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PTFE Capillary-Based DNA Amplification within an Oscillatory Thermal Cycling Device

Jyh J. Chen, Fu H. Yang, Ming H. Liao

***Abstract*—**This study describes a capillary-based device integrated with the heating and cooling modules for polymerase chain reaction (PCR). The device consists of the reaction polytetrafluoroethylene (PTFE) capillary, the aluminum blocks, and is equipped with two cartridge heaters, a thermoelectric (TE) cooler, a fan, and some thermocouples for temperature control. The cartridge heaters are placed into the heating blocks and maintained at two different temperatures to achieve the denaturation and the extension step. Some thermocouples inserted into the capillary are used to obtain the transient temperature profiles of the reaction sample during thermal cycles. A 483-bp DNA template is amplified successfully in the designed system and the traditional thermal cycler. This work should be interesting to persons involved in the high-temperature based reactions and genomics or cell analysis.

***Keywords*—**Polymerase chain reaction, thermal cycles, capillary, TE cooler.

I. Introduction

With recent advances in polymerase chain reaction (PCR) technology [1], an accurate and rapid method for quantization of the pathogen load has been realized during these years. And PCR, producing millions of copies from a specific DNA sequence, has become one of the most widely used DNA amplification techniques in molecular biology, forensic analysis, and medical diagnostics. Therefore, PCR makes the traditional disease diagnosis methods moving from conventional cell-culture processes to molecular level detection.

After PCR was invented, it was first performed in multiple water baths and then in programmable heat blocks or thermal cyclers. A specific DNA sequence is amplified by an enzymatic reaction using repetitive cycling among three temperatures. Each thermal cycle comprises denaturation, annealing, and extension steps. Due to a large thermal mass and associated slow heating and cooling rates in the conventional PCR machine, PCR might take as long as 2 hours. With the advancement in the microelectromechanical system (MEMS) technology, miniaturized PCR devices have been developed. Northrup et al. [2] presented a silicon-based PCR microchip. The battery-operated system showed a significant improvement over the commercial thermal cycling instrument. Since then, many types of PCR chips have been introduced. Various types of microreactors, including microchambers, microchannels, capillaries, and droplets, were employed for miniature DNA amplifications. These microreactors contained the advantages of the reduced reaction time as well as the low sample consumption.

Among the PCR micro-devices made by many researchers, they can be classified into two types. One is the miniature chamber-based PCR machine, and the other is the continuous flow PCR (CFPCR) device. For the CFPCR device, the reaction mixture moves through two or three different isothermal zones in a thin channel or capillary instead of cycling the temperature of whole device. It can be taken as the space-variant PCR machine. In the oscillatory CFPCR device, the slug of fluid is moved back and forth among the various constant-temperature zones. Chiou et al. [3] devised a thermocycling machine based on a capillary equipped with a bidirectional pressure-driven flow. This arrangement could approach a time period of 2.5 min for 30 cycle amplifications of a 500-bp DNA product. Frey et al. [4] presented a flow-through reactor by shuttling a submicroliter sample of the PCR mixture over three heaters. An externally actuated pneumatic pump and the width-variant microchannels allowed for an autonomous sample manipulation. Chen et al. [5] showed a bidirectional flow DNA amplification microreactor. The system was used for the amplification and real-time detection of a single-copy target gene from 24 human genomes.

In this paper we present a simple way of producing a capillary-based polytetrafluoroethylene (PTFE) PCR device. The temperature of the reaction sample is cycled among three temperature regions. Two cartridge heaters are placed into the aluminum blocks, and maintained at the required temperatures during the denaturation and the extension steps. A thermoelectric (TE) cooler with a heat sink and a fan is located onto the aluminum block in order to achieve the annealing temperature. A syringe contacted to the moving stage is used to shuttle the reaction sample among three blocks during PCR. Some thermocouples are inserted into the capillary to measure the sample temperatures, and the results can be utilized to assure the required PCR temperatures. A 483-bp DNA template is amplified successfully in the designed system.

