A SELF-CONTAINED MINIATURIZED PCR SYSTEM USING ELECTROMAGNETIC ACTUATORS

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ABSTRACT

In this paper, the development of a portable polymerase chain reaction (PCR) device is presented. The fully integrated self-contained system consists of four major parts: a disposable chamber chip with micro-channels and pumping membranes, a heater chip with micro-heaters and temperature sensors, a linear array of electromagnetic actuators, and a control/sensing circuit. The system can be fully operated with a 5V DC voltage, and does not require any external air compressor or bulky power supply. The size of the whole system is 67 mm × 66 mm × 25 mm, and is smaller than a PDA cell-phone. The miniaturized PCR system not only has the advantage of smaller size, less consumption of DNA solution, but also can effectively reduce the PCR process time into one-third of the time required by typical commercial PCR system.

INTRODUCTION

The concept of lab-on-a-chip (L.O.C) has made great impacts on biological and medical research by minimizing the system size, reducing the sample volume, and shortening the reaction time. The researches on miniaturized PCR chips for nucleic acid amplification have grown rapidly because of the widespread applications to DNA sequencing, medical diagnosis, disease assay, and so on. In terms of heating process, there are two typical types of PCR chips: the stationary chamber-based type and the dynamic continuous-flow-based type [1]. For the chamber-based device, the PCR process is performed by keeping DNA solution in a reaction chamber which is cycled between different temperatures [2]-[4]. In [5], virtual chambers, which are in fact DNA sample encapsulated with mineral oil, are proposed for PCR process to accelerated the cooling and heating due to smaller thermal capacity. For continuous-flow-based devices, miniaturized channels used to convey DNA solution through zones of different temperatures for specific reaction duration [6]-[8]. This approach does not require heating or cooling each zone to different temperature at different time, and therefore might speed up the whole PCR process.

In this work, we develop a self-contained miniaturized chamber-based PCR system which employs reliable electromagnetic actuators for pushing DNA solution between the reaction chambers made by PDMS polymer. The proposed device has three chambers of different temperatures. During PCR process, DNA solutions are pushed among these chambers by tiny electromagnetic actuators. A micro heater and a micro temperature sensor are monolithically fabricated in each chamber. A controller (MCU) is used for controlling the actuation sequences and the chamber temperatures.

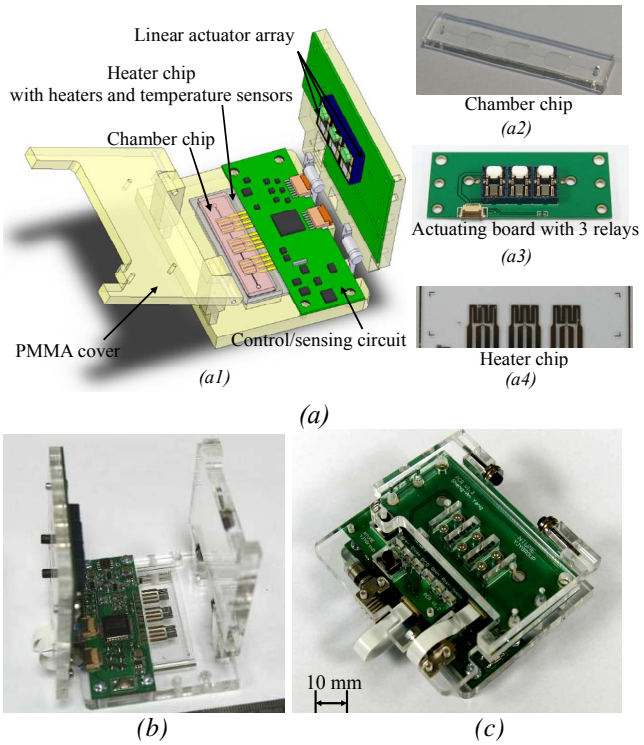

Figure 1: Miniaturized PCR system. The size of the integrated system is 67 mm × 66 mm × 25 mm.

