A polymer lab-on-a-chip for reverse transcription (RT)-PCR based point-of-care clinical diagnostics†

Soo Hyun Lee,*[ab] Sung-Woo Kim,[c] Ji Yoon Kang[b] and Chong H. Ahn[a]

Received 2nd July 2008, Accepted 23rd September 2008
First published as an Advance Article on the web 31st October 2008
DOI: 10.1039/b811131f

An innovative polymer lab-on-a-chip (LOC) for reverse transcription (RT)-polymerase chain reaction (PCR) has been designed, fabricated, and characterized for point-of-care testing (POCT) clinical diagnostics. In addition, a portable analyzer that consists of a non-contact infrared (IR) based temperature control system for RT-PCR process and an optical detection system for on-chip detection, has also been developed and used to monitor the RT-PCR LOC. The newly developed LOC and analyzer have been interfaced and optimized for performing RT-PCR procedures and chemiluminescence assays in sequence. As a clinical diagnostic application, human immunodeficiency virus (HIV) for the early diagnosis of acquired immune deficiency syndrome (AIDS) has been successfully detected and analyzed using the newly developed LOC and analyzer, where the primer sets for p24 and gp120 were used as the makers for HIV. The developed polymer LOC and analyzer for RT-PCR can be used for POCT for the analysis of HIV with the on-chip RT-PCR and chemiluminescence assays in shorter than one hour with minimized cross-contamination.

1. Introduction

Recently, there has been increasing demand for the development of point-of-care (POC) diagnostic kits or platforms using a polymer lab-on-a-chip. The innovative polymer lab-on-a-chip is an attractive device that can be used for POC clinical diagnostics. The polymers provide an excellent platform for the desired disposable lab-on-a-chip at low cost. Among the lab-on-a-chips (LOCs), the LOC for the polymerase chain reaction (PCR) has a great potential for POC clinical diagnostics. PCR technology, which was introduced by Kary B. Mullis in 1983, has been continuously developed to the current standard PCR technique that is used for the amplification of DNA.[1,2] Recently, biomedical microelectromechanical system (BioMEMS) technologies have been used for the development of PCR chips using materials such as silicon, glass[3,4] and polymers.[5–7] Generally, BioMEMS-based PCR systems can be divided into two categories, a steady fluid PCR[8–14] and a dynamic fluid PCR,[15–19] depending on the temperature of the cyclic reaction method used for the fluid sample. Several circular types of PCR chips, which were developed based on the BioMEMS technologies, have been reported recently.[20–23] Wang *et al.* introduced a circular type of PCR chip that utilized multiple membrane-activated micro pumps.[23] In addition, a functional lab-on-a-chip for RT-PCR was reported by Liao *et al.* that could be used for the development of a miniature RT-PCR system for the diagnosis of RNA-based viruses.[24] The miniature RT-PCR chip includes a micro temperature control module and a PDMS-based microfluidic control module. The microfluidic control module is capable of automating the RT-PCR process with minimum human intervention, but the chip does not have an on-chip detection scheme.

RT-PCR, where RT is added before the normal PCR step, is a well-known method used in molecular biology. Thus, complementary DNA (cDNA), which is used as a DNA template during PCR, is synthesized from messenger RNA (mRNA) by the RT reaction. This method is known to be the most sensitive technique for mRNA detection and quantification currently available. When compared to the two other commonly used techniques for quantifying mRNA levels, such as Northern blot analysis and the RNase protection assay, RT-PCR has the benefit of quantification of mRNA levels from much smaller amounts of sample; this technique is sensitive enough to accurately quantify the RNA from a single cell.

The AIDS epidemic is a worldwide concern. The HIV destroys the immune system and causes many life-threatening infections. Worldwide, however, only one in ten persons infected with HIV has been tested and knows what their HIV status is. For individuals there are many obstacles to being tested for HIV. One of them is the access to testing, so there is a great demand for the development of POC diagnostic kits or platforms for the diagnosis of the HIV. Currently POCT kits are available for the rapid HIV test;[25] these kits can be used to test HIV-1 in 20 minutes, using oral fluids, a finger-stick, whole blood, or plasma specimens. These POCT kits usually adopt the lateral-flow method referred to as an immunochromatographic strip (ICS). Although these kits provide a rapid and early detection, there are some limitations in terms of the sensitivity of these tests because the sensing mechanism is based on an immunoassay that uses

[a]*Microsystems and BioMEMS Laboratory, Department of Electrical and Computer Engineering, University of Cincinnati, Cincinnati, OH, 45221, USA. E-mail: chong.ahn@uc.edu*
[b]*NanoBio Research Center, Korea Institute of Science and Technology (KIST), PO BOX 131, Cheongryang, Seoul, 130-650, Korea. E-mail: dream.ideal@gmail.com; Fax: +82 2 958 6910; Tel: +82 2 958 6810*
[c]*Department of Neuroscience, Tufts University, Boston, MA, 02111, USA*
† Part of a special issue on Point-of-care Microfluidic Diagnostics; Guest Editors—Professor Kricka and Professor Sia.

antigens or antibodies. This limitation especially affects early diagnosis during the window period of HIV infection. On the other hand, the RT-PCR method has a much higher sensitivity for detection due to the PCR method that can amplify the desired product. Thus, the development of a lab-on-a-chip that can perform RT-PCR and detection would be desirable for a POC system that could be used in the clinical setting for the early detection of HIV infection.