II. Experimental Setup

An experimental setup of the designed system is shown in Fig. 1. The instrument has two heating modules and one cooling module, each constructed by assembling a grooved aluminum block, and a cartridge heater or a Peltier heating element. The capillary is fitted into the grooves formed on the surface of the blocks. The PCR mixture is in the form of a drop, which

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oscillates inside of the capillary. Three aluminum blocks define the denaturation, annealing, and extension regions. A syringe contacted with a moving stage shuttles the PCR sample along the capillary to accomplish the PCR process. Shuttling the sample back and forth is not easy. The sample has to be accelerated and very precisely stopped at the location of the specific regions. The location hysteresis effect must be reduced. By controlling the duration times of the sample drop among three blocks, the DNA sample can be amplified in this device.

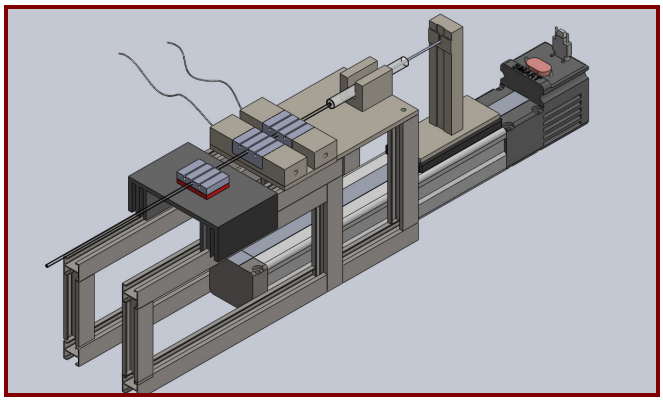

Fig. 1 The experimental setup of the designed system

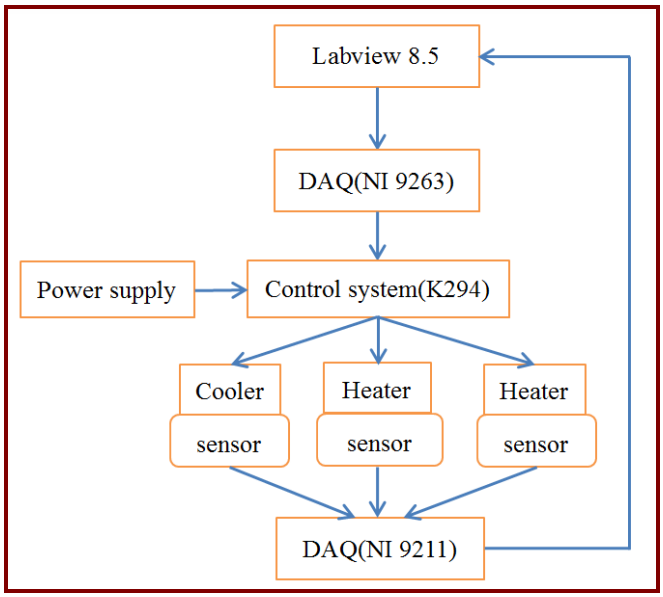

Fig. 2 The diagram of the temperature control system for the system

The diagram of the CFPCR microfluidics for the temperature measurement and the control system is illustrated in Fig. 2. It mainly consists of an AC power suppler, three PID controllers, two cartridge heaters, a TE cooler, a data acquisition (DAQ) system and some miniaturized K-type thermocouples. The experiments are carried out using a computer-controlled, LabVIEW-based (NI, National Instruments, Austin, TX) temperature control system. The cartridge heaters are inserted into the aluminum blocks to form the heating regions. The TE cooler is positioned onto the block to retain the sample at the annealing step. The thermocouples embedded within the blocks are connected to the DAQ card. The temperature acquired with the thermocouple is used as the feedback signal for the proportional/integral/derivative (PID) algorithm that is programmed in LabVIEW.

