Droplet-based micro oscillating-flow PCR chip

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Abstract

Polymerase chain reactions (PCR), thermally activated chemical reactions which are widely used for nucleic acid amplification, have recently received much attention in microelectromechanical systems and micro total analysis systems because a wide variety of DNA/RNA molecules can be enriched by PCR for further analyses. In the present work, a droplet-based micro oscillating-flow PCR chip was designed and fabricated by the silicon microfabrication technique. Three different temperature zones, which were stable at denaturation, extension and annealing temperatures and isolated from each other by a thin-wall linkage, were integrated with a single, simple and straight microchannel to form the chip's basic functional structure. The PCR mixture was injected into the chip as a single droplet and flowed through the three temperature zones in the main microchannel in an oscillating manner to achieve the temperature maintenance and transitions. The chip's thermal performance was theoretically analyzed and numerically simulated. The results indicated that the time needed for the temperature of the droplet to change to the target value is less than 1 s, and the root mean square error of temperature is less than 0.2 °C. A droplet of 1 μ l PCR mixture with standard HPV (Human Papilloma Virus)-DNA sample inside was amplified by the present chip and the results were analyzed by slab gel electrophoresis with separation of DNA markers in parallel. The electrophoresis results demonstrated that the micro oscillating-flow PCR chip successfully amplified the HPV-DNA, with a processing time of about 15 min which is significantly reduced compared to that for the conventional PCR instrument.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Microminiature devices have a great impact on clinical diagnosis [1] as they are low cost, small, portable, easy and fast to operate, use smaller volumes of sample and reagents, are adaptable for simultaneous multiple-assays, and suitable for system integration. The polymerase chain reaction [2], one of the most important molecular biological methods for the *in vitro* amplification of nucleic acid molecules, has been one of the active fields in microelectromechanical

systems (MEMS) and micro total analysis systems (μ -TAS), because nearly all molecular biological operations need PCR to enrich the nucleic acid samples in advance. The number of nucleic acids can be nearly doubled after one thermal cycle by controlling the system temperature in sequence to the denaturation temperature, the annealing temperature and the extension temperature with rapid transitions. After about 30–40 cycles, the DNA/RNA inside the PCR mixture will be rich enough for further analysis.

In the past ten years, two kinds of microchip for polymerase chain reaction, micro chamber PCR chips [3-12] and continuous-flow PCR chips [13–19], have been developed. The micro chamber PCR chip is derived directly from the thinwall tube usually used in the conventional PCR instrument. Thermal cycles of the PCR are realized by heating and cooling the PCR mixture in a reaction chamber with the characteristic size at the microscale. The greatly decreased thermal capacity compared to the thin-wall tube and the large surface-volume-ratio of the chip lead to a fast thermal cycle in the micro chamber PCR chip. The heating rates are $10 \, ^{\circ}\text{C s}^{-1}$ [3] and $30 \, ^{\circ}\text{C s}^{-1}$ [8], and some can reach 80 °C s⁻¹ [6, 7], while the cooling rates are 2.5 °C s⁻¹ [3], $4 \,^{\circ}\text{C s}^{-1}$ [8] and $40 \,^{\circ}\text{C s}^{-1}$ [6], even $74 \,^{\circ}\text{C s}^{-1}$ [7] by introducing Peltier elements into the chip. The thermal cycling program (including cycle numbers and reaction temperatures) for the PCR can be easily modified, depending on the DNA samples to be amplified in this stationary PCR chip. An intriguing innovation, the continuous-flow PCR chip [13], makes the PCR mixture continuously flow through a serpentine microchannel integrated with three thermally isolated reaction zones whose temperatures are held at the denaturation, annealing and extension temperatures to achieve nucleic acid replication. Since the continuous-flow PCR chip decouples the temporal and spatial factors, the substrate of the chip does not participate in the thermal transition process, thereby the thermal capacity is minimized to enable ultrafast reactions. Recently, Obeid et al [16] advanced a novel continuous-flow PCR chip with several outlets along the microchannel in the chip, and the cycle number of thermal cycling can be selected by controlling the outlet of the sample flow. Furthermore, samples continuously flowing through an annular microchannel driven by a pump [18] or magnetohydrodynamic actuation [19] gave a novel approach for the continuous-flow PCR chip and received a lot of attention. However, the potential contamination introduced by the previous reaction prevents the industrialization of the continuous-flow PCR chip.