DESIGN

Figure 1(a) shows schematic of the proposed PCR system. The detailed views of the components are shown in Figure 1(a2-a4). Figure 1(b) and 1(c) is the photographs of the assembled PCR system. The miniaturized PCR system consists of four major parts: a disposable *chamber chip* with micro-channels and pumping membranes, a *heater chip* with micro-heaters and temperature sensors, a *linear actuator array*, and a *control/sensing circuit*.

Figures 2(a) and 2(b) describe the operational principle of the device. These figures are the cross-sectional views of the $\overline{AA}$ line shown in Figure 2(c). Note that only the chamber chip, which consists of a *PDMS layer* and a thin *glass layer*, is shown in the figure. Figure 1(a2) is the picture of the fabricated chamber chip. The flexible membrane on chamber chip can be easily deformed by the compression force generated from a mini-actuator on top of each chamber. The chambers are connected by micro-channels.

As shown in Figure 2(a), these three chambers from left to right are the denaturation chamber, the extension chamber, and the annealing chamber. The temperature of each chamber is controlled by the heater chip (not shown) below the *glass layer* of the chamber chip. During the PCR process, two of the three actuators push the membranes of the corresponding chambers, and squeeze the DNA solution to the only un-squeezed chamber for

reaction at certain temperature for a specific time period. The DNA solution is pushed back and forth among these chambers for denaturation, annealing and extension reactions for 20 or 30 amplification cycles.

Furthermore, these three actuators can also generate specific peristaltic sequences, as shown in Figure 2(b), for pumping fluid. In other words, the device can also serve as a three-phase peristaltic micro pump. Moreover, the low-cost PDMS chip, which is designed to be disposable, is the only contaminated part where DNA solution is injected and manipulated. Figure 1(a3) shows the linear actuator array on which three mini-actuators are soldered. Commercially -available mini-relays (Panasonic TQ2-L2-5V) are employed as the mini-actuators.

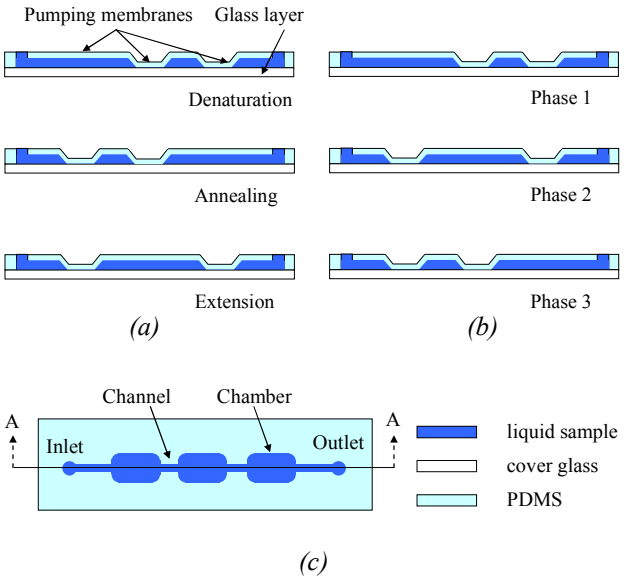

Figure 2: Working sequence of the actuators (a) for PCR amplification procedures; (b) as a three-phase peristaltic micro-pump. (c) The top view of the chamber chip.

For the *heater chip*, micro heaters and micro temperature sensors are realized by depositing and patterning platinum film on a glass substrate. Figure 1(a4) shows the fabricated heater chip. Real-time temperature of each chamber can be detected by the temperature sensor. By means of PID feedback control technique, the temperature in the chamber is maintained at the target level. Figure 3 shows the schematic illustration of a heater and a temperature sensor for each chamber. Since the temperature on the edge of the heater is usually lower than that in the center, the width of the serpentine wires near the edge of the chamber is narrower so that the heat generation is larger (under constant electric current). By this mean, temperature compensation around the edges can be achieved, and the temperature inside the whole chamber could be more uniform.