The cylindrical grooves with small openings of 3.2 mm in diameter are machined on the surface of the aluminum block. A standard 3 mm o.d. PTFE capillary with a wall thickness of 0.5 mm is used and positioned in the groove. A capillary length of 250 mm is applied for the three PCR steps. The position of the sample during PCR can be seen through the opening. One end of the capillary is connected to the syringe, and the other is opened to the air.

Each heat block is machined out of aluminum. The dimensions of the two different blocks are illustrated in Fig. 3. The high thermal conductivity of aluminum ensures good thermal uniformity within each block. The blocks shown at the left hand side of Fig. 3 are equipped with cartridge heaters and thermocouples connected to PID controllers to maintain denaturation and extension temperatures precisely. One Peltier heating element is used to keep the sample at the annealing temperature, and contacted the other block shown at the right hand side of Fig. 3 via an aluminum heat sink.

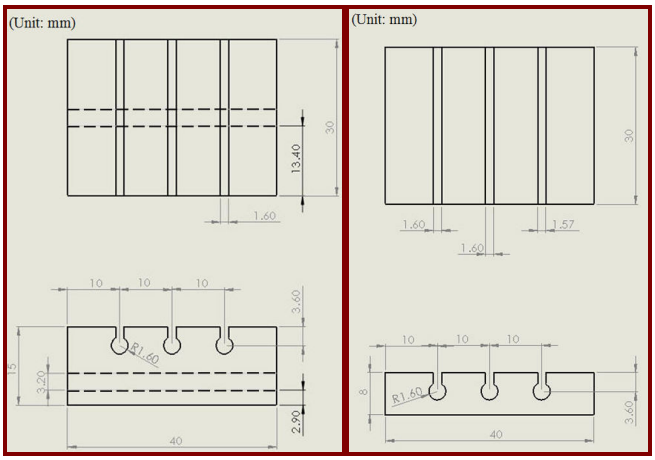

Fig. 3 The dimensions of the two different blocks

The transport of the sample is achieved by the syringe connected to the moving stage and from one region to the next (11 mm distance). Three thermostatic segments are linearly arranged along the motor rack (SmartT VL-ST60, Montrol, Taiwan) and utilized for DNA amplification. The bidirectional repeatability of the moving stage is less than 40 μm. This motor is connected to a microprocessor system and communicates with a computer through the RS-232 port. The moving speed is programmable by setting the moving distance and the moving time in the program.

In addition to the system setup, heating and cooling systems are the other main components of this oscillating CFPCR device. The heating system has cartridge heaters, aluminum blocks, thermocouples for heat sensing, a power supply unit with solid state relays (SSRs), and a NI control system to control the temperatures. To regulate the temperature, a thermocouple is

inserted in the grooved aluminum plate to measure the temperature and used as the feedback control. The aluminum for cooling is contacted with a Peltier cooling element. The hot side of the thermoelectric cooling element is in contact with the aluminum block, while the cold side is attached to an aluminum heat sink with a fan. A National Instruments control system is also used to control the temperature of the TE cooler.

For heating, each heat block is equipped with one bore (about 3.2 mm). The bore houses the resistance cartridge heater (3.175 mm diameter, 38 mm length, 14 W, C1J-9412, Watlow, USA), and a K-type thermocouple (K30-2-506, Watlow, USA). A thin layer of silicone heat transfer compound is applied between the heater blocks and the heaters to reduce the thermal contact resistance. The temperature difference of the block surface at three measured points is about ±1 K. The thermal cycling is controlled with a LabVIEW program through a control module. The temperature acquired with the thermocouple is used as the feedback signal for the PID controller programmed in LabVIEW. NI 9211 (National Instruments, USA) data acquisition card is utilized that controls the power output directly to the heaters. The controller applied constant (100%) power during the heating period. Three sets of PID gains corresponding to each step of a cycle are employed when the temperature approaches the prescribed, set temperatures. The whole system is insulated. The other thermocouples which are used to sense the temperature inside the capillary and two heating blocks are connected to a data acquisition system that converts the analog signal to the digital one. A computer receives the temperature signals through the NI 9211 interface and records the real-time temperature profiles. For cooling, the heat block is contacted with one TE cooler. The similar control scheme to the heater is utilized in the TE cooler.

