MICROFLUIDIC PCR CHIP INTEGRATED WITH MCU CONTROLLED TEMPERATURE SYSTEM

Dawoon Han[1], Mijung Choi[1], You-Cheol Jang[1], Kamrul Islam[1], Rohit Chand[1], Sandeep K. Jha[1], Sung Sik Ko[3], Ik-Jun Yeon[4], Yong-Sang Kim[1,2]

[1]Department of NanoScience and Engineering, Myongji University, Gyeonggi-do 449-728, Republic of Korea.
[2]Department of Electrical Engineering, Myongji University, Gyeonggi-do 449-728, Republic of Korea.
[3]Department of Sports & Health Care, Chungju National University, Chungbuk 380-702, Republic of Korea.
[4]Department of Environment Engineering , Chungju National University, Chungbuk 380-702, Republic of Korea.

Introduction

The polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA to several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. Typically, PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of 3 discrete temperature steps [1]. However, this procedure is laborious and time consuming with a conventional system and therefore requires miniaturization. The miniaturization offers a great potential of integrating different sample processing units on a single microchip, which are the essential objectives for realization of a lab-on-a-chip device [2]. Therefore, various groups have reported the development of on-chip PCR analytical systems [3]. Among them, the continuous-flow PCR chip possesses several advantages such as fast thermal cycling, series-amplifications, cost-effective fabrication, portability, and rapid detection.

Experimental

Fabrication of PCR chip

The microfluidic PCR chip is consisted of two parts. The PDMS microchannel was fabricated using negative molding method for sample flowing. Nagative photoresist (SU-8 2075, Micro Chem.) was spin-coated onto a bare silicon wafer. SU-8 was patterned to make a microchannel using photolithography (MA-6, Karl-suss) technique. The PDMS (DC-184, Dow Corning) mixture was poured on the SU-8 negative master pattern and cured for 4 hours at 75 °C. The PDMS was then peeled off and manual drilling was done to produce access holes. The width and depth of the microchannel are 500 and 200 μm respectively, and total length is 4625.83 mm for 25 cycles. ITO heaters were fabricated using conventional photolithography and wet etch process. Positive photoresist (AZ1512, Clariant) was spin-coated on ITO film deposited glass (Samsung Corning). AZ1512 was patterned to make electrode using photolithography. ITO film was etched using FeCl3/HCl solution for 1 hour and photoresist was removed. Fabricated PDMS microchannel and glass/heater chip were bonded each other after UV-ozone treatment during 40 min (Fig. 1).

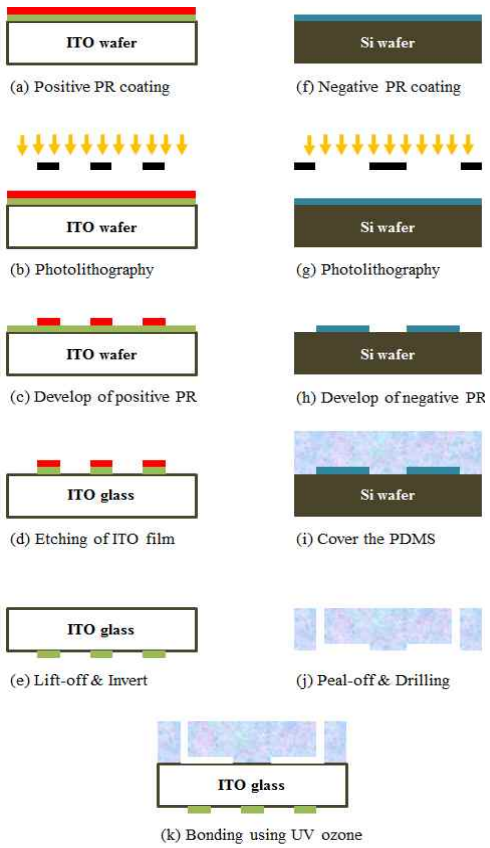

Fig. 1. Fabrication process for PCR chip.