Besides the aforementioned two kinds of micro PCR chips, some novel micro PCR chips have been developed. Rayleigh-Bénard convection was introduced to realize a two-temperature PCR [20, 21]. Convective flow was generated to achieve thermal cycling in a cavity with top and bottom surfaces maintained at the annealing-extension and denaturation temperature, respectively [20]. Heating from an infrared source in the center (to realize the denaturation temperature) and periphery of the chamber keeping constant low temperature (the annealing-extension temperature) was also adopted to produce the convection for Rayleigh-Bénard PCR [21]. By optimizing the geometrical structure of the cavity, steady laminar convection can be generated to realize DNA replication successfully. Recently, the droplet-based microfluidic technique was treated as a promising direction in miniature PCR instruments and received some researchers attention. Zhang et al [22] present a theoretical performance comparison between the continuous-flow PCR system and the droplet-based PCR system and the results demonstrated that the droplet-based microfluidic system was superior in terms of performance, ease of design and integration complexity. A bi-directional peristaltic pump was considered [23] to be

integrated with three reaction chambers into a droplet-based microfluidic PCR chip. A reactant droplet is designed to be driven by the pump to flow back and forth between three micro chambers thermally stabilized at the designed temperatures of PCR. Although it is just a theoretical analysis and numerical simulation, a novel concept for a PCR chip has been put forward which has the potential to combine the advantages of both the micro chamber PCR chip and the continuous-flow PCR chip.

In the present work, a droplet-based micro oscillatingflow PCR chip was designed and fabricated by the silicon microfabrication technique to miniaturize the flow process while maintaining the advantages of fixed temperature conditions as adopted in the continuous-flow PCR chip.

2. Principle of the droplet-based micro oscillating-flow PCR chip

Compared to the continuous-flow PCR chip, an additional space-time conversion was adopted in the present PCR chip. The main channel is a single, simple and straight microchannel with three independent temperature zones for the denaturation, annealing and extension temperatures integrated. The PCR process is performed in an oscillating-flow model, that is, the sample and reagents flow through the main channel back and forth several tens of times. Therefore, the space requirement for the multi-cycle chemical reactions is considerably reduced. At the same time, a changeable thermal cycling program can be realized by controlling the temperature of the three temperature zones and the length of time the droplet was at the temperature zone.

The schematic structure of the droplet-based micro oscillating-flow PCR chip is illustrated in figure 1. microliter-sized droplet of the PCR mixture is injected into the chip from the inlet/outlet hole and is driven by the pressure generated from an injector which is connected to the driven hole in the chip. The droplet flows through three sequential temperature (T1, T2 and T3) zones, which correspond to the denaturation, extension and annealing zones, respectively. The nucleic acid inside the droplet is thermally denatured and annealed as it passes through the T1 and T3 zones. When the droplet finishes the annealing reaction, the pressure difference is reversed to push it back through the extension process (in zone T2). A PCR thermal cycle is completed when the droplet leaves the extension zone (T2) and enters the denaturation zone, T1. The flow direction is again reversed as the droplet reaches the end of the denaturature zone. After a designated number of thermal cycles, such as 30-40 cycles, the droplet is pushed out of the chip from the inlet/outlet hole for further analysis.

3. Microfabrication and chip systems

A 300- μ m-thick silicon (100) wafer (3 inches) with a layer of silicon dioxide formed by the wet thermal oxidation on both sides has been used as the substrate. The dioxide layer was patterned by photolithography and wet etched by hydrofluoric acid to form the main microchannel mask (this side will be referred to as the M side hereafter). Photolithography was

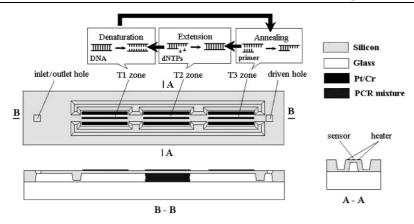


Figure 1. Schematic structure of the micro oscillating-flow PCR chip.

