

CONTINUOUS FLOW INFRARED POLYMERASE CHAIN REACTION (cfIR-PCR) USING AN INFRARED-MEDIATED HEATING SYSTEM AT CONSTANT POWER

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ABSTRACT

We report the development of a microfluidic platform for continuous flow PCR using an infrared-mediated heating system having the potential to provide rapid, accurate, and high-throughput genomic analysis. A single halogen lamp at constant power was used to rapidly establish a thermal gradient, creating distinct PCR zones on a plastic microchip. As the sample flowed through the microdevice, it passed through these zones, undergoing thermal cycling. Results show an increase in PCR product yield with each subsequent fraction as the surface becomes passivated. This simple IR-heating system allowed for the amplification of target DNA in approximately 40 minutes.

KEYWORDS: Infrared-mediated PCR, Continuous Flow PCR, High-throughput Genomic Analysis, Molecular Diagnostics

INTRODUCTION

Since the introduction of cf-PCR in 1998 [1], many research groups have studied cf-PCR using different designs, heating systems, and microchip materials (frequently aided by computer-aided thermal simulations) [2]. Most commonly, three distinct resistive heaters, heat blocks or rods are used to create temperature zones and the sample is moved into and out of each zone, undergoing thermal cycling. Alternatively, a single heat block or heat source [3] has been used to conduct cf-PCR through a naturally established thermal gradient, minimizing the number of thermal measurement and control systems required.

THEORY

Over the past decade, an infrared-mediated heating system has been developed in our group to facilitate non-contact PCR inside glass and polymeric microfluidic devices [4, 5]. However, as a stationary PCR format, the number of simultaneous PCR reactions per microdevice has been limited to seven samples [6] due to footprint limitations of the microfluidic architecture. In order to increase sample throughput using the IR-mediated heating system, we believed that continuous illumination of the lamp would establish a thermal gradient from the focal spot outward (Figure 1), allowing for cf-PCR in microfluidic devices by flowing the sample towards and away from the focal spot. For even heat distribution throughout the microdevice and stable temperature holds, a circular, 29-loop poly(methyl-methacrylate) (PMMA) microdevice was developed.

EXPERIMENTAL

The cfIR-PCR system was adapted from our existing IR-mediated heating systems normally used for stationary PCR. The microfluidic device was constructed with a round border that fit snugly into a manifold, ensuring that the microfluidic device was centered over the halogen lamp. The temperature of the inner zone of the microfluidic device was PID controlled to maintain temperature between 95–97 °C via a miniature thermocouple inserted in a reference microchamber. Three-layer microchips were fabricated in PMMA using a CO₂ laser ablation system (VersaLaser3.5, Universal Laser Systems, Inc.). The fluidic architecture was defined by cutting-through 0.2 mm PMMA and bonding this layer between two 1.5 mm pieces of PMMA. The 1.5 mm PMMA was chosen for the top and bottom layers for greater heat capacity (versus thinner PMMA), which is predicted to generate a more linear and gradual thermal gradient. The PCR reaction mixture consisted of 3 mM MgCl₂, 1X PCR buffer, 0.2 mM dNTPs, 4 μM primers for a 520 bp fragment of λ-phage DNA, 0.2 U/μL *Taq* polymerase, 0.25 μg/μL BSA, and 21.8 ng/μL λ DNA were infused into the microfluidic devices via syringe-drive flow (Aladdin-1000, World Precision Instruments) at 1.5 μL/min. Post-PCR samples were collected as 15 μL aliquots at the device outlet to show a time-course of DNA amplification.

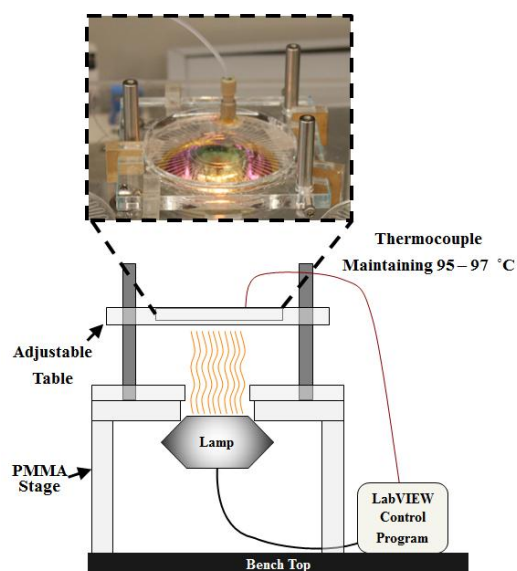


Figure 1: Apparatus for IR-mediated cf-PCR. Infrared heating system consists of a halogen lamp, controlled by an in-house LabVIEW program. Inset: the cfIR-PCR microchip is centered above the halogen bulb on an adjustable stage.

PCR products were separated and detected using a Bioanalyzer 2100 (Agilent), a commercially-available microchip electrophoresis instrument.

