highly-controllable etching characteristics of these materials enables the fabrication of extremely precise chamber forms. Although the thermal delay time [12] of glass substrates is longer than that of silicon-based materials, glass has a superior biocompatibility and can generally be fabricated at lower cost. Moreover, glass chips are more appropriate for pre-PCR and...
post-PCR DNA analysis tasks such as capillary electrophoresis separation.

Micro-PCR devices can be classified according to the positioning of their heaters and sensors. As in the traditional heating methods employed in large-scale PCR machines, the heaters of some micro-PCR devices are located outside the reaction chamber [6-10, 16]. However, some researchers have deliberately moved the heaters to within the PCR chamber in order to reduce the device size and to minimize the power consumption of the system [2-5,11-13,15]. Typically, the heaters reported in the literature are in the form of metal films located on silicon or glass substrates. Thermocouples are generally used to perform temperature sensing within the chamber since they tend to be highly reliable [2-3,7-10,16]. However, connecting thermocouples to the remainder of the PCR system is generally more complex and these devices provide relatively poor detection response performance. Accordingly, some researchers have proposed integrating sensors on the substrate itself as a means of reducing the size of the thermocycler while simultaneously permitting the direct detection of the temperature [4,5,11-13,15].

Maintaining a precise temperature distribution within the chamber is of fundamental concern in achieving a successful amplification of the DNA in the PCR process. LabVIEW is commonly used to control the temperature in the chamber by means of temperature feedback or by using the percentage/integrator/difference (PID) control method. Using these approaches, researchers have been successful in maintaining the temperature to within less than 0.5 °C of the target temperature [5,7-9,12,15]. So-called micro controllers (μC) are widely used in the field [10,13,16]. They provide a convenient means of developing portable, battery-operated PCR systems.

This study uses simple MEMS techniques to fabricate a fully portable PCR system containing a PCR thermocycler and a temperature controller operating under the control of a microprocessor programmed using predictive control principles adopted from the field of neural networks. The developed system provides rapid PCR amplification and features thin film heaters and reduced-volume chambers, disposable chambers fabricated from inexpensive PDMS material, accurate temperature control using predictive control applied by a neural network (NN) model, and a low power consumption which can be satisfied by rechargeable batteries. In optimizing the system design, this study successfully uses the developed PCR device to amplify a DNA fragment length from 240 base pairs to 1500 base pairs (bps). The portable PCR system is then employed to carry out clinical detections of *Salmonella*. It is found that the results obtained using the proposed systems are comparable to those achieved using a conventional PCR machine.

**Experimental**

The PCR chip developed in the present study was fabricated using MEMS (Micro-Electro-Mechanical System) techniques and operated under the control of a neural network in order to increase its efficacy.

The PCR chip was fabricated on soda-lime glass substrates (G-Tech Optoelectronics Corp., Taiwan) using conventional photolithography and metal layer lift-off techniques. A simplified schematic illustration of the MEMS fabrication process is provided in Fig. 1(a). To improve system throughput, a PCR chip module incorporating two integrated thermocyclers was fabricated (Fig. 1(b)). In the chip fabrication process, a 100 nm platinum (Pt) layer with a 12 nm titanium (Ti) adhesion layer was initially evaporated onto a glass substrate by means of an E-beam evaporation process. A lift-off operation was then used to pattern resistors to serve as sensors and heaters. The resistances of the sensors and heaters were designed to be 350 Ω and 20 Ω, respectively. The process was then used to pattern electrical leads in the form of a 250 nm gold (Au) layer with a 20 nm titanium (Ti) adhesion layer. Finally, the PCR chip was completed by bonding a 100 μm cover glass plate (Marienfeld Corp., Germany) over the base glass plate using UV glue as an electrical isolation and protection layer. The PCR chamber was formed by bonding an upper PDMS (Polydimethylsiloxane) plate with a drilled cavity of volume ~20 μL on top of the micro thermal platform. Typically, PDMS, like glass, is an excellent biocompatible material for biological applications. In this study, sample volumes were chosen between 5~20 μL. To avoid any potential for contamination of the DNA, the PDMS chamber was discarded following each PCR operation. Furthermore, some of the mineral oil can be used to effectively prevent the DNA samples from evaporating. The fabricated PCR chip was set on a heat sink to improve the cooling rate in the chamber and was then packaged to form the PCR chip module shown in Fig. 1(b).

As shown in Fig. 2, the portable PCR system developed in the present study comprises a thermo-cycling controller module and a PCR chip module. The physical dimensions of the system are also presented in this figure. The overall weight
of the system is 272.1 gram (with no batteries fitted). The thermo-cycling controller consists of four components, namely the heating power supply controller, the temperature detection circuit, the display LCD, and the keypad. Fig. 3 provides a schematic diagram of the various system components. Regarding the power circuit supply, a stable and reliable supply is required if the system is to remain in its designed condition. Except using power supplies for testing, batteries were also used for characterization of the developed system. Although a fixed power supply was used for testing purposes in the current study, the system is actually designed to be battery operated to be fully portable. It was found that the power circuit provided a stable power supply over the range 7~12 V and hence a 9 V battery was specified to drive the system.