Therefore, in this paper, we describe a disposable, polymer based, RT-PCR detection chip with embedded micro pinch-valves that has been designed and developed for point-of-care testing (POCT) clinical diagnostics. In addition, for RT and PCR amplification on the chip, an on-chip detection system using a chemiluminescence assay has been developed. For control of the RT-PCR LOC, a portable analyzer, which consists of a temperature control system for the RT and PCR, and an optical detection system for analysis of the chemiluminescence assay, has been developed. As a demonstration vehicle for application to clinical diagnostics, the detection of HIV, for the early diagnosis of the acquired immunodeficiency syndrome (AIDS), was performed using the polymer LOC for RT-PCR and its portable analyzer.

2. Design and fabrication

As a substrate material for the lab-on-a-chip, several polymer materials, polycarbonate (PC), polymethyl methacrylate (PMMA), polystyrene (PS), and cyclic olefin copolymer (COC) have been considered for the LOC. Among them, cyclic olefin copolymer (COC) was adopted for the LOC in this work, because it has excellent properties for supporting the LOC including good biocompatibility, excellent optical transparency, low background noise, extremely low moisture absorption, easy control of nonspecific absorption of the biochips, and a high glass transition temperature (T_g).[26] A schematic design of the

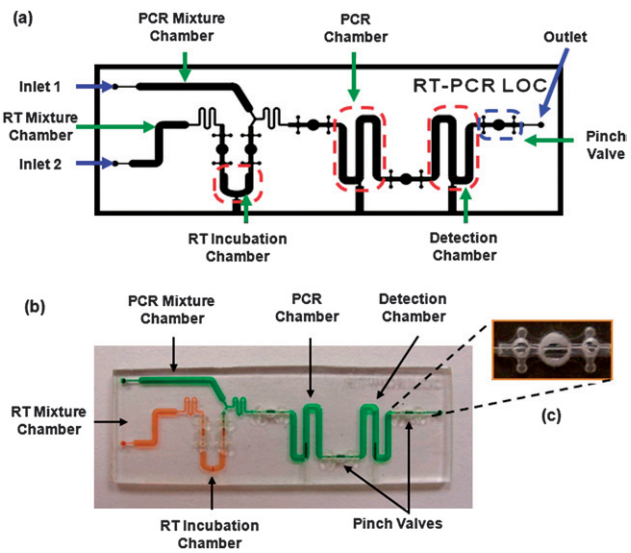

Fig. 1 LOC design for the RT-PCR: (a) design of the polymer RT-PCR lab-on-a-chip; (b) photograph of fabricated RT-PCR LOC including 5 different chambers; and (c) embedded micro pinch valves using silicone tubes.

RT-PCR lab-on-a-chip is shown in Fig. 1. This chip was fabricated with two different designs. One design has three holes in the RT incubation chamber, the PCR chamber and the detection chamber for inserting the thermocouple. The other design has no holes for the thermocouple. This chip includes five micro pinch valves to control the fluid used to perform the desired RT, PCR amplification and the detection process; it consists of top and bottom layers that are 54 mm × 18 mm and a cylindrical silicone tube (Dowing Corning Corporation, Midland, MI, USA). The volume of each chamber for the RT mixture, the PCR mixture, the RT incubation, the PCR, and the detection, with 0.2 mm thickness and 1 mm width, were designed to be 2 μl, 3 μl, 2 μl, 5 μl, and 5 μl, respectively.

For the top layer, a mold for the injection molding was fabricated using a computer numerical control (CNC) milling machine. This layer was designed to have five grooves each 350 μm thick for placement of an embedded silicone tube for the micro pinch valves. The bottom layer was fabricated using a Ni mold disk with micropatterns from the conventional lithography technique using the SU-8 2075 (Micro Chem Corporation, USA). After the photolithography, electroplating was performed to fabricate the micropatterns onto the Ni disk, used as a master mold for the injection molding. Finally, a 200 μm thick electroplated Ni pattern, on the Ni disk, was obtained. The plastic injection molding and thermal bonding technologies have been previously reported by our group for the fabrication of the microstructures on a COC substrate.[27] After cleaning the injection molded COC wafers, five silicone tubes, for the micro pinch valves, with a length of 6 mm, were placed between the top and bottom layers. Next, the top and bottom layers were thermally bonded together using a hot embossing machine (MTP-10, Tetrahedron Associates Inc., CA, USA). For the polymer thermal bonding process, the bonding temperature was heated up to the glass transition temperature (T_g) of the polymer. Because the COC has a glass transition temperature (T_g) of 135 °C, the thermal bonding process was performed around 135 °C under a pressure of 275 kPa. After 30 minutes, the hot plates were cooled down to 30 °C with the same pressure force. The working temperature range of the embedded silicone tube was −54 °C to 249 °C. Therefore, the thermal bonding process and PCR thermal cycles did not influence the embedded silicone tubes.

In order to functionally control all of the steps involved in the RT-PCR lab-on-a-chip, new micro pinch valves, using flexible silicone tubes, were embedded into the chip to maintain the high inner pressure, induced during PCR thermocycles, and to control the assay sequence.[27,28] Fig. 2 shows schematic views of the pinch valve mechanism including the RT-PCR LOC, the chip holder and the mechanical plunger. This valve was operated by an external mechanical plunger controlled manually to open and close the valves. In the developed pinch valve, actuator pins pinch the silicone tubes to close the channel. To perform the open and close functions, this valve does not require a high voltage[8] or a thermoelectric unit for the hydro gel valve.[29] Furthermore, the valve does not affect the biological reaction of the RT and PCR.