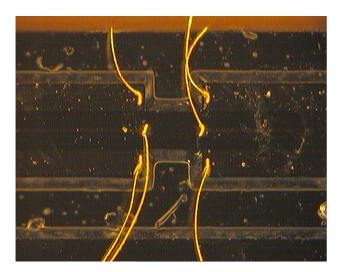


Figure 2. Corner structure with convex corner compensation.

done on the other side (I side) of the wafer to pattern the mask of the isolating grooves. The resistances were patterned by photolithography on the silicon dioxide layer of the I side. Sputter with Pt and lift off were used to make the resistors. The main microchannel and the isolating grooves were wet etched with ethylene diamine pyrocatechol and water (EDP). The etching depth was controlled to be about 280 μm . After removing the remnant silicon dioxide on the M side, the wafer was bonded with PYREX glass to form the close structure. In order to avoid undercutting during the wet anisotropic etching process, convex corner compensation was adopted. The final corner structure is shown in figure 2.

The main microchannel of the chip has a trapezoidal cross-section channel shaped by wet silicon etching with a channel top width of 1 mm and a height of 0.280 mm. The thermally stable regions are all 5 mm long and the linkage between the adjacent zones is 1 mm long with very thin lateral walls of about 20 μ m thickness. The thickness of the lateral wall was controlled by adjusting the etching time. The micro heater is 0.2 mm wide, 4.9 mm long and 0.6 μ m thick while the shape of the temperature sensor is nearly the same as the micro heater except its width is only 0.05 mm. From figure 3, the resistance of the Pt thin film temperature sensor used in the present work shows a good linearity in the entire temperature range. After

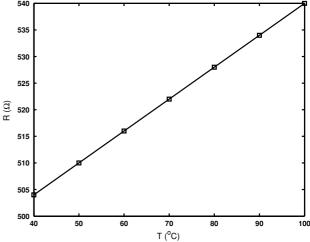


Figure 3. Resistance of the micro temperature sensor versus temperature.

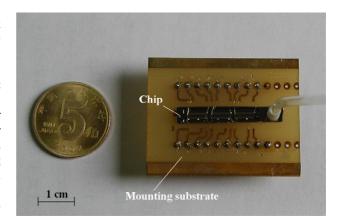


Figure 4. Photograph of the droplet-based micro oscillating-flow PCR chip with a coin.

calibration, the temperature at different thermal regions can be measured based on the sensor voltage.

Figure 4 is a photograph of the droplet-based micro oscillating-flow PCR chip with a coin for comparison. The whole chip system, which is shown in figure 5, consists of a power supply, a control board, an injector and a PCR chip. The temperature control system is composed of a resistor–voltage

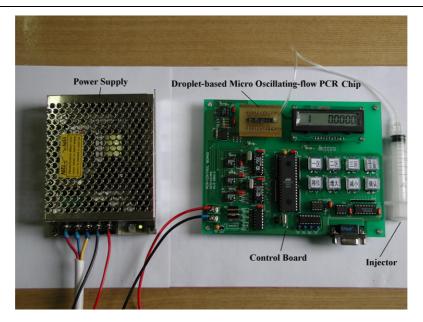


Figure 5. Photograph of the chip PCR system.

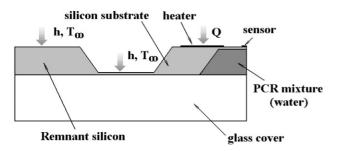


Figure 6. Two-dimensional physical model in the thermal design.

converter, an amplifier, an A/D sampling circuit, a microprocessor, an LCD screen, a key-press input circuit, nonvolatile memory and a driver for the micro heaters. The signal of the chip's temperature given by the micro temperature sensor was transferred into a voltage signal, and then amplified by the amplifier and changed into digital signals by the A/D sampling circuit. The digital signals were sampled and disposed of by the microprocessor. The corresponding voltage for the temperature was displayed on the LCD screen and saved in the memorizer. The measured voltage was compared with the design value input by the key-presses. The control signal based on the comparison results determined the state of the micro heaters via the driving circuit.

4. Thermal designs

Numerical simulation and lumped analysis were carried out to study the thermal performance of the present chip in order to give a design guide. Due to the geometrical symmetry of the chip, only half of the A–A section in figure 1 was taken into consideration, and the schematic structure of the simulative model is illustrated in figure 6.

In the figure, h is the convective heat transfer coefficient outside the chip, T_{∞} is the temperature of the air around the chip and Q is the input power. All the physical properties were assumed to be constant and the parameters are listed in

Table 1. Physical properties used in the thermal design.

