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Implementation of PCB-Based PCR Chip Using Double-Sided Tape

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Abstract

The fabrication of microfluidic channels in previously proposed polymerase chain reaction chips by using polydimethylsiloxane, polycarbonate, and poly (methyl methacrylate) is expensive. Hence, the channel fabrication using tapes was investigated in this study. This paper presents experimental results for DNA amplification performance and the adsorption of fluorescent substances with respect to various taping materials constituting microfluidic channels. Experiments on fluorescence adsorption showed the adhesive of double-sided tapes was the main cause of the adsorption. DNA amplification with microfluidic channels made of polypropylene and the double-sided tape showed comparable performances to that with the conventional tube in the general thermal cycler.

Keywords: *Micro-PCR chip, Double-sided tape, PCR, Acrylic adhesive, Microfluidic channel*

1. Introduction

Various inexpensive, disposable lab-on-a-chip systems (LOCs) are being developed for the miniaturization, integration and automation, and point-of-care diagnosis of routine biochemical processes [1, 2]. LOCs are used for various purposes in biotechnology, medical treatment and diagnosis, and basic research [3, 4]. The most urgent problems with the use of LOCs are that the fluid of cells and the aqueous solution of biomolecules must be processed stably and inexpensively, and that this process is more favorable as the amount of sample becomes smaller [1, 2, 5-7]. Because of these requirements, microfluidic channels have been actively developed to process small amounts of sample; generally, these channels are integrated through the expensive and difficult processes of etching, baking, and bonding the silicon, polymer, and glass materials [1, 2]. If tape is used, which is a thinner and flexible substitute for a microfluidic channel, more efficient and less expensive channel fabrication is possible [2].

Because tapes of various thicknesses are mass-produced, the thermal cycling required for DNA amplification can be effectively conducted by lowering the thermal resistance through selecting a thin tape. Furthermore, because the microfluidic channel is created by simply carving out the tape, fabrication is convenient. The market for LOCs is expanding greatly; however, because various types of tape are already mass-produced and their market is already very large, even if mass-production of the conventional method is established, the construction with tapes will be a much cheaper manufacturing method [2].

The material for the microfluidic channel of a polymerase chain reaction (PCR) chip will have a very large influence on PCR performance. First, during PCR, the temperature

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is raised close to 100℃; hence, the chip should be thermally stable and the part that is in close contact with the heater should be very thin. Furthermore, biomolecules should not stick to the material surface and should not adversely affect the biochemical reaction. Material-specific PCR performances have been reported [8], but because these experiments were conducted by inserting a piece of material into the PCR tube, they cannot be applied for the case in which fabrication is conducted with tapes. Furthermore, the previous study [8] does not report on the interaction between the material and the fluorescent substance inserted for fluorescence detection after DNA amplification. This study investigates the adsorption characteristics of fluorescence material as well as the PCR amplification performance according to the types of tapes being utilized for the chip construction. In Section 2, the design, structure, and control host system of micro-PCR chips using double-sided tape are discussed, and in Section 3, the PCR performances for different tape materials constituting the PCR chips are shown. The conclusion is presented in Section 4.

2. PCB-Based Micro-PCR Chip

2.1. PCR chip driving system

The control system of PCR chips should have not only the biochemical processing functions but also user interface capability. The functions for biochemical process comprise the protocol processing and the temperature control of the chip. The temperature can be increased or decreased by using the heater and cooling fan for heating and cooling, respectively, to the specified temperature after the present temperature is sensed. The protocol refers to repetitively performing the actions to be conducted at a specific temperature for a certain time period. The user interface function is designed to directly manage, edit, and control the basic biochemical protocols through the user interface. The two functions of biochemical process and user interface can be integrated as one process in the embedded system. However the biochemical process can be controlled more easily and efficiently by an embedded system, while the user interface can be done on PC.