RESULTS AND DISCUSSION

The spatial thermal gradient, generated by the IR lamp, of the whole microchip at a steady state was visualized using an IR-camera (FLIRi50) (Figure 2A) and a temperature measurement microchip, containing rows of 6 microchambers extending radially from the center in 8 directions, was fabricated (Figure 2B). A total of 48 microchambers were monitored simultaneously to map the temperatures at different distances from the center of the lamp. A 3D bar graph was generated from the temperatures recorded and shows that the temperature in the gradient ranges from $\sim 95^\circ\text{C}$ at 15 mm from the center down to $\sim 57^\circ\text{C}$ at 25 mm from the center (Figure 2C). These values were averaged to determine boundaries for each of the three zones required for PCR. It was determined that the slope of the thermal gradient was $\sim 4^\circ\text{C}/\text{mm}$, defining the denaturation zone from 15-17.5 mm, the extension zone from 17.5-22.5 mm, and the annealing zone from 22.5-25 mm (Figure 2D).

From this information, a circular continuous flow PMMA PCR microdevice was designed around this 2D temperature gradient and has 29 loops, consisting of three chambers for each of the three temperature zones (Figure 3A & B). Chamber volumes in each loop, which represents one PCR cycle, were adjusted by changing the microchannel dimensions, creating wider regions for slower flow. The ratio of volumes of the three zones was adjusted to a 1:2:2 ratio for the denature, anneal, and extension zones, respectively, and the volumes were calculated to be $0.26\ \mu\text{L}$: $0.5\ \mu\text{L}$: $0.5\ \mu\text{L}$ (Figure 3C). Dwell times of the sample in each of these zones was estimated to be proportional to these volumes and is a function of the flow rate (e.g., when flowing at $1.5\ \mu\text{L}/\text{min}$ the dwell times were calculated to be 10 sec:20 sec:20 sec for denaturation, annealing, and extension zones, respectively). The actual dwell times for each zone were measured by timing the migration of a dye plug infused through the microdevice via a syringe pump set to $1.5\ \mu\text{L}/\text{min}$ and were determined to be 9.2 ± 1.1 seconds: 20.8 ± 0.5 seconds: 20.7 ± 0.5 seconds. The dwell times can be adjusted by modulating the flow rate of the sample, varying the overall PCR cycling time. Furthermore, by designing different microfluidic architectures through the thermal gradient determined above, a range of annealing temperatures are achievable by moving the annealing zone chambers closer or further from the center of the microfluidic device.

Finally, PCR reagents for amplification of a 520 bp segment of λ -phage DNA were flowed through the cfIR-PCR microdevice and 15 μL fractions were collected at the outlet. Successful amplification of the fragment was achieved as evidenced by the presence of the target PCR product (Figure 3D). Interestingly, there was no evidence of PCR product in the first four fractions, however, starting with fraction 5, increasing amounts of PCR product were present in each subsequent fraction. This suggests that as PCR reagents flowed through microchannels for cfIR-PCR, the polymerase and/or BSA accumulated on the microchannel walls, passivating the surface and allowing for increased amounts of PCR product as the surface became more and more passivated. Future work involves the development of a pre-PCR passivation technique to prevent loss of valuable PCR reagents to the walls of the microchannels and ensure the recovery of PCR product in the first fraction.

CONCLUSION

In this study, we report the first approach of continuous flow PCR on a microfluidic device using a non-contact IR-mediated heating system at constant lamp power. This cfIR-PCR system was shown to be capable of generating increasing amounts of PCR product as the extent of surface passivation increased. Future studies will focus on PCR