Parameter	Illustration	Values
ρ_{Si}	Density of the silicon	2210 kg m ⁻³
k_{Si}	Thermal conductivity of the silicon	$128.6 \ \mathrm{W} \ \mathrm{mK}^{-1}$
$c_{p,Si}$	Heat capacity of silicon	$737.8 \text{ J kg}^{-1} \text{ K}^{-1}$
$ ho_{ m doplet}$	Density of the PCR mixture (water)	974.3 kg m^{-3}
k_{water}	Thermal conductivity of the PCR mixture (water)	0.6410 W mK ⁻¹
$C_{p, \text{water}}$	Heat capacity of PCR mixture (water)	$4202 \text{ J kg}^{-1} \text{ K}^{-1}$
$ ho_{ m glass}$	Density of the glass	2230.0 kg m^{-3}
$k_{ m glass}$	Thermal conductivity of the glass	$1.10~{ m W}~{ m mK}^{-1}$
$c_{p,\mathrm{glass}}$	Heat capacity of glass	$800~{ m J~kg^{-1}~K^{-1}}$

table 1. The properties of the PCR mixture were taken as those of water.

4.1. Thermal design of the temperature sensor

The temperature sensor was used to detect the average temperature of the silicon substrate in order to adjust the input power and to guarantee the constant-temperature control. Numerical simulation was adopted to study the temperature tracking capability of the sensor in the present layout.

Finite element analysis was used to simulate the transient thermal performance of the 2D model. In the simulation, the initial temperature of the whole chip and the environmental temperature T_{∞} were both 26 °C, and the convective heat transfer coefficient (h) outside the chip was 10 W K⁻¹ m⁻¹. The input power (Q) was 1 W. Based on the numerical simulation results, the sensor's temperature and the average temperature of the silicon substrate are compared in figure 7. The results demonstrated that the temperature difference between them was less than 0.6 °C. And because the sensor was close to the micro heaters (the highest temperature region in the chip), its temperature was always higher than the average temperature of the silicon substrate. The positive deviation was propitious to realize constant-temperature control without over-heating in the present chip.

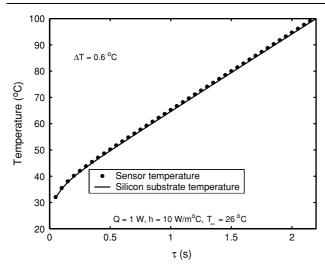


Figure 7. Temperature comparisons between the temperature sensor and the silicon substrate in the heating process.

4.2. Thermal isolation

In the design of the droplet-based micro oscillating-flow PCR chip, thermal isolation is essential to realize the programed temperature maintenance and transitions correctly and efficiently. The thermal isolation between the three temperature zones and the substrate must be considered to prevent heat loss and save the input power to meet the requirement of portable devices, and the thermal isolation between the adjacent temperature zones is much more important for the present chip to reduce thermal interferences and achieve robust constant-temperature control.

4.2.1. Thermal isolation between temperature zones and substrate. Deep grooves were fabricated as aforementioned to realize thermal isolation between the three temperature zones and the substrate. To figure out the optimized thermal performance, different widths and depths of the isolating grooves were taken into consideration. The top width of the groove, W, increased from 0, which referred to no groove, to 1.5 mm, and the depth, D, ranged from 0 (no groove) to 280 µm.

The simulation model was the same as shown in figure 6. In the simulation, constant power was input to heat the chip to a designed temperature and the time-dependent temperature variations of the sample inside the main channel with different D and W were studied. The thermal boundary condition and the initial condition were the same as those in section 4.1. The numerical results which were illustrated in figures 8 and 9 indicated that with increasing D and W, the time needed for the chip to reach the designed temperature decreased and the isolating microchannel cut down the heat loss considerably compared to that without grooves. Furthermore, based on the numerical results, the thermal isolation effects were much more sensitive to the variation of the depth of the grooves than that of its top width. Taking both the thermal isolation effects and the size of the chip into consideration, the depth of the isolating microchannel was chosen as 280 μm and the top width was 1 mm.

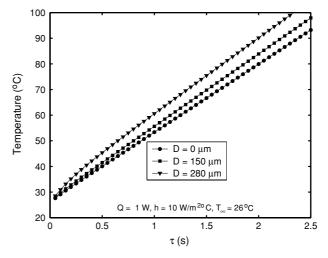


Figure 8. Temperature variations of the sample during the heating process with different groove depths.