Each embedded micro pinch valve had seven holes at the top and bottom layers as shown in Fig. 1 (c). The large hole at the center was for the external mechanical plunger and metal rod of the lab-on-a-chip holder. The pinch valve can be opened and closed through this hole. The two medium holes located at both

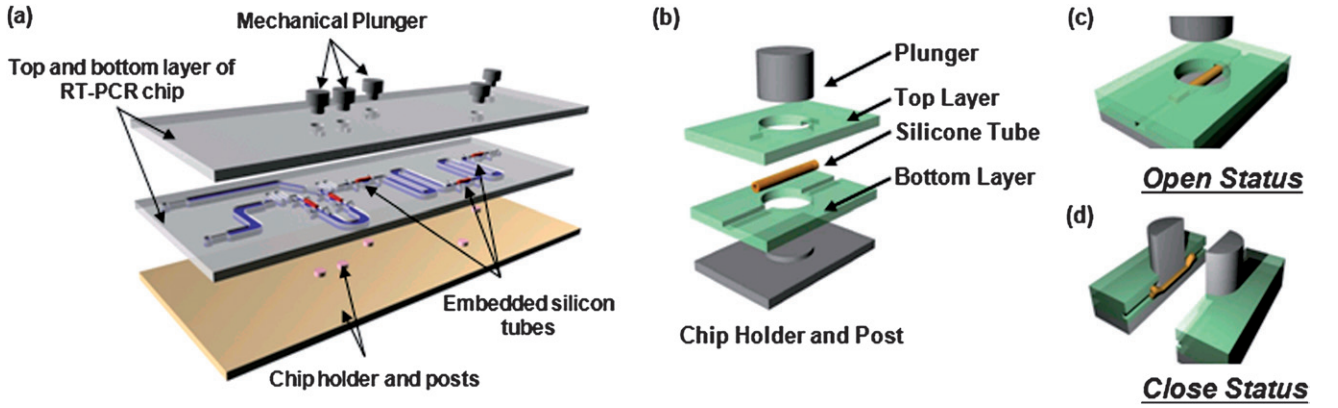

Fig. 2 Schematic illustrations of RT-PCR LOC and chip holder: (a) LOC chip, chip holder and mechanical plunger; (b) pinch valve to be assembled; (c) open status of the valve; and (d) close status of the valve.

ends of the center hole were filled with medical grade UV adhesive (Loctite Corp., USA) to seal both ends of the pinch valve. Four small holes around the two medium holes were used for venting of the UV adhesive. After dropping the UV adhesive into the medium holes, it filled the channels between the medium hole and the two small holes, and then passed through the gaps and channels by capillary force. Finally, the UV adhesive filling the channels and holes was cured for 30 seconds in the UV adhesive curing chamber (ZETA 7401 UV Chamber, Loctite Corp., USA). Through the sealing process, with the UV adhesive, the valve was fully enclosed and sealed to prevent leakage of the liquid sample, achieving almost zero dead volume through the valve.

3. Experiments

Instrumentation

To perform the RT-PCR and on-chip detection, a temperature control system with an infrared lamp[10,11] as the heat source, and an optical detection component with photodiodes and high-gain signal amplifiers were developed for a portable analyzer of the POCT. The dimensions of this analyzer were 25 cm in length, 10 cm wide and 20 cm high, with a weight of 2 kg. The temperature control system consisted of a signal conditioning circuit including three infrared halogen lamps, two DC cooling fans and thermocouples, and the Labview program to control the circuit using a data acquisition (DAQ) card (DAQ6025E, National Instruments Inc., USA). The used lamp was 80 W with 21 V. The signal conditioning circuit included a voltage regulator, an optoisolator triac driver and a triac. A pulse width modulated (PWM) signal, controlled by the Labview program, provided input from the triac driver, which achieved effective voltage control across the main triac. In order to control the lamp power and intensity, a PWM signal was also provided from one of the analog output channels of the DAQ card. If the measured temperature was lower than the setup point temperature, a relay was triggered to turn on the lamp's power supply. On the other hand, during the cooling cycle, another relay triggered the cooling fan to turn on and the lamp to turn off. A voltage regulator (LM341, National Semiconductor, USA) supported the triac driver with a DC of 5 V. The entire circuit requires a single 22 V of DC power supply with a current capacity of 180 mA. A total of five signals are generated from the DAQ card. One analog signal was a pulse width modulated signal to control the lamp intensity. Two digital signals controlled the on/off relay switches for the lamp and cooling fan. Two others were digital and analog for grounding. In order to perform real time monitoring of the temperature, the Labview program was developed as a negative feedback system using the proportional-integral-derivative (PID) control topology. The output from the negative feedback loop was the real time temperature, which was measured by a k-type thermocouple (Omega Engineering Inc., USA). Having RT-PCR LOC with three inserting holes for thermocouples, three different thermocouples were inserted into the RT incubation chamber, the PCR chamber and the detection chamber, respectively. Each thermocouple directly contacted the solution to measure the real temperature. During the RT, PCR and detection process, all temperature profiles were recorded through Labview program. After recording the real temperature profiles, RT-PCR LOC without inserting holes for thermocouples has been characterized using the recorded temperature profiles.

The optical detection system consisted of a lens, a high sensitivity photodiode and high gain amplifier circuits that converted the current from the photodiode to the output voltage. The S2386-8K (Hamamatsu Co., Japan), a photodiode that had only 0.05 nA dark current, was used to detect the light signal from the chemiluminescence assay of the RT-PCR lab-on-a-chip. A high gain amplifier circuit (C-9051, Hamamatsu Co., Japan) was also used, which had a conversion gain of 1.0×10^8 V/A.