MCU Controlled temperature system

A schematic diagram of the ASIC control system is shown in Fig. 2. In the control system, an ATmega128 microcontroller (ATMEL Corp.) acts as a 8-bit analog to digital converter (ADC) and an 8-bit pulse-width-modulation (PWM) module controls the micro sensing and heating elements. The function of the ADC is to convert the signal received from the detection circuit

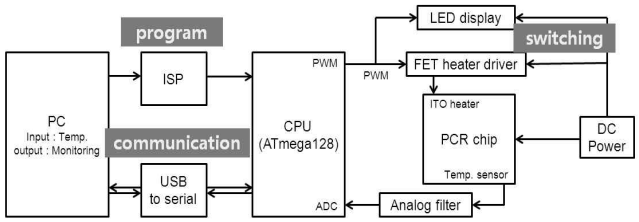

Fig. 2. Schematic diagram of MCU controlled temperature system

using an 8 kHz sampling rate. Meanwhile, the PWM module heats the DNA samples at a heating rate which is governed by the specified duty cycle value. The output of the PWM is connected to a MOSFET (metal-oxide-semiconductor field-effect transistor, IRF3205), which acts as a switch to control the flow of current through the heating sensor (LM 35DZ), thereby providing a means to control the heating efficiency of the micro thermocycler. As described above, the microcontroller provides digital signals to LED, which in turn cause the input voltage devide each regions to keep each temperatures.

Results and Discussion

A miniaturized PCR microfluidic device was fabricated in this study using standard photolithographic technique. The PCR microchip composed of PDMS based microchannel for sample injection and retention. The indium-tin-oxide (ITO) microheater was fabricated on glass substrate for thermal cycling. The PDMS microchannel was fabricated by negative molding method. The width and depth of fabricated microchannel are 250 μm and 200 μm, respectively. The ITO heaters were a choice for thermal cycling as it offers linear calibration range for temperature upon application of DC power. The thin film heaters were mounted on a glass substrate by photolithography and wet etching techniques. The three different temperature zones namely denaturation, annealing, and extension for the PCR reaction were controlled with computer interface. As it was crucial to precisely control the cycling of temperature on the PCR microchip, therefore, we combined fabricated microchip with temperature sensors (LM35Z) and computer program. The computer system was operated by 8-bit microcontroller (ATmega128). They were utilized for the system control and sensing information. In order to obtain the temperature information from the device heating sensor, a low DC bias was used. The sensor produced feedback control for each temperature zones (Fig. 3). The fabricated microchip was further used for on-chip amplification of genomic DNA from bacteria E. coli. The agarose gel electrophoresis of PCR amplicon from this experiment is shown in Fig. 4. The result indicates that the PCR products obtained after thermocycling of the bacterial PCR mixture (lane 2 and 3) shows similar with band as the PCR products obtained after thermocycling in a conventional PCR instrument (lane 1 and 4), which proves the success of our proposed device.

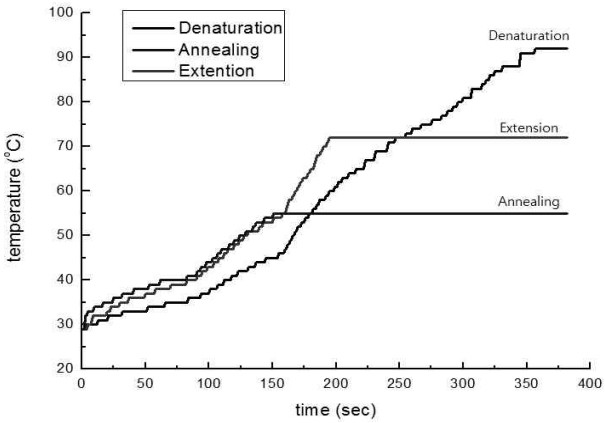

Fig. 3. Temperature calibration
(Denaturation - 92 ℃, Annealing - 74 ℃, Extension - 55 ℃)

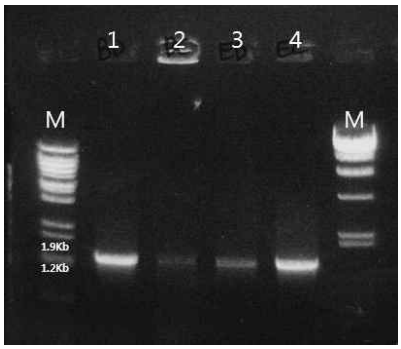

Fig. 4. Gel-doc showing *E.coli* and *B.subtillis* 1.5 kbp DNA amplification

Conclusion

We have demonstrated PCR microchip based on ITO glass substrate. The ITO is transparent material and has good thermal conductivity under influence of DC bias, so it is convenient for use as microheater on PCR chip. The temperature on ITO microheater was regulated by thermostatic action by using an on-line computer system. As a result, microfluidic PCR chip could be used in amplification of target DNA in a similar way to a conventional PCR machine.

References

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