The control/sensing circuit controls the actuators and the heaters, and read the signals from the temperature sensors. The self-contained system can be fully operated with a 5V DC voltage, and does not require any external air compressor or bulky power supply. Six LEDs are also placed on the circuit board to indicate the operating status.

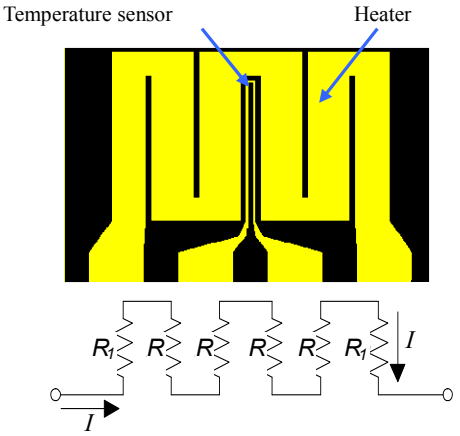

Figure 3: Design of a heater incorporated with a temperature sensor. The equivalent circuit of the heater illustrates the effect of temperature compensation.

FABRICATION

Chamber Chip

Figure 4(a) illustrates the fabrication process of the chamber chip. The chamber chip with micro-channel and micro-chamber structures is fabricated using a SU-8 mold. First, SU-8 (GM 1070) is spin-coated and patterned on a silicon wafer as the mold for the channel and chamber structures with a thickness of 100 μm. Then, PDMS replicates are created using the mold. On each PDMS replicate, two holes are punched as the inlet and outlet for loading DNA solution. Finally, the PDMS layer and a cover glass are bonded together by oxygen plasma treatment of 90 seconds.

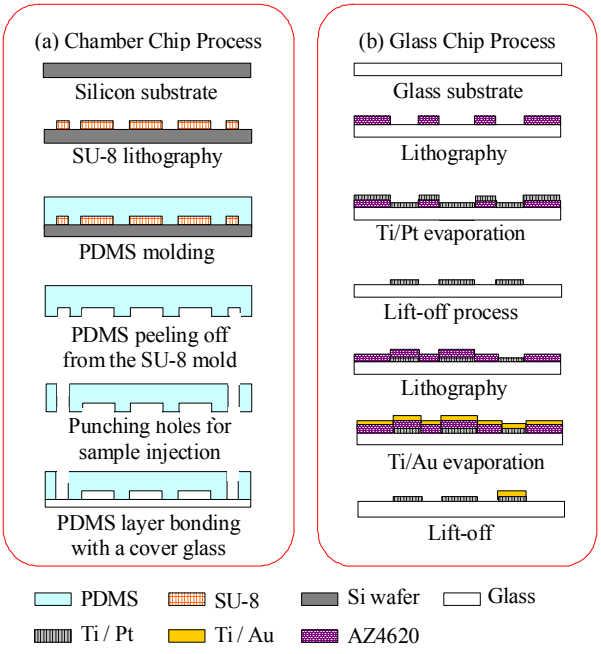

Figure 4: Fabrication process of the PDMS chip and the glass chip.

Heater Chip

Figure 4(b) describes the fabrication process of the heater chip. Micro-heaters and temperature sensors are

fabricated on a glass substrate by thin-film deposition and lift-off process. First, lithography of AZ4620 as photo-resist is carried out. A 80 nm thin platinum (Pt) layer is deposited with a 20 nm titanium (Ti) adhesion layer by e-beam evaporation. The platinum pattern is defined by lift-off process in acetone solution for the temperature sensors and the micro-heaters. Again, the lithography process and lift-off process are carried out for defining the electrical leads with a 400 nm evaporated aurum (Au) layer and a 20 nm Ti adhesion layer.

RESULTS

Figure 5 illustrates the measured transient temperatures at the centers of the three chambers detected by an infrared thermometer. These three chambers are heated to 94 ℃, 54 ℃, and 72 ℃ for denaturation, annealing, and extension, respectively. The temperatures in the annealing and extension chambers reach the target levels within 15 seconds, while the denaturation chamber reaches its target level around 40 seconds. PWM method is used to feed power to the heater.