RT and PCR procedures

As a demonstration vehicle for on-chip RT-PCR LOC clinical diagnosis, HIV was attempted. The reliable markers, p24 and gp120, a major core protein and an external envelope protein encoded by the HIV gag gene and envelope gene, respectively, were used for HIV detection. HEK 293 cells, a source of RNA, were generated by the transformation of human embryonic kidney cell cultures. The total RNA was isolated by the Trizol method and kept at −70 °C. For the reverse transcription reaction, 2 μl of reverse transcription mixture was used, which contains 0.4 μl of 5× RT buffer, 0.1 μl of 0.5 μg/μl oligo (dT) 25,

0.1 μl of 100 ng/μl total RNA, 0.1 μl of 10 mM dNTPs, 0.2 μl of 0.1 M DTT, 0.1 μl of Rnase out, 0.1 μl of SuperScript RTase, and 0.1 μl of 10 pM/μl forward and reverse primers, respectively. The forward and reverse primers for p24 were, respectively, biotin-GCCAG ATGAG AGAAC CAAGG -3′ and 5′- GGGTT CCTTT GGTCC TTGTC -3′. The forward and reverse primers for gp120 were, respectively, biotin-CACCT CAGTC ATTAC ACAAG CC -3′ and 5′- CTGGC CTAAT TCCAT GTGTG C -3′. Each forward primer for p24 and gp120 was conjugated with biotin for the chemiluminescence assay.

3 μl of the PCR mixture contained 0.5 μl of 10× PCR buffer, 0.1 μl of 10 mM dNTPs, 1.5 μl of double-deionized water, 0.45 μl of 250 μg/ml bovine serum albumin (BSA), 0.05 μl of 0.5 U/μl Taq DNA polymerase, and 0.2 μl of 10 pM/μl forward and reverse primers, respectively.

To verify the performance of the RT-PCR LOC and temperature control system, the conventional PCR system (Eppendorf PCR Matercycler, Fisher Scientific, USA) was used for reference. For the RT-PCR process, 2 μl of RT mixture and 3 μl of PCR mixture were loaded into each chamber of the RT-PCR LOC. After the RT mixture was transferred into the RT incubation chamber, it was incubated at 40 °C for 2 minutes using an infrared heating method. Then, 2 μl of RT mixture and 3 μl of PCR mixture were injected into the PCR chamber concurrently with different flow rates controlled by the syringe pumps (Pump 33, Harvard Apparatus, Holliston, MA, USA), followed by thermal cycles with the PCR mixture. It was initially heated to 95 °C for 60 s and cycled for 30 cycles at 94 °C for 10 s, 56 °C for 10 s and 72 °C for 10 s, and maintained at 72 °C for 60 s for the final extension.

After the on-chip RT-PCR, the RT-PCR products were injected into the wells of a 1.5% agarose (BP160-500, Fisher Bioreagents, USA) gel matrix after mixing with loading buffer (Molecular Biology Reagent, SIGMA, Missouri, USA), for electrophoretic size separation. The samples were loaded, then the agarose gel matrix was placed into a bath filled with 1× Tris-borate-EDTA (TBE) buffer with 180 V applied at the ends of the bath for 30 minutes.

Detection

In view of the bioreaction mechanism, for the on-chip chemiluminescence assay, single stranded probe DNA should be immobilized first at the detection chamber, as shown in Fig. 3, before loading the sample onto the chip. To functionalize the surface of the detection chamber, with single stranded probe DNA, without modifying other surfaces, each immobilized sample was injected and suctioned out through the outlet port.

Initially, 1× PBS buffer solution was injected into the detection chamber and suctioned out for cleaning. After drying with air, 0.5 μg/μl of streptavidin was introduced and incubated for 5 minutes. Next, 1× PBS buffer solution was again injected into the chamber for washing. To block non-specific adsorption over the surface, 1 μg/μl of BSA solution was injected and incubated for 5 minutes. After one more washing step with 1× PBS buffer, biotin conjugated with single stranded probe DNA (biotin-CTG TCC AAA GGT ATC CTT TGA GCC AAT TCC for p24; biotin-CCA ACC GCG AGA AGA TGA CCC AGA TCA TGT for gp120) was injected and incubated for 10 minutes. To complete the immobilization step, the detection chamber was dried with pressurized air.

After the RT and PCR amplification, 5 μl of the amplified DNA sample from the PCR chamber was transferred to the detection chamber in order to perform the on-chip detection. After closing the valves at the both ends of the detection chamber, it was heated to 95 °C to denature the amplified DNA for 1 minute and then cooled to 60 °C to anneal with the single stranded probe DNA that was immobilized in advance for 1 minute. After washing with 1× PBS buffer, 1 μg/mL of horseradish peroxidase (HRP) labeled with streptavidin was injected from the outlet and incubated for 5 minutes. After rinsing with 1× PBS buffer, the SuperSignal® femto chemiluminescent substrate solution was added to fill the detection chamber (SuperSignal® femto maximum sensitivity substrate, Rockford, IL, USA). For the substrate solution, an equal quantity of the SuperSignal® ELISA Femto Luminol/Enhancer solution and SuperSignal® ELISA Femto Stable Peroxide solution were mixed. Next, the emitted chemiluminescent light was measured using the Typhoon™ 8600 (GE Healthcare, USA) scanner, and the newly developed portable analyzer for comparison of the assay results.

