Proceedings of the 2nd IEEE International
Conference on Nano/Micro Engineered and Molecular Systems
January 16 - 19, 2007, Bangkok, Thailand

ITO-coated glass/polydimethylsiloxane continuous-flow PCR chip

Seung-Ryong Joung, Jaewan Kim, Y. J. Choi, C. J. Kang and Yong-Sang Kim*

***Abstract*—We propose a continuous-flow polymerase chain reaction (PCR) chip using indium-tin-oxide (ITO)-coated glass/ polydimethylsiloxane (PDMS) materials for DNA amplification. The continuous-flow PCR chip enables fast thermal cycling and series amplification, which are difficult to achieve in a conventional PCR or micro-chamber PCR chip. Six heaters of ITO thin films were fabricated on glass for the thermal cycling of the flowing PCR sample. The PDMS microchannel was fabricated using a negative molding method. The width and depth of the microchannel are 250 μm and 200 μm, respectively, with a total channel length of 1340 mm. The PCR chip can perform 20 cycles of amplifications. The ratio of the channel lengths for three different temperature zones, namely denaturation, annealing, and extension, is 2:2:3, respectively. Using the fabricated continuous-flow PCR chip, two DNA plasmids (720-bp pKS-GFP and 300-bp PG-noswsi) were successfully amplified.**

***Keywords*—polymerase chain reaction, continuous-flow PCR chip, PDMS, glass, indium-tin-oxide film**

I. Introduction

POLYMERASE Chain Reaction (PCR) is a well-established method for amplifying specific regions of a DNA. PCR is a widely utilized scientific tool in molecular biology specifically in DNA sequencing, genotyping, new drug discovery and environmental application. Conventional PCR devices are suitable for amplifying DNA, but they are sizable devices and require long cycling times. Recently, miniaturized PCR devices have attracted great interest because they have many advantages over conventional PCR devices, such as portability, low cost, fast thermal cycling speed, reduced reagents/sample consumption [1,2]. Nowadays, most miniaturized PCR devices can be classified into two types, micro-chamber PCR chips [3], and continuous-flow PCR chips [4-6]. Specially, continuous-flow PCR chips have more advantages since a series of amplifications is achievable and integration of this chip with other microfluidic systems is possible. Figure 1 shows the schematic of continuous-flow PCR chip.

We propose continuous-flow PCR chip using ITO heater integrated glass and polydimethylsiloxane (PDMS) for DNA amplification. The continuous-flow PCR chip is composed of two parts namely PDMS microchannel for sample injection and ITO heater/glass chip for thermal cycling. The PDMS is widely used material for microfluidic devices because it can be easily and repeatedly fabricated by the molding method. Indium-tin-oxide (ITO) heater electrodes integrated on the glass chip for thermal cycling enable portable device realization without huge temperature control system. Furthermore, PDMS, ITO electrode and glass are all transparent material, so they are capable for fluorescence measurement and real-time monitoring. The continuous-flow PCR chip also can be applied for a 'lab-on-a-chip' or a micro total analysis system (μ-TAS).

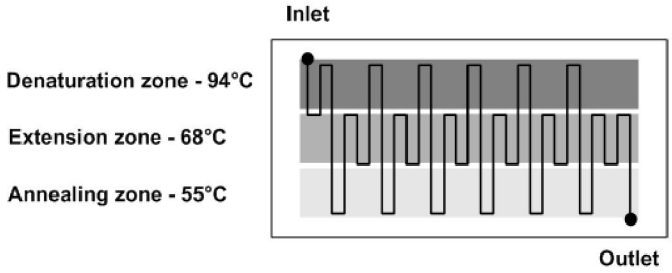

Fig. 1. Schematic of continuous-flow PCR chip.

II. Experimental

A. Fabrication of continuous-flow PCR chip

The PDMS microchannel was fabricated using negative molding method for sample delivery. Negative photoresist (SU-8 2075, Micro Chem Inc., Newton, MA, USA) was spin-coated on a bare silicon wafer. SU-8 was patterned to make microchannel using photolithography (MA-6, Karl-suss) technique. The PDMS (DC-184, Dow corning) mixture was poured on the SU-8 negative master template and cured 4 hours at 72°C. The PDMS was then peeled off and access holes were drilled manually. The width and depth of the microchannel are 250 μm and 200 μm, respectively, with a total channel length of 1340 mm, which is equivalent for 20 thermal cycles. The ratio of the channel lengths for three different temperature zones

This work is supported by grant No. M10600000274-06J0000-27410 from the Korean Ministry of Science and Technology for National Research Laboratory Program.