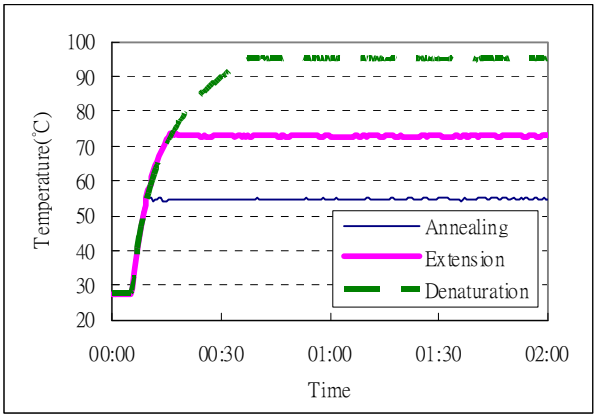

Figure 5: Temperature measured during heating by an infrared thermometer of the 3 chambers respectively.

Figure 6 shows the temperature distribution captured by an infrared imager. Figure 6(a) shows the infrared image in which all three chambers are heated simultaneously at the designated temperatures. Figure 6(b), (c), and (d) are the infrared images when one of chambers is heated to its corresponding temperature. Even though thermal interaction occurs between the three chambers, the temperatures of the three chambers are successfully controlled at the target level.

The measured flow rates at different frequencies are presented in Figure 7 by using the working sequence illustrated in Figure 2(b). Obviously, the flow rate reaches a maximum value of 25 μL/ min as the operating frequency is around 40 Hz, and then decay rapidly as the frequency increases.

During the PCR process, the chamber chip, which is loaded with DNA solution, is fastened above the heater chip by folding the PMMA covers which is integrated with the linear actuator array, as shown in Figure 1(c). By using the miniaturized system, successful PCR is performed for the MCF-7/adr cell line (122 bp segment). Primer sets of GAPDH (forward primer: 5'-AGT CAA CGG ATT TGG TCG TA-3'; reverse primer: 5'-GAA ACA TGT AAA CCA TGT AG-3') are chosen. The 8 μL DNA solutions, which contains DNA samples, double distilled water (ddH_2O), and Gotaq® Green Master Mix, is injected into the PDMS chip for the PCR amplification. Firstly, the DNA solution is warmed up at 94 ℃ for 90 seconds. Then, 30 thermal cycles of different temperatures are performed. In each cycles, the set point temperatures are 94 ℃ for 25 seconds, 54 ℃ for 25 seconds and 72 ℃ for 30 seconds. Finally, temperature of 72 ℃ is kept for 5 minutes.

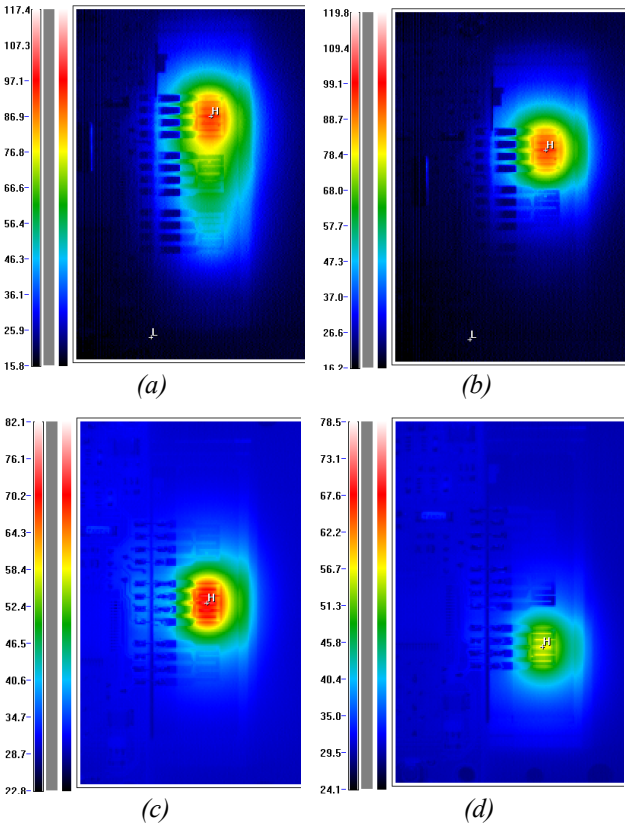

Figure 6: Infrared thermal image of the three chambers.