4. Results and discussion

Micro pinch valves

The performance of the micro pinch valves using embedded silicone tubes was assessed by measuring the maximum tolerance for the pressure across the valve. One end of the valve was connected to a syringe pump and a pressure sensor using a T-shaped connector. The plunger was pushed down over the

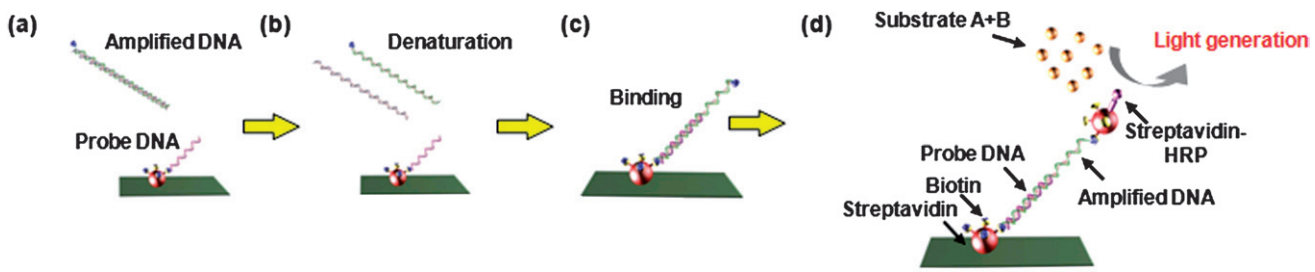

Fig. 3 Schematic of the detection chamber process: (a) loading the amplified DNA sample conjugated with biotin; (b) heating to the temperature for denaturation to make single stranded DNA; (c) single stranded DNA will be conjugated with probe DNA at the annealing temperature; and (d) the principle of chemiluminescence assay.

embedded silicone tube close the pinch valve. A syringe pump (Pump 33, Harvard Apparatus, Holliston, MA, USA) was connected to the inlet of the pinch valve and then pressurized; a differential pressure sensor (ASDX030D4, Honeywell, MN, USA) was used to measure the differential pressure across the valve. This pressure sensor can measure the pressure up to 207 kPa. The output voltage from the pressure sensor was collected by the Labview program using a data acquisition device (NI USB-6008, National Instrument Corporation, USA) in real-time. The flow rate of the syringe pump was 6 ml/min.

Based on the experimental results, this pinch valve showed excellent performances with no detectable leakage up to 137 kPa for air (data not shown). When the fluid filled closed chamber was heated, the inner pressure of the chamber increased. In order to confirm whether the measured pressure from the pinch valve was adequate for the PCR thermocycles, the increments in the inner pressure caused by the thermal effects was calculated. From the simulation results, 84.6 kPa of pressure developed during the heating from 25 °C to 95 °C, in the case of water, under a fixed volume. Thus, the pinch valve embedded into the RT-PCR LOC had enough of a margin to endure the generated pressure during the PCR thermocycles.

RT-PCR amplification for HIV diagnostics

The measured temperature profile from the RT-PCR LOC was plotted during the PCR thermocycles and is shown in Fig. 4. The heating from 56 °C to 72 °C took 5 s at a 3.2 °C/s heating rate. For the heating step from 72 °C to 94 °C, generally 8 s are needed, at a rate of 2.75 °C/s. Thus, the average heating rate for the two steps was 2.975 °C/s and the cooling rate from 94 °C to 56 °C was 1.81 °C/s. The cooling rate was slower than the heating rate due to the poor cooling capability using fans.

Homogeneity of the temperature at each temperature stage is desirable for a successful PCR reaction. As shown in Fig. 4(b), the measured temperature profile with the thermocouple had a very reasonable range, which was less than ± 1 °C for its temperature control. This result implies that the PID controller and negative feedback control system for the Labview program controlled the circuit and the intensity of the infrared lamp well. The distance between the infrared lamp and the chip is an important parameter to obtain a satisfactory temperature profile and to accomplish the PCR. Because the infrared lamp was housed in a concave reflector, the light from the lamp was focused on a certain point. Therefore, the PCR chamber of the lab-on-a-chip was located on the focal length of the lamp in order to achieve the maximum heating rate. If the light is not focused on the PCR chamber, it takes a longer time to reach the denaturation temperature and the uniformity of the lamp intensity is not guaranteed. With the poor temperature profile, the PCR reaction can not be achieved.

Before performing the on-chip detection, gel electrophoresis was performed first to demonstrate the products from the RT and PCR amplification process. As shown in Fig. 5, 200 bp and 210 bp of DNA for p24 and gp120, respectively, were successfully amplified with the polymer LOC by RT and PCR. Lanes 1 and 6 show a 50 bp DNA ladder (BIONEXUS, Inc., CA, USA) as a reference. Lane 2 is the RT-PCR product from a conventional thermal cycler and lane 4 is the RT-PCR product processed by the LOC. Lanes 3 and 5 are negative controls without DNA templates from the conventional thermal cycler or the LOC, respectively.

The results of the experiments demonstrated that RT and PCR were successfully performed. The amplification capacity using the disposable LOC for RT-PCR was confirmed. Specifically, the markers p24 and gp120, which are the biomarkers for HIV, were used for the diagnosis of HIV. The advantage of the RT-PCR on the polymer LOC included a short processing time, which was