In the proposed system, the heating and sensing controller operates under the control of an ATMEGA8535 microcontroller (ATMEC Corpor., USA). This single chip provides a 10-bit analog to digital converter (ADC) and an 8-bit pulse-width-modulation (PWM) module. The function of the ADC is to convert the signal received from the detection circuit using an 8 kHz sampling rate. Meanwhile, the PWM module is used to heat the samples on the PCR chip at a heating rate which is governed by the specified value of the duty cycle. The output of the PWM is connected to a MOSFET, which acts as a switch to control the heating efficiency of the micro thermocycler. Regarding temperature control, thermal management of the PCR chip is executed by means of a predictive control mechanism provided by a neural network.

Fig. 4 illustrates the arrangement of the implemented temperature controller. In this diagram, \( Y_t \) represents the set of temperature values input through the keypad based on the required duplication conditions of the target DNA. Meanwhile, \( Y_p \) denotes the temperature signal corresponding to the temperature detected by the on-chip sensors. This feedback signal is supplied to the neural network, which subsequently outputs the signal \( Y_m \). \( U \) is the optimization value of the neural network and is given an initial value in close relation to \( Y_t \) once onset of the DNA amplification. \( U \) actually specifies the duty cycle of the PWM module and is used to control the switching on and off of the heating elements through the MOSFET switch. In other words, \( U \) is used to control the heating temperature in the reaction chamber of the PCR chip. The current neural network (NN) model comprises three layers. These neurons provide the neural network output, \( Y_m \). The width of the duty cycle is then adjusted according to the difference between the input signal, \( Y_t \), and the output signal, \( Y_m \). It is noted that the larger the difference between the two signals, the more the width is changed.

**Results & Discussion**

Experiments were performed to test the performance of the portable system in terms of the heating rate, the temperature stability at preset points, the power consumption at each step of the PCR process, the difference between the temperatures detected by a thermocouple and by the integrated temperature sensors, the difference between the conventional PCR machine and the portable PCR device and the PCR products following DNA amplification. The heating power, and hence the heating rate of the device, was controlled by adjusting the magnitude of the supply voltage. With a 7 V supply voltage, the heating rate detected by a thermocouple was found to be approximately 7 °C per second and the cooling rate, to be approximately 5.5 °C per second. Increasing the supply voltage to 12 V was found to increase the heating rate to a value of greater than 20 °C per second. However, such a high heating rate requires more power from the system. These values provide an acceptable trade-off between power consumption and sample heating rate performance.

In the conventional PCR cycle, the sample is maintained at each temperature step of the thermal cycle for about 60 seconds. To test the stability of the designed thermal platform,
different constant PWM duty cycles were specified to heat DNA samples in the chamber. The corresponding temperature profiles are presented in Fig. 5. It can be seen that with a constant duty cycle, a time of more than 100 sec is required for the temperature in the chamber to stabilize. In Fig.5, we get the optimal duty cycle values for heating. For example, we use the specific duty cycle values, which are 24/64, 9/64, 14/64, in PCR three thermo steps in the study. Furthermore, using NNs method in the system brings us a better control results. In Fig. 6, we compare the NNs-controlled program with non-NNs one, which use the same control circuit. The temperature variation is less than 0.2 °C with NNs, and it is much better than the circuit with non-NNs in the same system.

Fig. 7 shows the temperature stability results for each temperature setting point over a duration of 60 sec when the portable system was operated with 9 V. It is noted that the plotted data correspond in part to the previous thermal stage and to the transition period between two successive thermal stages as well as to the current constant temperature stage itself. The maximum temperature variation at each of the set points is found to be better than ±0.2 °C. Furthermore, it can be shown that the rate of temperature increase in the reaction chamber is approximately 11.5 °C/sec, while the cooling rate is 5.5 °C/sec. When using the integrated temperature sensors for detection purposes, the transition periods are found to be less than 0.5 sec. However, when the thermocouples are used, a time of 1~2 sec is required to acquire the target temperature due to the thermal delay between the platform and the DNA sample arising from thermal conduction and convection effects. When proceeding to the next thermal stage in the PCR cycle, the required value of the PWM module duty cycle is obtained by tuning the preceding value using the NN model. For the target temperature being precisely controlled, the temperature in the chamber must be calibrated. Before using the practical temperature value acquired with the sensor, taking a value by calibration in the sample will represent the set temperature in this step. In the present study, the sensing voltage was taken as an indication of the temperature value in the sample. Fig. 7 shows the temperature curves derived from the sample and the feedback signal received from the sensor. The temperatures of the sample and the sensor differ because of the longer response time of the thermocouple than the integrated sensor and also because of the thermo delay from conduction and convection in the PCR sample and the electrical isolation and protection layer.

