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High-Throughput Polymerase Chain Reaction in Parallel Circular Loops Using Magnetic Actuation

Yi Sun,[†] Nam-Trung Nguyen,[‡] and Yien Chian Kwok*,[†]

National Institute of Education, Nanyang Technological University, 01 Nanyang Walk, Singapore 637616, and School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798

We report here a novel multichannel closed-loop magnetically actuated microchip for high-throughput polymerase chain reaction (PCR). This is achieved by designing a series of concentric circular channels on one microchip and exploiting a magnetic force to drive DNA samples flowing continuously through the closed loops. The magnetic force arises from an external permanent magnet through ferrofluid plugs inside the microchannels. The magnet enables simultaneous actuation of DNA samples in all the channels. As the samples go around the loops, they pass through three preset temperature zones. Parameters of PCR, such as incubation time, temperatures, and number of cycles, can be fully controlled and adjusted. High reproducibility was achieved for different channels in the same run and for the same channels in consecutive runs. Genetically modified organisms (GMOs) were amplified simultaneously using the developed device. This simple, reliable, and high-throughput PCR microchip would find wide applications in forensic, clinical, and biological fields.

In the past few years, much attention has been paid to the development of miniaturized PCR devices.[1–3] Two basic types of PCR devices have been reported, namely microchamber PCR devices and continuous flow (CF) PCR devices.[4] In the former, thermal cycles of the PCR are realized by heating and cooling the PCR mixture in a reaction chamber with a characteristic size at the microscale;[5] while in the latter, the PCR mixture continuously flow through a serpentine microchannel integrated with three thermally isolated reaction zones.[6] The two approaches were actively investigated and optimized during the last years. However, with respect to high-throughput massive parallelization, both methods are struggling with unfavorable factors.[7] The use of multichamber microthermocyclers can increase the throughput significantly, but the drawbacks are high fabrication costs, inhomogeneous temperature fields between chambers, and risk of cross-contamination.[8,9] In continuous-flow systems, parallelization is not easily realized as it increases the chip footprint and most likely one pump is required for each microchannel. Multiple analyses can only be performed in a serial fashion, and substantial efforts are necessary to avoid cross-contamination.[10]

To facilitate the widespread use of microfluidic PCR technology in a variety of fields, a reliable and straightforward protocol suitable for parallelization is urgently needed. In this paper, we present a novel parallel PCR approach by arranging several concentric circular microchannels on a single microchip and exploiting a magnetic force as the pumping mechanism. We describe here the design, the fabrication, and the amplification results of this parallel PCR microchip. The microchip combines the cycling flexibility of the stationary chamber PCR and the quick temperature transitions associated with the continuous-flow PCR. Most importantly, the small footprint and potential simultaneous pumping makes it the right candidate for multichannel PCR analysis.

The system consists of a multichannel PCR microchip, a temperature control system, and an external permanent magnet (Figure 1a). The parallel PCR microchip has a very simple structure, which consists of four concentric circular microchannels for PCR reaction and four pairs of straight channels tangent to the loops for sample injection and collection. To avoid cross-contamination, a three-layer lamination concept was implemented where concentric circles and inlet/outlet channels were located on separate layers connected via holes on the middle layer (Figure 1b). The three layers were fabricated in the polymethyl methacrylate (PMMA) substrate by laser ablation and were then bonded together by a low-pressure, high-temperature thermal bonding technique under a pressure of 20 kPa at 165 °C.[11] The outer dimension of the microchip was 30 mm × 30 mm (Figure 1c). The four concentric loops are 900 μm wide and 400 μm deep, with diameters ranging from 15 to 20 mm, corresponding to linear

* Corresponding author. Phone: +65-6790-3836. Fax: +65-6896-9414. E-mail: yienchian.kwok@nie.edu.sg.

† National Institute of Education.

‡ School of Mechanical and Aerospace Engineering.

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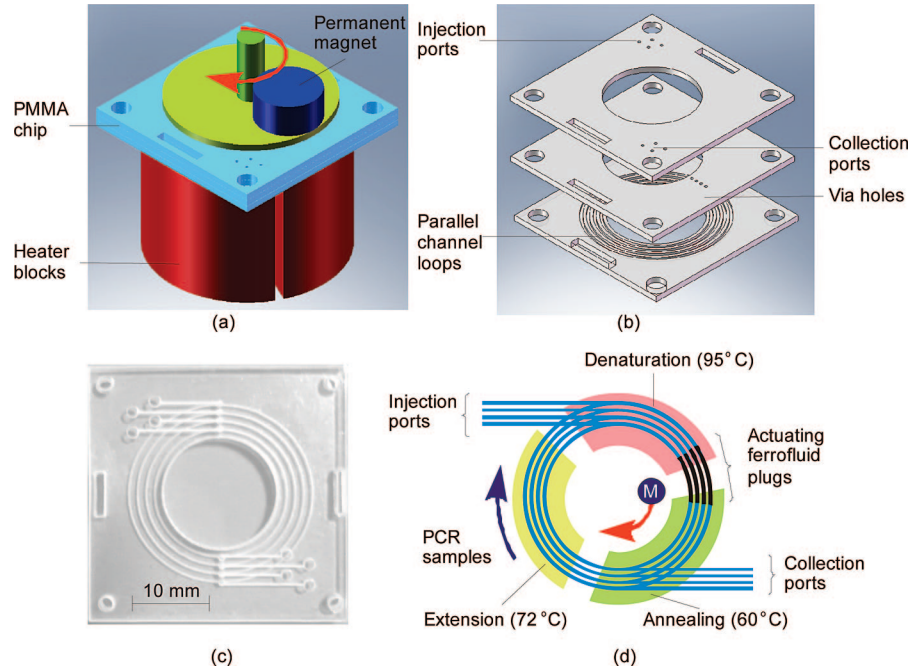