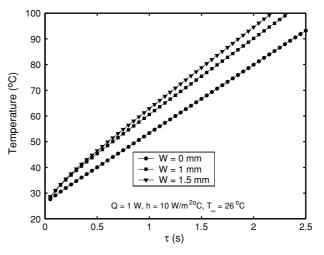


Figure 9. Temperature variations of the sample during the heating process with different groove widths.

4.2.2. Thermal isolation between adjacent temperature zones. To minimize the axial conduction between the neighboring temperature zones, the thickness of the lateral wall of the linkage between two temperature zones was designed to be about 20 μ m. Therefore the thermal resistance is about 249.60 K W⁻¹. When the temperature difference between the adjacent temperature zones was 20 °C, the axial heat flux was about 10% of the input power (about 1 W) while without any thermal isolation methods the interference heat flux was of the same magnitude as the input power.

To examine the thermal isolation effects of the thin-wall linkage between the adjacent temperature zones, a simplified lumped network was used to study the transient thermal performances of the chip. Due to the sharp contrast of the thermal conductivities between silicon and the chip support, it was believed that the thermal interference between the adjacent temperature zones was mainly caused by the axial heat conduction in the silicon substrate and the efforts given by the chip supports were neglected, which was expressed as

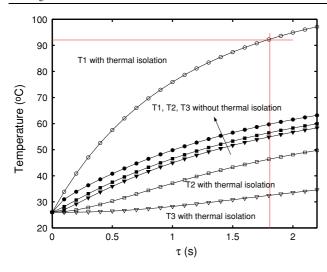


Figure 10. Temperatures of three zones versus time with and without thermal isolation in the linkage when only the T1 zone was heated.

in equations (1)–(3),

$$m_1 c_p \frac{\mathrm{d}T_1}{\mathrm{d}\tau} = Q A_{h,1} + h A_{c,1} (T_\infty - T_1) + \frac{T_2 - T_1}{R_{12}},\tag{1}$$

$$m_2 c_p \frac{\mathrm{d}T_2}{\mathrm{d}\tau} = h A_{c,2} (T_\infty - T_2) + \frac{T_1 - T_2}{R_{21}} + \frac{T_3 - T_2}{R_{23}},$$
 (2)

$$m_3 c_p \frac{\mathrm{d}T_3}{\mathrm{d}\tau} = h A_{c,3} (T_\infty - T_3) + \frac{T_2 - T_3}{R_{32}},$$
 (3)

where m_i and T_i are the mass and temperature of the lumped node, T_{∞} denotes the environmental temperature and c_p stands for the heat capacity of the subsystem. Q and $A_{h,i}$ refer to the input power and the heating area, h and $A_{c,i}$ are the convective heat transfer coefficient and the cooling area, $R_{i,j}$ expresses the thermal resistance between the adjacent temperature zones, and the subscripts i and j indicate the index of the three temperature zones (T1, T2 and T3). In the lumped model, the first temperature zone (T1) was heated to 93 °C, with the two downstream temperature zones (T2 and T3) being exposed to the surroundings for cooling without any power input.

The results of the lumped capacity analysis are shown in figure 10. Taking the thin-wall linkage as the thermal isolation between the adjacent temperature zones, when the first temperature zone reached 93 °C, which needed about 1.8 s, the temperatures of the two downstream temperature zones were 47.2 °C and 33.0 °C, respectively. A great temperature difference existed between the adjacent temperature zones of T1 (with power input) and T2 (without power input) demonstrating that the thin-wall linkage achieved excellent thermal isolation and produced very small thermal interference between the adjacent temperature zones. contrast, without thermal isolation treatment of the linkage, the temperatures of the three temperature zones increased much more slowly because the input power can transfer unblocked from the heating zone (T1) to the cooling zones (T2 and T3). Furthermore, great thermal interference was introduced and the temperatures between the adjacent zones had very small differences; for instance, the temperatures for the three zones at 1.8 s were 59.7 °C, 57.4 °C and 55.8 °C, respectively.

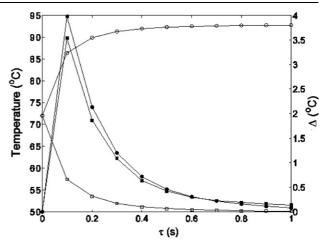


Figure 11. Transient thermal performance of the droplet in the heating and cooling processes (○: average temperature in the heating process, •: the root mean square error in the heating process, □: average temperature in the cooling process, ■: the root mean square error in the cooling process).