The PCR system used to assess the performance of our device is designed for exponential amplification of the specific regions of a DNA template. To compare DNA amplification using the designed device, a standard protocol PCR product is prepared using MJ research PTC-200. PCR conditions is set as follows: 3 min at 95°C for initial denaturation, subsequent 30 cycles of 30 s at 95°C, 30 s at 58°C, and 30 s at 72°C, 3 min for final extension. The complete 30 PCR cycles on the commercial PCR machine takes about 2 hours.

The mixture of DNA sample is prepared in 25 µl under following conditions: 15 ng DNA template, 1 µl high purified deoxynucleotide triphosphates (dNTPs) (10 mM each of dATP, dTTP, dCTP, and dGTP,), 10× PCR buffer (200 mM KCl, 200 mM Tris-HCl, 15 mM $MgCl_2$), 0.1 µg bovine serum albumin (BSA), 1 µl of each primer, 0.5 µl thermostable *Tag* polymerase (5 U/µl), and double-deionized (dd) H_2O. BSA is used to dynamically coating the capillary inner surface. A specific amount of the PCR volume plug is flanked by mineral oil to avoid droplet evaporation during thermocycling.

The PCR mix is introduced into the capillary from the inlet. Following sample loading, a home-made syringe pump that controls the sample oscillation is connected to the port near the denaturation temperature zone. The other port is opened to the air. The PCR sample moves back and forth to complete the PCR process. The denaturation, annealing, and extension steps are accomplished by keeping the sample at the specific heating or cooling region for certain time durations. Characterization of the PCR products is realized by gel electrophoresis. The photograph of the assembled oscillatory CFPCR device is illustrated in Fig. 4.

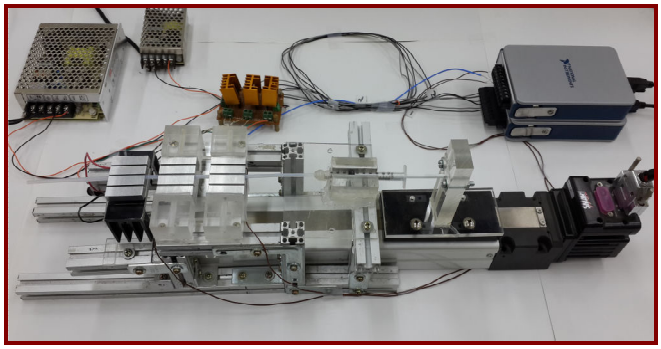

Fig. 4 The photograph of the assembled oscillatory CFPCR device

III. Results and Discussion

Since PCR is quite thermal-sensitive, before carrying out any amplification experiments it is necessary to assess the performance of the temperature control in the oscillatory CFPCR device. During the experiments, no bubble formation is observed at the three temperature regimes involved in the PCR, and the sample evaporation problem can be negligible. Fig. 5 shows the transient temperature profiles of the sample when it stays still at three isothermal regimes. The arrangement of three regions from left to right in Fig. 5 is annealing, extension, and denaturation region, respectively. The duration times for sample staying at the annealing, extension, and denaturation regions are 10 s, 20 s, and 15 s, respectively. And the moving speed of the sample from one region to another region is 14.4 mm/s. For the temperature profile of the sample drop at the denaturation step, the temperature of the sample increases as it moves from the extension region to the denaturation region. The duration times for denaturation step ranged from 94°C to 95°C is about 5 s. The sample temperature falls from the denaturation step to the annealing step. The duration times for annealing step ranged from 50°C to 60°C is about 7 s. Then the temperature of the sample increases again as the sample moves from the annealing step to the extension step. The duration times for extension step ranged from 68°C to 72°C is about 13 s. The surface temperatures of the heating blocks measured by the infrared imager are also obtained. It is clear that three different temperature zones are formed. In each zone, the respective temperature is almost constant, and the maximum surface temperature difference among these blocks is less than 3 °C. This is likely because of the proper thermal isolation by air gaps. As the cycling temperatures are precisely controlled, desirable temperature kinetics for PCR could be obtained. It should be noted that other DNA templates with various temperature requirements can also be amplified by simply changing the

temperature setting.