From the above reasoning, we adapted a system of local-host structure as shown in Figure 1. In the Figure, the PCR chip's temperature was controlled by setting appropriate heating and cooling temperatures through temperature measurement and the periodic control procedure for the heater and fan. The figure also showed a block diagram of the control system. The microcontroller in the local system was PIC18F4550 (Microchip Technology, Inc.), which included an analog-to-digital converter (ADC), pulse width modulation (PWM), and USB interface. To heat and cool the PCR chip, the PWM and a field effect transistor (FET) were used to control the heater and the fan. Using the voltage divider and ADC, the temperature value corresponding to the thermistor resistance of the PCR chip was obtained. USB 2.0 was employed to connect the local system to a PC. The temperature value was recorded in the local system every 5 ms, and the host read the temperature values from the local system and calculated the PWM value based on the proportional-integral-derivative (PID) controller mechanism. In other words, in the host, the temperature control and functions related to PCR protocol performance were processed utilizing the excellent graphic user interface (GUI) environment and the file management capability of PC.

The temperature control speed of the PCR chip was approximately 10℃/s, therefore the processing period was set to control the temperature in less than 1/20 s (5 ms) for a margin of error less than 0.5℃. Because it was difficult to achieve proper resolution with the normal timer provided by the windows OS, a high precision event timer (HPET) was used to achieve a resolution smaller than 1 ms, which was to facilitate various multimedia applications. Note that the local-host system using the PC has the advantage of reducing the total cost because major functions can be performed in the host [9-12].

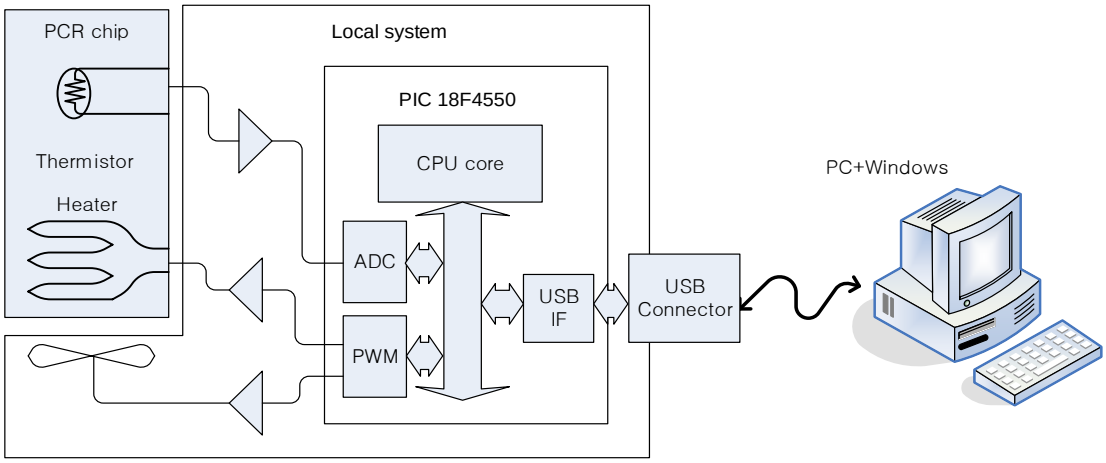
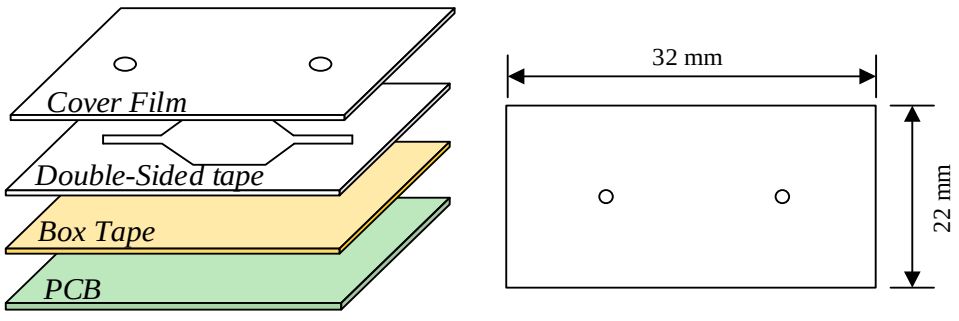