4.3. Transient thermal performance of the droplet

Thermal performance of the droplet in the working process was numerically simulated by finite element analysis. The physical model for the numerical simulation was the same as that adopted in section 4.1. The thermal boundary condition and the initial condition were also the same as those in section 4.1, except that in this simulation, input power was cut off but the temperature at the position of the sensor was kept constant (93 °C for the heating process and 50 °C for the cooling process). Due to the great importance of the temperature uniformity of the PCR mixture to the nucleic acid amplification, root mean square errors of the temperature in the droplet were selected to describe the temperature uniformity.

The variation of the average temperature (T) and the root mean square errors (Δ) of the droplet temperature during heating and cooling processes is shown in figure 11. The numerical results indicated that, no matter what the heating or cooling process, the average temperature will be close to the target value after less than 1 s. The maximum temperature differences from the target values are $0.12\,^{\circ}\mathrm{C}$ for the heating process, and $0.21\,^{\circ}\mathrm{C}$ for the cooling process respectively. Furthermore, as illustrated in figure 11, the root mean square errors (Δ) of the droplet temperature will increase rapidly at the moment the droplet enters the target thermal region and then drops quickly. After about 1 s, the droplet temperature will be very uniform and the root mean square errors are less than $0.2\,^{\circ}\mathrm{C}$ ($0.14\,^{\circ}\mathrm{C}$ for the heating process and $0.08\,^{\circ}\mathrm{C}$ for the cooling process).

5. Experiments

To verify the applicability of the present chip, HPV (Human Papilloma Virus)-DNA was amplified by the present chip PCR system and the amplification results were analyzed by slab gel electrophoresis. In order to avoid any unwanted difficulty, the positive reference of the HPV-DNA kitsTM (Fosun Company Inc.) was chosen as the DNA templates in the experiments.

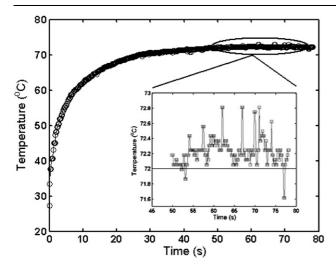


Figure 12. Constant-temperature controls in the extension zone with input power of 0.8 W.

5.1. Surface treatments

Before doing the PCR experiments, the inner surface of the main microchannel in the present chip was silanized with dichlorodimethylsilane (10% dichlorodimethylsilane in acetone) to reduce the adsorption of enzyme on the native silicon surface, and then the chip was washed ten times with double distilled deionized water and dried over night.

5.2. Constant-temperature controls in the experiment

Maintaining a constant temperature is essential to the present chip. Constant-temperature controls in the chip (extension zone for example) are experimentally verified with the results shown in figure 12. It is clear that when the temperature zone enters the steady region, the fluctuation of the real temperature is less than 1 $^{\circ}$ C and the average value is slightly higher than the designed one with a difference of about 0.2 $^{\circ}$ C.

5.3. Droplet handlings

In the experiment, a small droplet (about 0.1 μ l) of paraffin oil was injected into the chip from the inlet/outlet hole by a singlechannel pipetteTM (Eppendorf Company) in advance. Then a microliter-sized PCR mixture (prepared under the guide of the kits) was injected on the paraffin oil. Finally, another paraffin oil droplet was used to seal the PCR mixture. The droplet was pushed and pulled by pressures generated by an injector which was connected to the driven hole by the two-component epoxy glue. When the droplet entered a temperature zone, the reading of the LCD for this temperature zone showed a sudden change, which is shown in figure 13, and this was used to determine the position of the droplet and helped to realize robust droplet handling. When the droplet entered the T2 zone from the T1 zone, the temperature of the T2 zone gave a sudden rise because of a high temperature exciter, the entering droplet with denaturation temperature. In contrast, when the droplet departed the T2 zone to the T3 zone, a 'thermal short circuit' between T2 and T3 zones caused by the droplet (the length of the droplet is larger than the linkage between the two

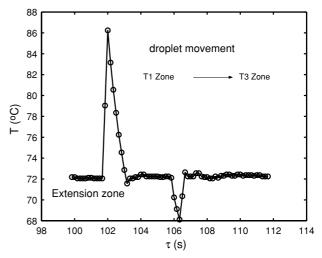


Figure 13. Temperature changed in the extension zone when the droplet entered from the denaturation zone, stayed in the extension zone and left to the annealing zone.

temperature zones) resulted in a sudden temperature drop in the T2 zone.