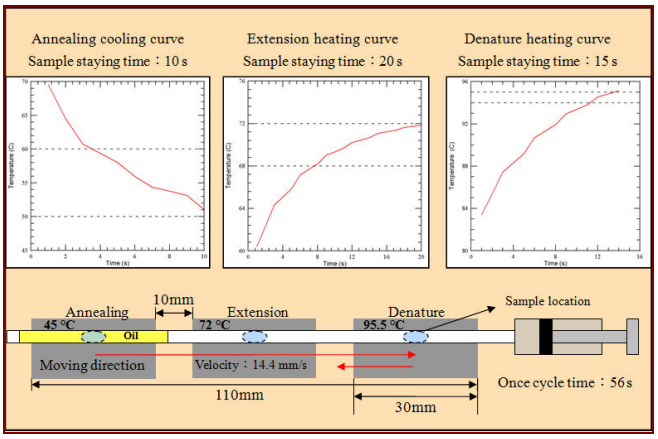

Fig. 5 The temperature profiles of the sample

During the PCR process, the instability of the PCR sample drop not only hinders the precise position control of the sample, but also decreases the reaction efficiency. In our experiments, the PTFE capillary is uncoated with any surfactant. The reaction mixture inside an oil plug is introduced into the capillary. When the sample mixture of 20 µl is shuttled along the capillary at a low speed (less than 15 mm/s), the oil and water plugs move simultaneously. The PTFE surface has a highly hydrophobic nature, allowing the mineral oil to wet the inner surface of the capillary preferentially over the aqueous phase [6]. Accordingly, the mineral oil gradually encapsulated the aqueous phase to form a water/oil droplet. When the plug is transported along the capillary, the droplet remained intact throughout the reaction shown in Fig. 6.

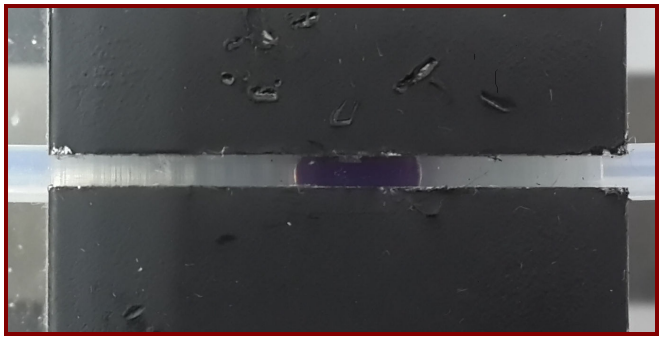

Fig. 6 The drop inside the capillary

Compared with the other types (unidirectional or closed-loop) of the continuous-flow PCR systems, the oscillatory CFPCR system can provide a flexible cycle number by only modifying the pumping program, without need to fabricate a new device. It is one of the advantages for performing the DNA amplification in the oscillatory CFPCR system. And the reaction sample can be easily moved among the isothermal regions by the external pump or the moving stage. Therefore, the effect of cycle number on the oscillatory CFPCR is studied. Fig. 7 displays this effect, where the moving speed of 14.4 mm/s is used and tested. As seen from Fig. 7, the most efficient cycle number seemed to be around 30. With the decrease of cycle number from 30 to 25, the PCR yield is gradually reduced. When the cycle number is 25, the amplification product is small in yield but could still be distinguished by the gel electrophoresis pattern.