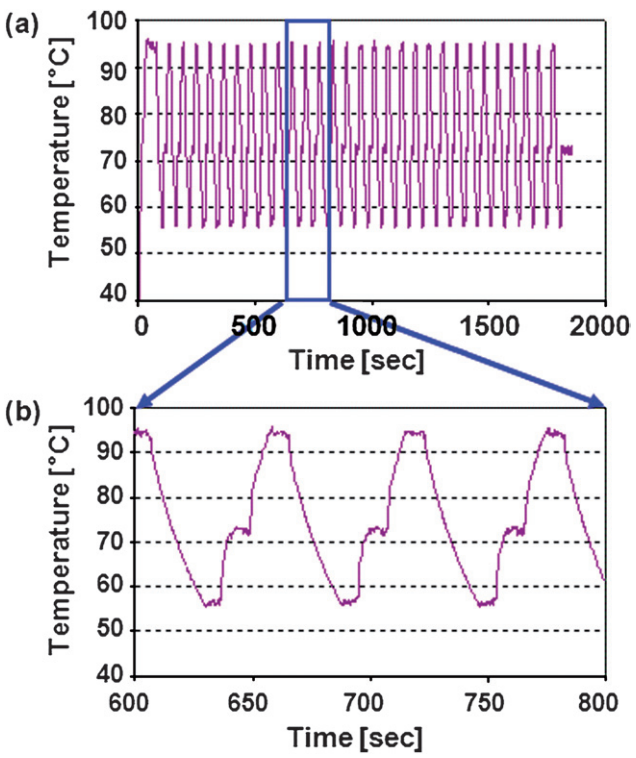

Fig. 4 Measured temperature profile of the RT-PCR LOC during PCR thermocycles: 94 °C for 10 s, 56 °C for 10 s and 72 °C for 10 s.

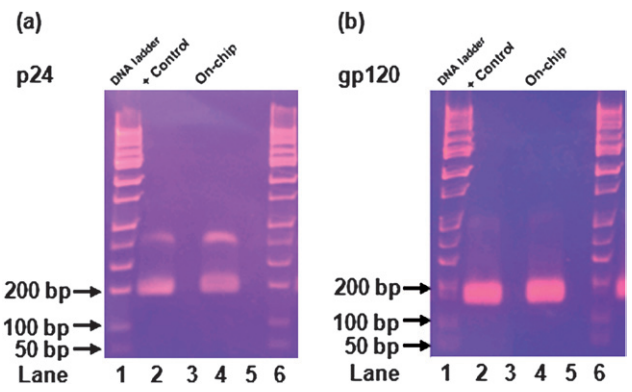

Fig. 5 Agarose gel electrophoresis of the PCR product for (a) p24 and (b) gp120: Lane 1 and 6 show 50 bp DNA ladder for reference; Lane 2 shows p24 and gp120 PCR product with conventional PCR thermocycler; Lane 3 is a negative control without DNA template; Lane 4 shows the PCR product for p24 and gp120 on the RT-PCR LOC; and Lane 5 is a negative control without DNA template on the RT-PCR LOC.

less than 35 minutes including a RT time of two minutes. In addition, because of the successive RT and PCR on the chip, there was no contamination and minimized sample loss, allowing the seamless amplification and detection of DNA.

On-chip detection using chemiluminescence assay

In order to perform on-chip detection with the chemiluminescence assay, the amplified DNA sample was introduced into the detection chamber after the RT and PCR. The amplified DNA was bound to a single stranded probe DNA attached onto the surface of the detection chamber by denaturation and annealing of the DNA. In advance of this process, immobilization of the streptavidin–biotin–single stranded probe DNA was tested on the COC surface without an adhesion layer using streptavidin–FITC (fluorescein isothiocyanate). Various concentrations of streptavidin–FITC in the range of 0.01, 0.05, 0.1, 0.2, and 0.5 μg/μl were injected and incubated for 5 minutes in the chamber. After rinsing with 1× PBS buffer and then drying with pressurized air, the fluorescent intensities were measured with a Typhoon™ 8600 scanner and then analyzed by ImageQuant™ software (GE Healthcare, USA). The measured intensities from the FITC dye were directly proportional to the concentration of the streptavidin–FITC (data not shown). Therefore, this result insured that streptavidin-FITC was adsorbed on the hydrophobic surface of the COC chamber without an additional adhesion layer.

In addition, we tested the temperature effects on the binding between streptavidin and biotin–single stranded probe DNA using streptavidin–biotin–FITC. If a high temperature, such as 95 °C, disrupts the binding between them, fluorescent dye conjugated with biotin should flow out of the detection chamber, after washing with 1× PBS buffer. For this experiment, five chips were prepared and 0.5 μg/μl of streptavidin–biotin–FITC solution was injected and incubated for 5 minutes in the detection chamber. One chip was placed at room temperature, but the four other chips were incubated for 5 minutes at different temperature 65, 75, 85, and 95 °C. After cooling the chips to room temperature, the solution was ejected by pressurized air. After rinsing with 1× PBS buffer, the measured fluorescent intensities from the samples incubated at different temperatures gave off a reasonable quantity of fluorescent light. No significant differences were detected in the fluorescence intensities among samples at 65, 75, 85, and 95 °C. Based on the results, the streptavidin–biotin–ss DNA was confirmed to be immobilized in the detection chamber within the temperature range used during denaturation and annealing.

Based on the experimental confirmation, the on-chip detection using a chemiluminescence assay was performed in sequence after the RT and PCR amplification process. Before loading the RT and PCR mixture onto the chip, single stranded probe DNA was immobilized on the surface of the detection chamber. In order to demonstrate the binding between the amplified DNA from the RT-PCR and the streptavidin–biotin–probe DNA which were already attached on the detection chamber through the denaturation and annealing, as shown in Fig. 3(b) and (c), the detection chamber was heated to 65, 75, 85, and 95 °C, to denature the DNA for 1 minute and then cooled to the annealing temperature for 1 minute. After the chemiluminescence assay, as