Figure 1. PCR Chip and Its Control System

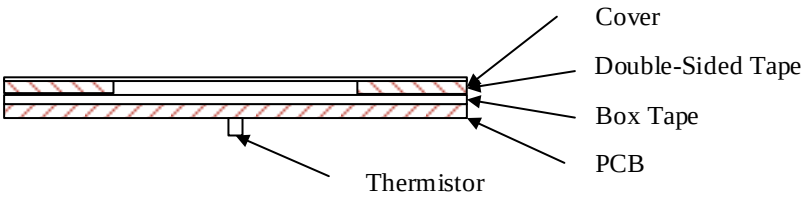
2.2. PCR chip structure

Our previously reported micro-PCR chips were divided into upper and lower PCB parts; the heater and sensor were attached to the lower part, while the thermal pad was attached to the upper part of the PCB. Thus, they were fabricated to transfer heat or cooling to the channel by going through the substrate. Furthermore, the holes on the cover were divided into an in/out holes and an air hole. However, the proposed micro-PCR chip was modified to have a four-layer structure, as shown in Figure 2. The PCB was at the very bottom; the heating circuit, which was used for heating, and the thermal sensor, which senses temperature, were attached to the PCB [13-15].

When the channel was constructed directly on the upper surface of the PCB base, results were not always properly obtained because the DNA or fluorescence reagent were stuck to the PCB. Therefore, a box tape made of polypropylene was attached onto the top of the PCB base. Usually, the channel was fabricated by using polydimethylsiloxane, and it was formed by soft lithography fully utilizing the characteristics of polydimethylsiloxane. However, to lower the cost and prevent from deforming at high temperatures, a 400-μm double-sided tape was attached to the upper surface of the box tape. Before attaching, it was carved to secure the chamber space as shown in Figure 2 by a cutting plotter. The cover was constructed by using polyethylene terephthalate film in the previously proposed systems; however, in this study, polypropylene film was used, which was a material for tubes prevalent in bio laboratories.



(a) Assembly diagram (b) Channel Cover



(b) Cross section

Figure 2. Proposed Micro-PCR-Based PCR Chip

Many types of double-sided tape were available; however, to be used in the proposed chip, the tape had to be able to withstand a high temperature of 95℃ and the resultant high pressure during the PCR process. Furthermore the fluorescence reagent should not be adsorbed on to the construction materials for the fluorescence DNA detection system to be integrated later. In this experiment various tapes were tested for the adsorption performance. They were the box tape, #9495MP (3M, USA) and T-#7720(510) (Tapeworld, Korea), as listed in Table 1. Two kinds of tapes for the channel construction had an acrylic adhesive as shown in the 2nd and the 3rd rows in the table, but their carriers were made of polyester for #9545MP and polycarbonate for T-#7720(510). The polypropylene which was the material for the cover of our chip was also tested together. Note that the polypropylene was the common material for PCR tubes.

The final selected tape after adsorption test was employed for the channel construction for PCR performance verification was 400-μm-thick T-#7720(510) tape.

Table 1. Box and Double-Sided Tapes Used in the Experiment

Heading level	Manufacturer	Tape Number	Adhesive Type
Box Tape	3M	#309 Mini Clear	Acrylic Adhesive
Double-Sided Tape	3M	#9495MP (Double Coated Tape:0.1mm)	Hi-Performance Acrylic
Double-Sided Tape	Tapeworld (Korea)	T-#7720(510) (Double Coated Tape:0.2mm)	High Adhesion Acrylic

3. Experiments and Results

When bovine serum albumin (BSA) was not added in the PCR mix, the increase of the adsorption had been reported. For fluorescence adsorption test of various tapes, therefore, the effect of the addition of BSA was also investigated. Each tape under investigation was cut to get three pieces. One of three pieces was not immersed in the PCR mix for comparison purpose, and another piece was dipped into the mix for a while, and the last piece was submerged in the mix for the whole PCR cycling process.