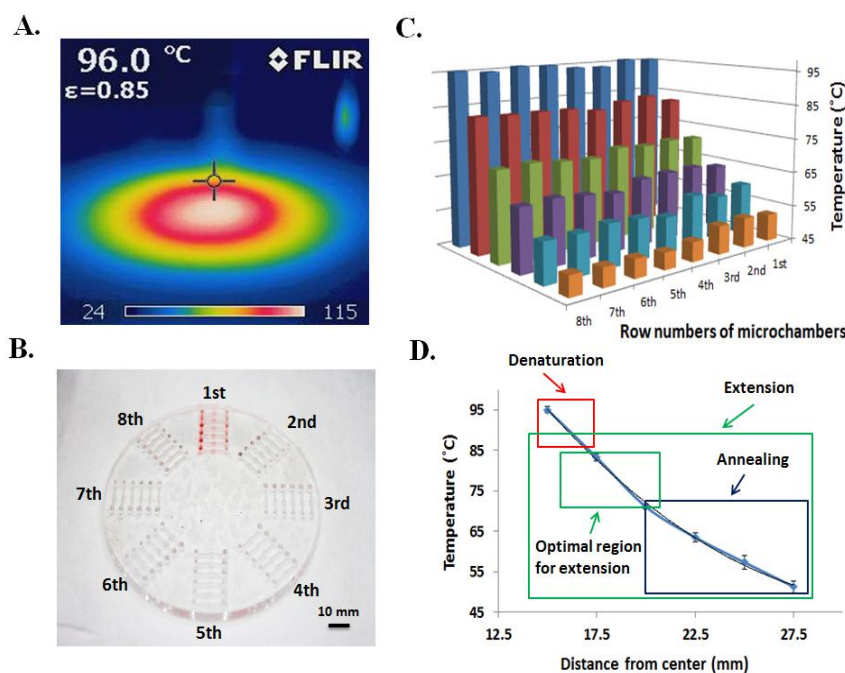


Figure 2: Temperature measurement (TM) microchip and temperature distribution. A) Thermal image of the TM microchip taken with an IR-camera while heating. B) Photograph of the TM microchip. C) 3D bar graph showing the time-averaged temperature (i.e., steady state) in each chamber of the TM microchip. D) Plot showing the distance from the center of the microchip versus the temperature, defining the functional zones for each of the three steps required for PCR.

device passivation and heat transfer studies between the microdevice and flowing PCR mixtures. Importantly, this simple approach could be used for high-throughput, segmented-flow applications such as digital, droplet PCR and/or single cell analysis.

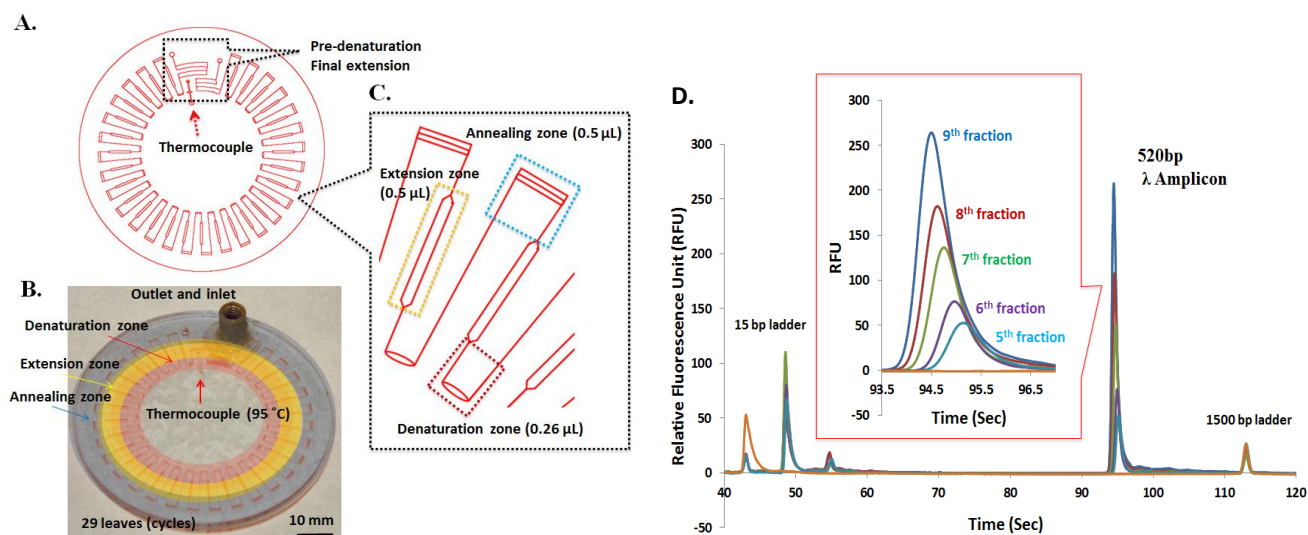


Figure 3: Configuration of and results obtained from the cfIR-PCR microchip A) Schematic of the cfIR-PCR microchip showing the loops for thermal cycling. B) Photograph of the cfIR-PCR microchip highlighting the location of the denature (red), extension (yellow), and anneal (blue) zones. Channels are filled with red dye for contrast. C) Close-up view of two of the 29 loops, corresponding to PCR cycles. D) Overlaid electropherograms of λ -phage PCR product, amplified in the cfIR-PCR microdevice. The first four fractions collected did not contain detectable product.

REFERENCES

1. Kopp, M.U., et al., *Chemical amplification: Continuous-flow PCR on a chip*. Science, 1998. **280**(5366): p. 1046-1048.
2. Hashimoto, M., et al., *Rapid PCR in a continuous flow device*. Lab Chip, 2004. **4**(6): p. 638-45.
3. Crews, N., et al., *Continuous-flow thermal gradient PCR*. Biomed Microdevices, 2008. **10**(2): p. 187-95.
4. Giordano, B.C., et al., *Polymerase chain reaction in polymeric microchips: DNA amplification in less than 240 seconds*. Analytical Biochemistry, 2001. **291**(1): p. 124-132.
5. Easley, C.J., et al., *A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability*. Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(51): p. 19272-19277.
6. Lounsbury, J.A., et al., *A multi-chamber PMMA microdevice for simultaneous amplification of up to seven individual samples using infrared-mediated PCR*. Proceedings – μ TAS 2011: The 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences 2011, Seattle, WA; p. 750-752.

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