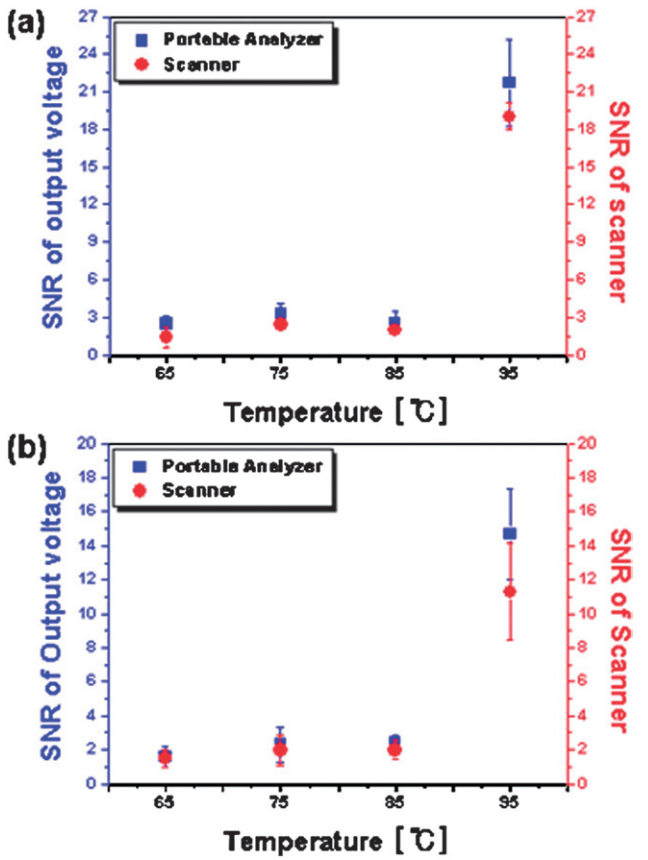

Fig. 6 Comparison of normalized chemiluminescent intensities from the newly developed portable analyzer (left side) and conventional scanner (right side) at different denaturation temperatures for (a) p24; and (b) gp120.

described above in the detection protocol, the emitted chemiluminescent light was measured by a Typhoon™ 8600 scanner and also the newly developed portable analyzer for the comparison of the assay results. Fig. 6 shows the signal to noise ratio (SNR) for p24 and gp120 markers, according to the different denaturation temperatures. In order to normalize the intensity of the chemiluminescence assay, the net signal was calculated by subtracting the background signal from the intensity of each temperature. Then, each net signal was divided by the value of the subtracted background signal from the control in order to obtain the SNR. Comparing the measured data from the conventional scanner and the newly developed portable analyzer, they both showed the same tendencies for the SNR. When the temperature was 95 °C, the values of the SNR for the p24 and gp120 markers were always higher than at other temperatures since there was more amplified DNA denatured at 95 °C. The optical detection part of the portable analyzer showed a comparable SNR to the conventional scanner, which provides assurance of the accuracy of the on-chip detection using the chemiluminescence assay. Thus, the newly developed optical detection system can be used as a part of a portable analyzer for POCT in the clinical diagnosis setting, using the polymer LOC for RT-PCR. This system has a great potential to be used for the POCT diagnosis.

5. Conclusions

A fully disposable RT-PCR lab-on-a-chip with embedded micro pinch valves has been designed and fabricated on polymer substrates, and fully characterized for the RT-PCR process of the early and rapid detection of HIV. The developed pinch valves have demonstrated their practical feasibility for numerous labs-on-a-chip or μTAS that require on/off fluidic flow in a passive mode. Furthermore, it can be easily integrated with other microfluidic components or systems to construct a complete functional diagnostic system, including the RT-PCR assay or complicated immunoassays. A portable analyzer for the polymer RT-PCR LOC has also been developed and characterized for the on-chip detection using a chemiluminescence assay for POCT clinical diagnostics in less than 1 hour. As one of the demonstration vehicles of the POCT, the rapid detection of HIV has been successfully performed for the early diagnosis of AIDS using the developed LOC and analyzer. The polymer LOC for RT-PCR and the portable analyzer developed in this work has envisaged the rapid diagnosis of infectious diseases in a POCT platform.

Acknowledgements

The authors thank Dr Jungyoup Han for his assistance in device testing at the University of Cincinnati. The ERC Nano/Micro Fabrication Center and the Center for Genome Information at the University of Cincinnati are also acknowledged.