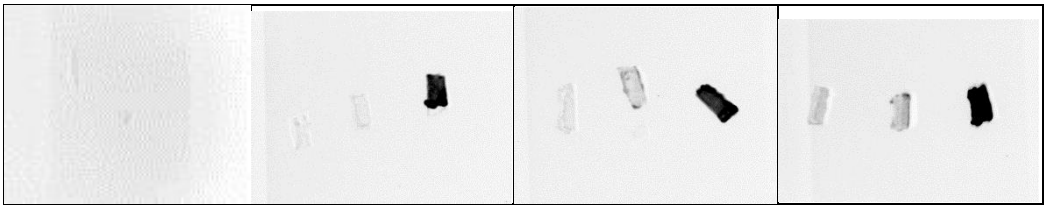
Figure 3 shows the fluorescence adsorption result when BSA was not added in the PCR mix. The contrast of each images in the figure were enhanced to improve the visualization. The results for the mix with BSA was shown in Figure 4. In the case of polypropylene, which was the material of the cover, fluorescent color was not distinguishable. However the fluorescent color of the other tapes could be visible indicating the fluorescence adsorption. The fluorescent color of the adhesive tapes seemed to be less dark with BSA than without it. However it was hard to conclude that BSA reduced the adsorption of the fluorescent material to the adhesive surface considering the different condition of taking pictures.

Attaching several layers made the fluorescent colors more vivid. This indicated the fluorescence material adsorbed mostly on the adhesive part rather than on the carrier materi-

al of the double-sided tape. If the adsorption of the fluorescence material adsorption on the adhesive can be cleared, double-sided tape will be a good material for the channel construction.

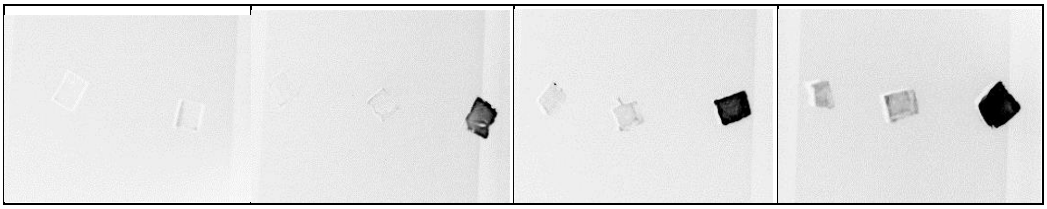
The upper surface of box tape itself was made of polypropylene and the adhesive side was not part of the channel. Therefore the box tape had no problem for utilizing the bottom of the channel.

The fluorescence adsorption performances of various types of the double-sided tapes were similar. And the channel height of our chip was 400μm, only 2 layers of T_#7720 were enough for the channel construction. Therefore it was chosen for our chip. PCR protocol was composed of 10 min preheating at 95℃ followed by 40 cycles of two temperatures; 15 s at 95℃, and 1 min at 60℃. The target DNA of this experiment was that of the Ureaplasma urealyticum (UU) (Labgenomics Co., Ltd.). It had the concentration of 1 ng/μL and mixed with 8 μL of SYBR, 1 pM of primer F, and 1 pM of primer R. Bovine serum albumin (BSA) of 1 ng/μL was added to reduce the adsorption, as the failure rate was not negligible without it.