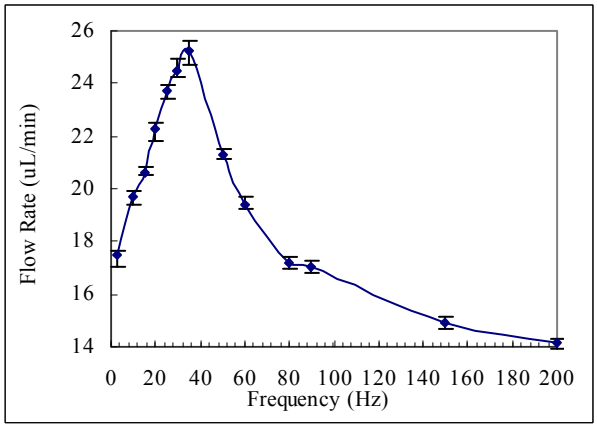

Figure 7: The measured flow rate of the peristaltic pump actuated by three electromagnetic actuators.

Figure 8 shows the photograph of slab gel electrophoresis of the PCR products. Lane M is the DNA ladder. Lane 1 is the result by a commercial PCR machine (Mastercycler gradient, Eppendorf®). The result of our miniaturized PCR system is shown in Lane 2. For both cases, the 122 bp PCR products are clearly observed, and no other specific products are found. Also, the results by our system are consistent with the result by the commercial

PCR machine. Our PCR system takes 46 min and 40 sec for the whole PCR process, while the commercial PCR machine takes about 2 hours and 30 minutes. Note that the required DNA sample for commercial PCR machine is 25 μL. Therefore, our PCR system only required less than 1/3 of the DNA sample.

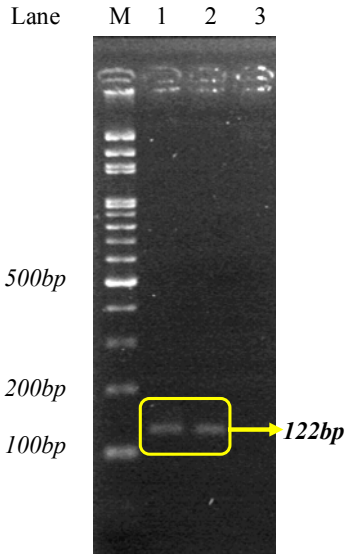

Figure 8: The observed fluorescence photograph of slab gel-electrophoresis of the PCR products.

CONCLUSION

A self-contained PCR system is developed in this work. Three chambers are designed for different reactions at specific temperatures. DNA solution is driven by electromagnetic mini-actuators between these chambers. Micromachined heaters and temperature sensors are also implemented in the system. The PWM method is used to feed the power to the heaters, and the PID technique is employed to control the temperatures at the target levels. Successful PCR amplification is performed. The DNA sample required by the miniaturized PCR device is less than one third of that required by a commercial PCR machine. Also, the proposed PCR system takes about 47 minutes for the whole PCR reaction, which is about one third of that by a commercial PCR machine. The self-contained system, whose size is 67 mm × 66 mm × 25 mm, can be fully operated with a 5V DC voltage, and does not require any external air compressor or bulky power supply.

ACKNOLEDGEMENTS

This project is sponsored by the National Science Council, Taiwan, ROC (contract no: NSC 96-2323-B-002-009). The authors would like to thank Prof. A.-B Wang for providing the infrared imaging system.

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