Seung-Ryong Joung, Jaewan Kim, Y. J. Choi, C. J. Kang, Yong-Sang Kim are with Department of Nano Science & Engineering, Myongji University, Korea.

Yong-Sang Kim is with Department of Electrical Engineering, Myongji University, Korea.

Jaewan Kim, Y. J. Choi, C. J. Kang is with Department of Physics, Myongji University, Korea.

*Contacting Author: Yong-Sang Kim, Department of Electrical Engineering, Myongji University, Korea (Phone: +82-31-330-6365; Fax: +82-31-321-0271; email:kys@mju.ac.kr).

1-4244-0610-2/07/$20.00 ©2007 IEEE

namely denaturation, annealing, and extension, is 2:2:3, respectively. Indium-tin-oxide heaters were fabricated using a conventional photolithography and wet etching process. Positive photoresist (AZ1512, Clariant, Switzerland) was spin-coated on ITO film deposited glass (Samsung Corning co., Korea). ITO film was etched using $FeCl_3$/HCl solution for 2 hours before the photoresist was removed. For electrical isolation, PDMS was spin coated on the glass/heater chip and baked at 95 °C for 30 min. Fabricated PDMS microchannel and glass/heater chip were bonded with each other under UV-ozone treatment for 40 min. Figure 2 shows the fabrication process of continuous-flow PCR chip. Figure 3 is a photograph of fabricated continuous-flow PCR chip.

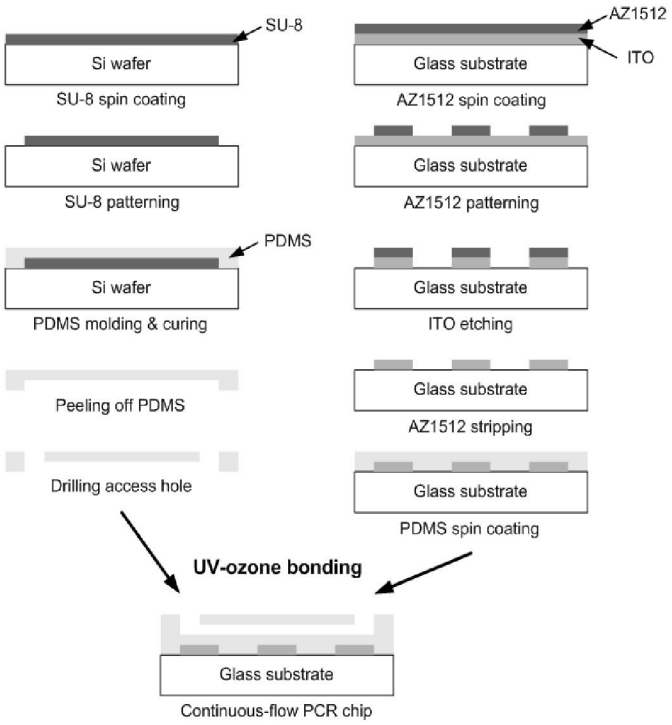

Fig. 2. Fabrication process of continuous-flow PCR chip

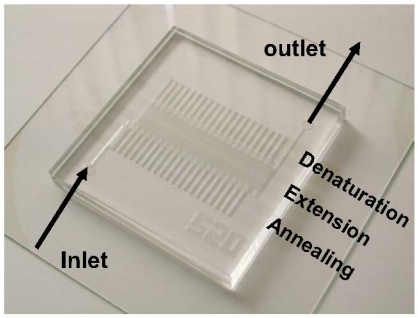

Fig. 3. Photograph of fabricated continuous-flow PCR chip

B. PCR reagent

Two DNA plasmids,700 bp pKS-GFP and 300 bp PG-noswsi, were amplified using the fabricated PCR chip. Each reaction mixture contained EF-Tap DNA polymerase 2.5 U/μl, 10 mM each dNTP, and 10× EF-Taq reaction buffer (SG6202, Solgent, Korea). The primer set used for pKS-GFP is composed of 10 pmole/ml each of GFP-F (forward; 5'-AG CC ATG GC GAGCAAGGGC-3') and GFP-R (reverse; 5'-TTACTTGTACAGCTCGTCCA-3'). On the other hand, the primer set used for 300 bp PG-noswsi amplification is 10 pmole/ml of each component T7 (forward; 5'-TAATACGACTCACTATAGGG-3') and SP6 (reverse; 5'-CTATTTAGGTGACACTATAGA-3').