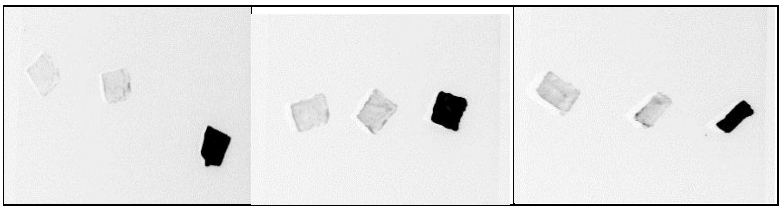


(a) polypropylene (b) box tape (c) T-#7720 (d) 2 layers of T-#7720

Figure 3. Fluorescence Adsorption Test Images when BSA was not Added (The Leftmost Piece in the Image was not Immersed in the PCR Mix, and the Center Piece was Immersed for a While, and the Rightmost Piece was Submerged in the PCR Mix During the Whole PCR Process).



(a) polypropylene (b) box tape (c) T-#7720 (d) 2 layers of T-#7720



(e) #9495MP (f) 2 layers of #9495MP (g) 3 layers of 9495MP

Figure 4. Fluorescence Adsorption Test Images when BSA was Added (The Leftmost Piece in the Image was not Immersed in the PCR Mix, and the Center Piece was Immersed for a While, and the Rightmost Piece was Submerged in the PCR Mix During the Whole PCR Process)

Figure 5 presents one of the PCR experimental results using with our microchip system. It was compared with that with the conventional PCR thermal cycler (General PCR column in the figure). The figure and the many other results showed the amplification per-

formance using the proposed PCR chip system was comparable to that of the conventional PCR process.

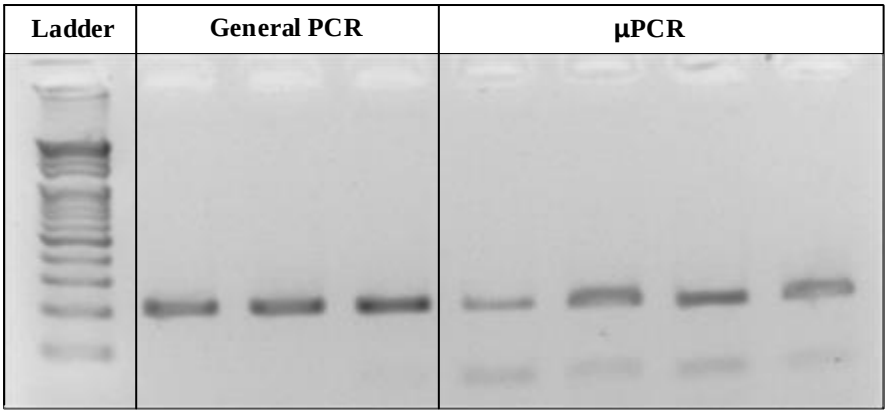


Figure 5. Agarose Gel (2.0%) Electrophoresis Image of the PCR Product

4. Conclusion

The channel construction method using double-sided tapes for the PCB-based PCR chip was investigated. The proposed chip was constructed by covering the PCB substrate with a box tape and constructing channel its upper surface. The channel was constructed by attaching the double-side tape carved for the channel and covering with a polypropylene film. The various channel construction materials such as polypropylene, the box tape, double-sided tapes were tested for the adsorption of the fluorescence materials. The polypropylene of the cover and the bottom of the channel did not inhibit PCR amplification if BSA was added to the PCR mix. The experimental results presented the adhesive of double-sided tapes was the main cause of the adsorption and the tendencies were similar to both types of double-sided tapes. Therefore thicker tape was chosen because only double layers was enough for accommodating the height of the proposed chip. The resultant PCR chip was verified to have the comparable performance to the convention PCR process using tubes and the PCR thermal cycler.

As the production process for PCB and tapes are well-developed, the chip cost can be greatly reduced. However the fluorescence adsorption on the adhesive of double-sided tapes should be seriously considered for integrating the fluorescence detection into the chip. The smaller adhesive as possible will be preferable according the presented experimental results.