References

1 R. C. Weast, *CRC Handbook of Chemistry and Physics*, CRC Press, Boca raton, FL, 1983.
2 K. B. Mullis, F. Ferre, R. A. Gibbs, *The Polymerase Chain Reaction*, Birkhaeuser, Boston, 1994.
3 I. Schneegaß, R. Bräutigam and J. M. Köhler, Miniaturized flowthrough PCR with different template types in a silicon chip thermocycler, *Lab Chip*, 2001, **1**, 42–49.
4 Z. Zhao, Z. Cui, D. Cui and S. Xia, Monolithically integrated PCR biochip for DNA amplification, *Sens. Actuators, A*, 2003, **108**, 162–167.
5 Y. Liu, C. B. Rauch and R. L. Stevens, DNA Amplification and Hybridization Assays in Integrated Plastic Monolithic Devices, *Anal. Chem.*, 2002, **74**, 3063–3070.
6 C. G. Koh, W. Tan, M. Zhao and A. J. Ricco, Integrating Polymerase Chain Reaction, Valving, and Electrophoresis in a Plastic Device for Bacterial Detection, *Anal. Chem.*, 2003, **75**, 4591–4598.
7 R. H. Liu, J. Yang and R. Lenigk, Self-Contained, Fully Integrated Biochip for Sample Preparation, Polymerase Chain Reaction Amplification, and DNA Microarray Detection, *Anal. Chem.*, 2004, **76**, 1824–1831.
8 D. S. Yoon, Y. Lee, Y. Lee, H. J. Cho, S. W. Sung, K. W. Oh, J. Cha and G. Lim, Precise temperature control and rapid Thermal cycling in a micromachined DNA polymerase chain reaction chip, *J. Micromech. Microeng.*, 2002, **12**, 813–823.
9 M. A. Northrup, C. Gonzalez, D. Hadley, R. F. Hills, P. Landre, S. Lehew, R. Saiki, J. J. Sninsky, R. Watson and R. Watson Jr, A MEMS-based Miniature DNA Analysis system, *The 8th International Conference on Solid State Sensors and Actuators and Eurosensors IX*, 1995, 764–767.
10 R. P. Oda, M. A. Strausbauch, A. F. R. Huhmer, N. Borson, S. R. Jurrens and J. Craighead, Infrared-mediated thermocycling for ultrafast polymerase chain reaction amplification of DNA, *Anal. Chem.*, 1998, **70**, 4361–4368.
11 A. F. R. Huhmer and J. P. Landers, Noncontact infrared-mediated thermocycling for effective polymerase chain reaction amplification of DNA in nanoliter volumes, *Anal. Chem.*, 2000, **72**, 5507–5512.
12 Y. C. Lin, M. Y. Huang, K. C. Young, T. T. Chang and C. Y. Wu, A rapid micropolymerase chain reaction system for hepatitis C virus amplification, *Sens. Actuators, B*, 2000, **71**, 2–8.
13 A. Gulliksen, L. Solli, F. Karlsen, H. Rogne, E. Hovig and T. Nordstrøm, Real-time nucleic acid sequence-based amplification in nanoliter volumes, *Anal. Chem.*, 2004, **76**, 9–14.
14 T. M. H. Lee, M. C. Carles and I. M. Hsing, Microfabricated PCR-electrochemical device for simultaneous DNA amplification and detection, *Lab Chip*, 2003, **3**, 100–105.
15 N. Crews, C. Wittwer and B. Gale, Thermal Gradient PCR in a Continuous-Flow Microchip, Microfluidics, *BioMEMS, and Medical Microsystems V Proceedings of SPIE*, 2007, **6465**, 646504.
16 M. U. Kopp, A. J. de Mello and A. Manz, Chemical amplification: continuous-flow PCR on a chip, *Science*, 1998, **280**, 1046–1048.
17 P. J. Obeid and T. K. Christopoulos, Continuous-flow DNA and RNA amplification chip combined with laser-induced fluorescence detection, *Anal. Chim. Acta*, 2003, **494**, 1–9.
18 P. Belgrader, C. J. Elkin, S. B. Brown, S. N. Nasarabadi, R. G. Langlois and F. P. Milanovich, A reusable flow-through polymerase chain reaction instrument for the continuous monitoring of infectious biological agents, *Anal. Chem.*, 2003, **75**, 3446–3450.
19 X. Zhou, D. Liu, R. Zhong, Z. Dai, D. Wu and H. Wang, Detection of SARS-coronavirus by a microfluidic chip system, *Electrophoresis*, 2004, **25**, 3032–3039.
20 J. Liu, M. Enzelberger and S. Quake, A nanoliter rotary device for polymerase chain reaction, *Electrophoresis*, 2002, **23**, 1531–1536.
21 J. West, B. Karamata, B. Lillis, J. P. Gleeson, J. Alderman and J. K. Collins, Application of magnetohydrodynamic actuation to continuous flow chemistry, *Lab Chip*, 2002, **2**, 224–230.
22 M. Curcio and J. Roeraade, Continuous segmented-flow polymerase chain reaction for high-throughput miniaturized DNA amplification, *Anal. Chem.*, 2003, **75**, 1–7.
23 C. Wang, Y. Chen, C. Liao, H. Lee and G. Lee, Micromachined Flow-through Polymerase Chain Reaction Chip Utilizing Multiple Membrane-activated Micropumps, *19th IEEE International Conference on MEMS.*, 2006, 374–377.
24 C. Liao, G. Lee, H. Liu, T. Hsieh and C. H. Luo, Miniature RT-PCR system for diagnosis of RNA-based viruses, *Nucleic Acids Research*, 2005, **33**(No. 18), e156.
25 C. G. Timothy, S. P. Bharat, K. P. Susan and J. S. McDougal, Performance of the OraQuick and Hema-Strip rapid HIV antibody detection assays by non-laboratorians, *Journal of Clinical Virology*, 2004, **30**, 229–232.
26 W. J. Huang, F. C. Chang and P. P. J. Chu, Functionalization and chemical modification of cyclo olefin copolymers (COC), *Polymer*, 2000, **41**, 6095–6101.
27 J. Han, C. Gao, J. Do, S. H. Lee, J. Kai, S. Lee, L. Ramasamy, J. Nevin, G. Beaucage, J. Y. Lee and C. H. Ahn, A functional disposable lab-on-a-chip with embedded micro pinch valves for whole blood analysis, *The 9th International Conference on Miniaturized System for Chemistry and Life Sciences*, 2005, 43–45.
28 K. W. Oh, R. Rong and C. H. Ahn, Miniaturization of pinch-type valves and pumps for practical micro total analysis system integration, *J. Micromech. Microeng.*, 2005, **15**, 2449–2455.
29 J. Wang, Z. Chen, M. Mauk, K. Hong, M. Li, S. Yang and H. H. Bau, Self-Actuated, Thermo-Responsive Hydrogel Valves for Lab on a Chip, *Biomed. Microdevices*, 2005, **7**, 313–322.