C. Surface treatment

The increased high surface to volume ratio of PCR chip renders the enzymes in PCR mixture to stick along the hydrophobic walls of the PDMS microchannel. Also, countless bubbles are formed at the denaturation zone. Thus, special caution must be paid to the condition of microchannel or injection of PCR mixture. To prevent enzyme adsorption, methacryloyloxyethyl phosphorylcholine (MPC) polymer was modified onto the hydrophobic PDMS microchannel surface [7]. But prior to that, a sequential cleaning procedure was performed, wherein each solution was allowed to flow along the PDMS microchannel in the following order: Brij solution (1 ml of Brij 35 % solution per liter) for 5 min, HNO_3 (0.1 N hydrochloric acid solution) for 5 min, SDS (1 g of sodium dodecyl sulfate per liter) for 5 min, NaOH (0.1 N sodium hydroxide solution) for 5 min, and finally SDS for 20 min. Afterwhich, the microchannel was rinsed with deionized water (D. I. water). Then, MPC polymer was flown for surface modification during 30 min. And surfactant (Tween 80 1%) was added in the PCR mixture for the purpose of dynamic coating.

D. Experimental setting

The temperature of each zone was set 94 °C for denaturation, 55 °C for annealing, 68 °C for extension. All of the reagents and PCR mixture were injected into the microchannel using syringe pump (KDS100, Kd Scientific). PCR product collected was 5 μl for electrophoresis. The amplified PCR products were analyzed by 1 % agarose gel electrophoresis with ethidium bromide.

III. Results and Discussion

A. Temperature calibration

ITO heaters were calibrated for liquid temperature control. Using ITO heater, D. I. water was heated in the PDMS microchannel. Thermocouple (CHAL-0003, Omega) was injected into the microchannel during UV-ozone bonding for temperature measurement. Figure 4 shows the temperature calibration result of ITO heater. Temperature of D. I. water increases linearly with an applied power. We can get 94 °C for denaturation with an applied power of about 1100 mW. Therefore, we can control the temperature at a particular

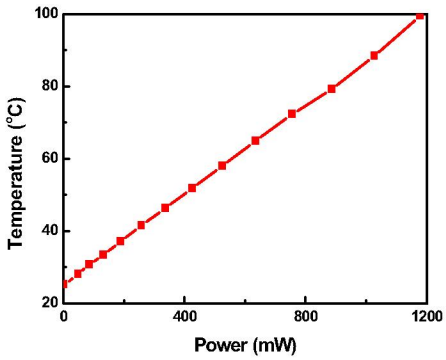

Fig. 4. Temperature calibration of ITO heater.

amplification zone accurately.

B. *PCR experiment*

Amplified PCR product was collected at the end of the microchannel for gel electrophoresis analysis. Surface treatment is one of the most important factors of continuous-flow PCR. Generally, PDMS surface enzyme adsorption causes a poor PCR reaction. Adsorption of the polymerase onto the PDMS microchannel was terminated by

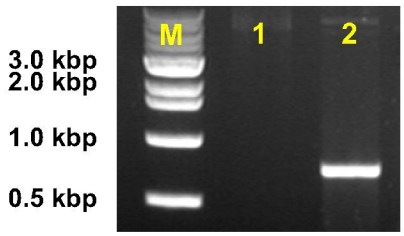

Fig. 5. Influence of surface treatment. Lane M : size marker, lane 1 : none surface treated continuous-flow PCR result, lane 2 : MPC polymer coated microchannel using and Tween 80 1 % added continuous-flow PCR result.

MPC polymer modification and Tween 80 dynamic coating. Figure 5 shows electrophoresis result of continuous-flow PCR product.

It represents the influence of surface treatment. No amplified product was observed at lane 1, which was performed in an untreated PDMS microchannel. On the other hand, successful amplified 700 bp pKS GFP product with no none-specific band was observed at lane 2 which was performed with MPC-Tween 80 modified PDMS microchannel. DNA amplification was performed with various flow rates. The flow rate through the microchannel determines the residence time of a fluid element

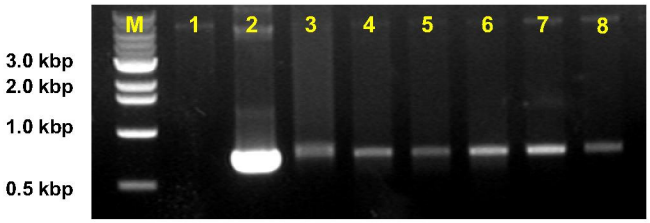

Fig. 6. Influence of the liquid flow rate. Lane M : size marker, lane 1 : negative control, lane 2 : conventional PCR result, lane 3 ~ 8 : continuous-flow PCR results. (lane 3 : 0.8 μl/min, lane 4 : 1.6 μl/min, lane 5 : 2.4 μl/min, lane 6 : 3.2 μl/min, lane 7 : 4.0 μl/min, lane 8 : 4.8 μl/min).

in a specific temperature zone. Figure 6 shows the effect of the liquid flow rate.