To commercialize the micro-PCR chip in future, fluorescence detection and biomolecule adsorption experiments, DNA amplification experiments, and experimentation using various DNA will be necessary for fabricating microfluidic channels with double-sided tape.

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References

[1]. D. Patkoa, Z. Martonfalvic, B. Kovacsa, F. Vondervisztа, M. Kellermayerc, R. Horvatha, Sensors and Actuators B, vol. 196, no. 352, **(2014).**

[2]. M. Focke, D. Kosse, C. Muller, H. Reinecke, R. Zengerle and F. Stettenab, Lab on a Chip, vol. 10, no. 1365, **(2010).**
[3]. J. J. Ramsden, "Biomedical Surfaces", Artech House, Norwood, MA, **(2007)**.
[4]. M. Malmsten, "Biopolymers at Interfaces", Taylor & Francis, New York, **(2003)**.
[5]. C. Zhang and D. Xing, Nucleic Acids Res., vol. 35, no. 4223, **(2007).**
[6]. C. Zhang, D. Xing and Y. Li, Biotechnology Advances, vol. 25, no.483, **(2007).**
[7]. D. P. Herzog, IVD technology, **(2006).**
[8]. R. Kodziusa, K. Xiaoc, J. Wud, X. Yid, X. Gongd, I. G. Fouldsb and W. Wen, Sensors and Actuators B, vol. 161, no. 349**(2012).**
[9]. C. Plaisant, A. Rose, B. Shneiderman and A. J. Vanniamparampil, IEEE Software, vol. 14, no. 66, **(1997).**
[10]. J. D. Kim, Y. U. Lee and S. Kim, IEICE Trans. Fundamentals, vol. E86-A, no. 859, **(2003).**
[11]. J. D. Kim, J. Kim and G. Lee, Efficient control system for PCR chips. Proceedings of SPIE, vol. 7929, **(2011)** February 14, San Francisco, USA.
[12]. C. Y. Park, J. D. Kim, J. H. Ku, Y. S. Kim, H. J. Song and J. W. Kim, "PCB-based PCR Chip", Sensor Letters, vol. 10, no. 1197, **(2012).**
[13]. T. M. Hsieh, C. H. Luo, F. C. Huang, J. H. Wang, L. J. Chien and G. B Lee, Sensors and Actuators B, vol. 130, no. 848, **(2008).**
[14]. K. Shen, X. Chen, M. Guo and J. Cheng, Sensors and Actuators B, vol. 105, no. 251, **(2005).**
[15]. K. Lians, S. O'Rourke, D. Sadler, M. Eliacin, C. Gamboa, R. Terbrueggen and M. Chason, Sensors and Actuators B, vol. 138, no. 21, **(2009).**
[16]. P. C. Chen, D. S. Parka, B. H. You, N. Kim, T. Park, S. A. Soper, D. E. Nikitopoulos and M. C. Murphy, Sensors and actuator, vol. B149, no. 291, **(2010).**
[17]. K. H. Chung, Y. H. Choi and M. Y. Jung, "Natural Convenction PCR in a Disposable Polymer Chip", IEEE SENSORS Conference, **(2009)**.
[18]. Q. Zout, Y. Miao, Y. Chen, U. Sridhar, C. S. Cheong, T. Chai, Y. Tie, C. H. L. The, T. M. Lim and C. K. Heng, Sensors and Actuators A, vol. 102, no. 114, **(2002).**
[19]. J. H. Wang, L. J. Chien, T. M. Hsieh, C. H. Luob, W. P. Chou, P. H. Chen, P. J. Chen, D.S. Lee and G. B. Lee, Sensors and Actuators B, vol. 141, no. 329, **(2009).**
[20]. D. N. Kim, Y. Lee and W. G. Koh, Sensors and Actuators B, vol. 137, no. 305, **(2009).**
[21]. C. E. William, "User interface design for the engineer", Electro/94 International Conference Proceedings, Combined Volumes, vol. 415, **(1994)**.

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