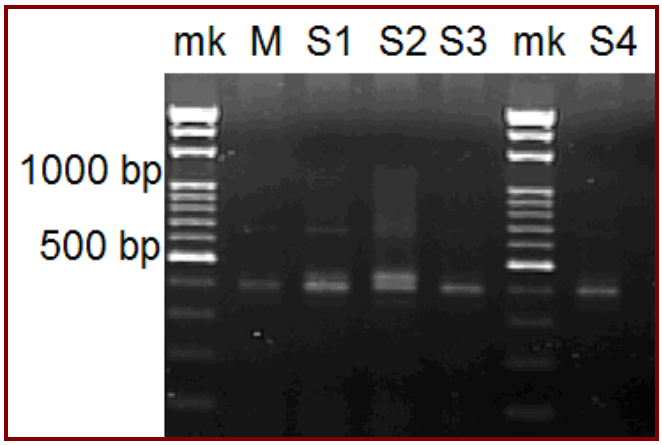

Fig. 7 An agarose gel electrophoresis image of PCR products amplified from a DNA template. All the PCR amplifications are carried out in the commercial PCR machine and our device. Lane mk is a marker ladder. Lane M corresponds to the products of the commercial PCR machine. Lanes S1, S2, S3, and S4 correspond, respectively, to the products of 20 µl (30 cycles), 20 µl (25 cycles), 10 µl (30 cycles), and 10 µl (25 cycles)

Fig. 8 shows a gel image which includes a marker, a control representing the amplicon from a commercial thermal cycler, and 3 amplicons from a 30-cycle nanoliter CFPCR device at the mixture volume amounts of 20 µl, 10 µl, and 5 µl. The relative intensity of the amplicons produced at the different volume amounts compared to the control amplicon; the intensity yields decrease for those obtained from the commercial thermal cycler, from our device of 20 µl, 10 µl, and 5 µl. Successful amplification of the 483 bp DNA fragment is achieved at volume amounts down to 5 µl.

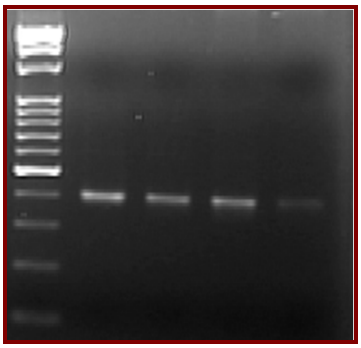

Fig. 8 An agarose gel electrophoresis image of PCR products amplified from a DNA template. All the PCR amplifications are carried out in the commercial PCR machine and our device. Lanes from left to right are a marker ladder, the products from the commercial PCR machine, and the products from our device of 20 µl, 10 µl, and 5 µl, respectively

IV. Conclusion

In this article, a novel PCR device is tested. All three temperature steps have sufficient heating duration to perform PCR. A syringe contacted to the moving stage is used to shuttle the reaction sample among three blocks during PCR. A 483-bp DNA template is amplified successfully in the designed system.

Acknowledgment

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References

[1] R. K. Saiki, S. Scharf, F. Faloona, K.B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim, "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia," *Science*, vol. 230, pp. 1950–1954, 1985.

[2] M. A. Northrup, M. T. Ching, R. M. White, and R. T. Wltson, "DNA amplification in a microfabricated reaction chamber," in *Proceeding of the 7th international conference of solid state sensors and actuators*, pp 924–926, 1993.

[3] J. Chiou, P. Matsudaira, A. Sonin, and D. Ehrlich, "A closed-cycle capillary polymerase chain reaction machine," Anal. Chem., vol. 73, pp. 2018–2021, 2001.

[4] O. Frey, S. Bonneick, A. Hierlemann, and J. Lichtenberg, "Autonomous microfluidic multi-channel chip for real-time PCR with integrated liquid handling," *Biomed. Microdevices*, vol. 9, pp. 711–718, 2007.

[5] L. Chen, J. West, P. A. Auroux, A. Manz, and P. J. R. Day, "Ultrasensitive PCR and real-time detection from human genomic samples using a bidirectional flow microreactor," *Anal. Chem.*, vol. 79, pp. 9185–9190, 2007.

[6] D. Liu, G. Liang, X. Lei, B. Chen, W. Wang, and X. Zhou, "Highly efficient capillary polymerase chain reaction using an oscillation droplet microreactor," *Anal. Chim. Acta*, vol. 718, pp. 58-63, 2012.

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