When the PCR process finished, the droplet was put out of the chip and collected by a pipette. Due to the loss in the handling process, about 1 μ l, including the PCR mixture and paraffin oil, was used for the detection at last.

Recently, droplet handling techniques in microsystems have been well studied, such as the thermopneumatic pump [24] and electrowetting-based actuation [25]. By introducing these methods into the present chip to integrate other microfluidic components with the present kernel thermal cycling structure, a micro clinical diagnosis system can be realized.

5.4. PCR experiment

The PCR mixture experienced a 2 min pre-denaturation process for perfect melting, and then cycled for 35 cycles: 4 s at 93 °C (denaturation), 4 s at 50 °C (annealing) and 4 s at 72 °C (extension). Finally, before the droplet was pushed out of the chip, the sample underwent about 4 min of postextension processing in the extension zone. Therefore, it took about 15 min to finish the whole amplification process. The same PCR experiment was done by Rotor-GeneTM with a total 19 μ l PCR mixture in the thin-wall tubes for comparison. It needed about 2 h and 10 min for Rotor-GeneTM to finish the amplification process. The results amplified by the present chip PCR system and the conventional PCR instruments (Rotor-GeneTM) were analyzed by slab gel electrophoresis. The electrophoresis results are shown in figure 14. In figure 14, lane 0 refers to HPV-DNA amplified by Rotor-GeneTM, lanes 1 and 3 are the amplification results of HPV-DNA amplified by the present chip PCR, lane 4 is result of water (negative control), and lanes M are the molecular markers. The clear and correct bars indicate that the droplet-based micro oscillatingflow PCR chip amplified the HPV-DNA successfully and the processing time needed for the chip PCR system was about one ninth of that for the conventional PCR instrument.

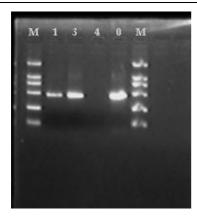


Figure 14. Photograph of slab gel electrophoresis for the present PCR chip and the conventional PCR instrument (Rotor-GeneTM).

6. Conclusion

A novel biochip, for polymerase chain reaction, a dropletbased micro oscillating-flow PCR chip, was designed, fabricated and tested. By adopting an additional spacetime conversion, the droplet-like PCR mixture experienced the denaturation, annealing and extension processes in an oscillating manner in a single, simple and straight microchannel integrated with three constant-temperature zones. The theoretical and numerical results showed that the thermal isolation of the temperature zones from the substrate can be achieved by deep grooves and the heat conduction between the adjacent temperature zones was considerably decreased by a very thin lateral wall of the linkage. Based on the numerical simulation, less than 1 s was needed for the droplet (the PCR mixture) to reach the target temperature with acceptable differences in the temperature transition process. The root mean square error of the droplet temperature was very small. Therefore, fast temperature transition and uniform temperature distribution of the sample and reagents were realized in the present chip. HPV-DNA was successfully amplified by the chip system, and the slab gel electrophoresis analysis of the amplification results testified to the applicability of the PCR chip. The time for the whole PCR cycling by the present chip was only about 15 min.

The droplet-based micro oscillating-flow PCR chip combines the advantages of the steady-state processing with those of the continuous processing. This chip is small and has superior thermal and fluidic characteristics. Moreover, it holds great promise for practical applications, especially for chips that require complex thermal conditions, such as changes in the resorting time or the reaction temperature during the thermal cycling.