Positive control was carried out with a conventional PCR machine at lane 2. Reaction conditions were 30 cycles of 30 s for denaturation at 94 °C, 30 s for annealing at 55 °C, 1 min for extension at 68 °C. Total reaction time was 90 min. Continuous-flow PCR was performed at 0.8 – 4.8 μl/min with reaction times at 35 – 210 sec/cycle. Total reaction time with 20 cycles was 9 – 70 min. The intensity of the band represented a PCR product amount. At low flow rates, that is longer residence time, the amplification of the PCR product was not guaranteed. Only at an increased flow rate of 4.0 μl/min did the product was well intensified. This indicated the fast thermal cycling ability of continuous-flow PCR chip. However, too fast flow rate generates less PCR product, which was evident from the sample performed at 4.8μl/min shown at lane 8. Therefore, optimum flow rate of this particular continuous-flow PCR chip is 4.0μl/min and total reaction time is 14 min for 20 cycles, which is 4 times faster than conventional PCR machine. Other

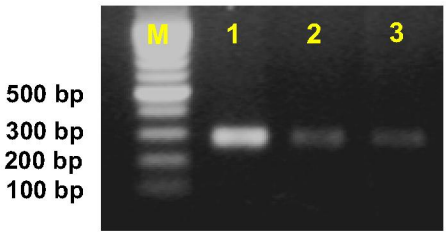

Fig. 7. Result of PG-noswsi amplification. Lane M : size marker, lane 1 : 4.0 μl/min, lane 2 : 6.0 μl/min, lane 3 : 8.0 μl/min.

DNA fragment 300 bp PG-noswsi was amplified in the same manner. Figure 7 shows result of PG-noswis amplification.

DNA amplifications were performed at 4.0 – 8.0 μl/min with reaction time at 21 – 42 sec/cycle, which were successfully amplified as well. Total reaction time with 20 cycles would last for 7 to 14 min. The optimum flow rate was 4.0μl/min and total reaction time was 14 min for 20 cycles.

IV. Conclusion

We propose a continuous-flow PCR chip using ITO-coated glass/ PDMS materials for DNA amplification. The continuous-flow PCR chip showed several advantages such as fast thermal cycling, series of amplifications, cost-effective fabrication, portability, and fluorescence detection. Using the continuous-flow PCR chip, 720bp pKS-GFP and 300 bp PG-noswsi plasmid DNAs were successfully amplified. The continuous-flow PCR chip is also applicable for a 'lab-on-a-chip' or a micro total analysis system (μ-TAS).

References

[1] I. Schneegaß, J. M. Kohler, "Flow-through polymerase chain reactions in chip thermocycler," Molecular Biotechnology, vol. 82, pp. 101-121, 2001.

[2] Q. Xiang, B. Xu, R. Fu, and D. Li "Real time PCR on disposable PDMS chip with a miniaturized thermal cycler," Biomedical Microdevices, vol. 7:4, pp. 273-279, 2005.

[3] M. U. Kopp, A. J. de Mello, A. Manz, "Chemical amplification : continuous-flow PCR on a chip," Science, vol. 280, pp. 1046-1047, 1998.

[4] I. Schneegaβ, R. Brautigam and J. M. Kohler, "Miniaturized flow-through PCR with different template types in a silicon chip thermocycler," Lab on a chip, vol. 1, pp. 42-49, 2001.

[5] J. A. Kim, J. Y. Lee, S. S. Seong, S. H. Cha, S. H. Lee, J. J. Kim, T. H. Park, "Fabricated and characterization of a PDMS-glass hybrid continuous-flow PCR chip," Biochemical Eng. Journal, vol. 29, pp. 91-97, 2006.

[6] T. Fukuba, T. Naganuma and T. Fujii, "Microfabricated flow-through PCR device for in situ gene analysis in extreme environments," MicroTAS, pp. 725-728, 2003.

[7] T. Fukuba, T. Yamamoto, T. Naganuma, T. Fujii, "Microfabricated flow-through device for DNA amplification-towards in situ gene analysis," Chemical Eng. Journal, vol. 101, pp. 151-156, 2004.