The total power of the system, which is given by the power consumption of the on-chip heaters and that of the controller module, is shown in Fig. 8. The LabVIEW DAQ module (PCI-6014, National Instruments, USA) was used to record the power consumption of the system during each thermal step of the PCR cycle when the device was operated using a battery-operated supply. In the study, a LabVIEW program provides measurements where the sampling rate is 5. The results indicate that the total heating power of the device at the temperature setting points is ~1.4 W at 94 °C, ~0.9 W at 72 °C, and ~0.5 W at 57 °C. Furthermore, the total power consumption of the controller was found to be 0.28 W, i.e. including that of the LCD display and the keypad. During the PCR process, the average power of the system was determined to be 1.18 W with a maximum heating power of ~2.6 W. When the thermo step changes, the system provides maximum available power for heating in temperature rising, and
Table 1. Compare the specifications between the conventional PCR machine and the portable device.

<table>
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<tr>
<th></th>
<th>Conventional PCR machine</th>
<th>Portable PCR system</th>
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<tbody>
<tr>
<td>Heating rate (°C/second)</td>
<td>3</td>
<td>20.5 (by integrated sensor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5 (by thermocouple)</td>
</tr>
<tr>
<td>Cooling rate (°C/second)</td>
<td>2</td>
<td>7 (by integrated sensor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5 (by thermocouple)</td>
</tr>
<tr>
<td>Power consumption (Watt)</td>
<td>250*</td>
<td>1.18**, 2.08***</td>
</tr>
<tr>
<td>Dimensions (w × d × h)</td>
<td>180 × 300 × 230</td>
<td>98 × 74 × 40 (control module)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74 × 48 × 25 (chip module)</td>
</tr>
<tr>
<td>Weight (gram)</td>
<td>7500</td>
<td>184.8 (control module)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87.3 (chip module)</td>
</tr>
</tbody>
</table>

minimum available power for cooling, shown in Fig. 8. The use of a 9 V alkaline battery is sufficient to operate the system at the correct conditions. However, this battery restricts the operation time of the device to a maximum of 40 minutes, which is sufficient time to duplicate the DNA of base pairs less than 500, and this time is insufficient to duplicate the DNA by any more than 500 bps. However, replacing the 9 V battery with eight 1.2 Volt 1800 mA*hr batteries (GP NiMH Rechargeable Battery, Gold Peak Group, H.K.) in the series, the system was capable of amplifying DNA to longer base pairs (>500), which can be successfully detected by slab-gel electrophoresis. With this power supply, the portable system can be operated for more than 10 hours.

The temperature profiles of the conventional PCR machine (PCR Sprint, Thermo Hybaid, U.K.) and the portable PCR device detected in the PCR samples by a thermocouple are shown in Fig. 9. For an equivalent set of PCR parameters, the operation times of the portable system are found to be 98 seconds per cycle less than that of the conventional PCR machine. In general, the total operation times of the portable system is 1 hour less than that of the conventional PCR machine. Table 1 shows the differences between the conventional PCR machine and the portable device. There are advantages with the portable device in terms of operation time, power, dimensions, and weight. Although the sets of operating PCR of the portable device are fewer than that of the
conventional PCR machine, the portable device is provided with portability. Furthermore, increasing the number of the sets is realized by improvement of the PCR chip module in this system.

Initial testing of the PCR system performance was carried out by conducting the amplification of a 273 base pair fragment of S. pneumoniae autolysin (lyt A) genes. Twenty cycles performed at 94°C for 10 sec, 57°C for 10 sec, and 72°C for 10 sec were performed following a hot start at 94°C for 30 sec. The final step was an extension at 72°C for 60 sec. The total operation time of the designed PCR was less than 15 min, after which the duplicated DNA could be successfully detected by a slab gel electrophoresis technique. In this study, detection of Salmonella and the simultaneous detection of the antimicrobial resistant of Salmonella were performed using the proposed portable system. The corresponding slab gel electrophoresis images for the microorganism detections and their antimicrobial resistant are shown in Fig. 10. In the detection of Salmonella, the PCR product’s concentration is 1.11 μg/μl with the proposed micro PCR system, 1.37 μg/μl with positive control. In the simultaneous detection of the antimicrobial resistant of Salmonella, the PCR product’s concentration is 2.36 μg/μl with the proposed micro PCR system, 2.24 μg/μl with positive control. The results demonstrate that the performance of the portable PCR system is comparable to that of the conventional PCR machine.

Conclusion

This paper has presented a battery-operated portable PCR device comprising a microfabricated PCR chip and a miniature programmable controller. The micro-PCR device incorporates a PDMS chamber positioned on a glass thermocycler and is fabricated using a simple and reliable micro fabrication technique. The deliberate choice of PDMS and glass as materials ensures that the micro-PCR device can be readily integrated with other micro devices such as CE or pre-PCR process chips. After each PCR process, the reaction chamber is discarded to avoid any potential contamination of the DNA products. When operated by eight 1.2 Volt 1800 mA*hr batteries in series, the mini controller consumes about 1.2 Watts per second during the PCR process. This particular battery configuration provides sufficient power to operate the system for more than 10 hours, which is sufficient for general DNA amplification purposes. The proposed system has been used successfully and repeatedly to duplicate several DNAs used in clinical studies of bacteria detection. It has been shown that the performance of the proposed PCR system is comparable to that of a conventional PCR machine. Hence, the device presented in this study is suitable for use in the lab for clinical detection purposes.

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