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References

- Kricka L J and Wilding P 1996 Micromachining: a new direction for clinical analyzers *Pure Appl. Chem.* 68 1831–6
- [2] Mullis K B, Ferré F and Gibbs R A 1994 *The Polymerase Chain Reaction* (Boston, MA: Birkhäuser)
- [3] Woolley A T, Hadley D, Landre P, deMello A J, Mathies R and Northrup M A 1996 Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device Anal. Chem. 69 4081–6
- [4] Shoffner M A, Cheng J, Hvichia G E, Kricka L J and Wilding P 1996 Chip PCR: I. Surface passivation of microfabricated silicon-glass chips for PCR *Nucleic Acids Res.* 24 375–9
- [5] Cheng J, Shoffner M A, Hvichia G E, Kricka L J and Wilding P 1996 Chip PCR: II. Investigation of different PCR amplification systems in microfabricated silicon-glass chips *Nucleic Acids Res.* 24 380–5
- [6] Poser S, Schulz T, Dillner U, Baier V, Köhler J M, Schimkat D, Mayer G and Siebert A 1997 Chip elements for fast thermocycling Sensors Actuators A 62 672–5
- [7] Daniel J H, Iqbal S, Millington R B, Moore D F, Lowe C R, Leslie D L, Lee M A and Pearce M J 1998 Silicon microchambers for DNA amplification Sensors Actuators A 71 81–8
- [8] Northrup M A, Benett B, Hadley D, Landre P, Lehew S, Richards J and Stratton P 1998 A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers Anal. Chem. 70 918–22
- [9] Lee C Y, Lee G B, Liu H H and Hwang F C 2002 MEMS-based temperature control systems for DNA Amplification Int. J. Nonlinear Sci. Numer. Simul. 3 215–8
- [10] Lin Y C, Yang C C and Huang M Y 2000 Simulation and experimental validation of micro polymerase chain reaction chips Sensors Actuators B 71 127–33
- [11] Zhao Z, Cui D F and Wang L 2002 Design and microfabrication of biochemical reaction chip Int. J. Nonlinear Sci. Numer. Simul. 3 219–22
- [12] Yoon D S, Lee Y S, Lee Y S, Cho H J, Sung S W, Oh K W, Cha J H and Lim G B 2002 Precise temperature control and rapid thermal cycling in a micromachined DNA polymerase chain reaction chip J. Micromech. Microeng. 12 813–23
- [13] Kopp M U, de Melloo A J and Manz A 1998 Chemical amplification: continuous-flow PCR on a chip Science 28 1046–8
- [14] Schneegaβ I and Köhler J M 2001 Flow-through polymerase chain reactions in chip thermo cyclers *Rev. Mol. Biotechnol.* 82 101–21
- [15] Zhang Q T, Wang W H, Zhang H S and Wang Y L 2002 Temperature analysis of continuous-flow micro PCR based on FEA Sensors Actuators B 82 75–81
- [16] Obeid P J, Christopoulos T K, Crabtree H J and Backhouse C J 2003 Microfabricated device for DNA and RNA amplification by continuous-flow polymerase chain reaction and reverse transcription-polymerase chain reaction with cycle number selection Anal. Chem. 75 288–95
- [17] Sun K, Yamaguchi A, Ishida Y, Matsuo S and Misawa H 2002 A heater-integrated transparent microchannel chip for continuous-flow PCR Sensors Actuators B 84 283–9
- [18] Liu J, Enzelberger M and Quake S 2002 A nanoliter rotary device for polymerase chain reaction *Electrophoresis* 23 1531–6
- [19] West J, Karamata B, Lillis B, Gleeson J P, Alderman J, Collins J K, Lane W, Mathewson A and Berney H 2002 Application of magnetohydrodynamic actuation to continuous flow chemistry *Lab Chip* 2 224–30
- [20] Krishnan M, Ugaz V M and Burns M A 2002 PCR in a Rayleigh–Bénard convection cell Science 298 793

- [21] Braun D, Goddard N L and Libchaber A 2003 Exponential DNA replication by laminar convection *Phys. Rev. Lett.* 91 158103
- [22] Zhang T H, Chakrabarty K and Fair R B 2002 System performance evaluation with system C for two PCR microelectrofluidic systems *Technical Proc. 2002 Int. Conf.* on Modeling and Simulation of Microsystems (San Juan, Puerto Rico, USA, 22–25 April) pp 48–53
- [23] Bu M Q, Melvin T, Ensell G, Wilkinson J S and Evans A G 2003 Design and theoretical evaluation of a novel
- microfluidic device to be used for PCR *J. Micromech. Microeng.* **13** S125–30
- [24] Handique K, Burke D T, Mastrangelo C H and Burns M A 2001 On-chip thermopneumatic pressure for discrete drop pumping *Anal. Chem.* 73 1831–8
- [25] Cho S K, Moon H J and Kim C J 2003 Creating, transporting, cutting and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits J. *Microelectromech. Syst.* 12 70–80