Figure 1. (a) Schematic of the PCR system. (b) Three-layer lamination concept. The first layer has four pairs of straight inlet/outlet channels; the middle layer has eight small via holes; and the third layer has four concentric loops. The layers were fabricated separately in PMMA and thermally bonded together. (c) Photograph of the fabricated microchip. The outer dimension was 30 mm × 30 mm. The four concentric circles are 900 μm wide and 400 μm deep, with diameters ranging from 15 to 20 mm. (d) The microchip was placed on top of three heating blocks. Samples as well as ferrofluid plugs were injected into the different loops. When exposed to the moving magnetic field, the ferrofluid plugs are drawn around the microchannels and continuously propel the PCR samples through the three temperature zones.

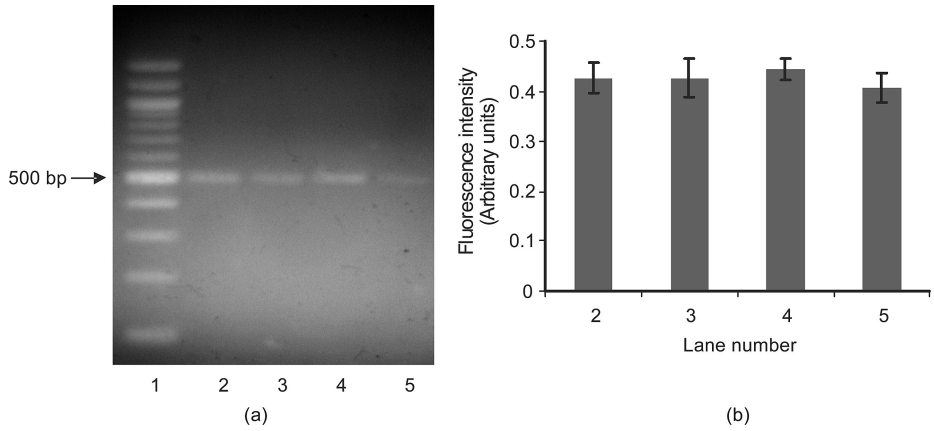

Figure 2. (a) UV image of PCR products from different channel loops. Lane 1, 100 bp DNA ladder; lanes 2−5, 500 bp PCR products from the outermost loop (lane 2) to the innermost loop (lane 5). PCR was performed at velocity of 0.23 rad/s, and 25 cycles were completed within 13 min. (b) Band intensities normalized with respect to the 500 bp fragment of the 100 bp ladder. Triplicate amplifications were done to test the reproducibility. The standard deviations between the four channels in the same run, and that between three consecutive runs in the same channels were 2% and 4%, respectively.

lengths ranging from 47 to 63 mm. The microchip was placed on top of three heating blocks. Circularly arranged copper blocks separated by small air gaps ensured the formation of three distinct and steady temperature zones (Figure 1d). The circular arrangement of the three zones allows the real realization of the sequence of denaturation, annealing, and extension instead of denaturation, extension, and annealing in most CF PCR devices, effectively avoiding the possible formation of the DNA double strands.

Multiple PCR reaction mixtures were loaded into different circular loops together with small oil-based ferrofluid plugs (Figure 1d). Ferrofluid is a stable colloidal suspension of subdomain magnetic particles in a carrier fluid which can be moved through the microchannels and adopt any geometry. The carrier liquid is synthetic ester oil, which is immiscible with the aqueous PCR reaction mixture. When exposed to a gradient field, the whole plug responds as a homogeneous magnetic liquid, moving to the region of the highest flux. The forces acting on the magnetic fluid are proportional to the gradient of the external field and the magnetization value of the fluid; as a result, this actuation concept can be miniaturized due to the possible high magnetic field gradient in microscale. Ferrofluid plugs will follow the path of the moving magnetic field as long as the net force generated by the magnetic field acting on the ferrofluid is greater than the force imposed by the pressure gradient. All these features allow the use of ferrofluid plugs to drive the PCR reaction mixtures in closed-loop microchannels. The translating magnetic field here

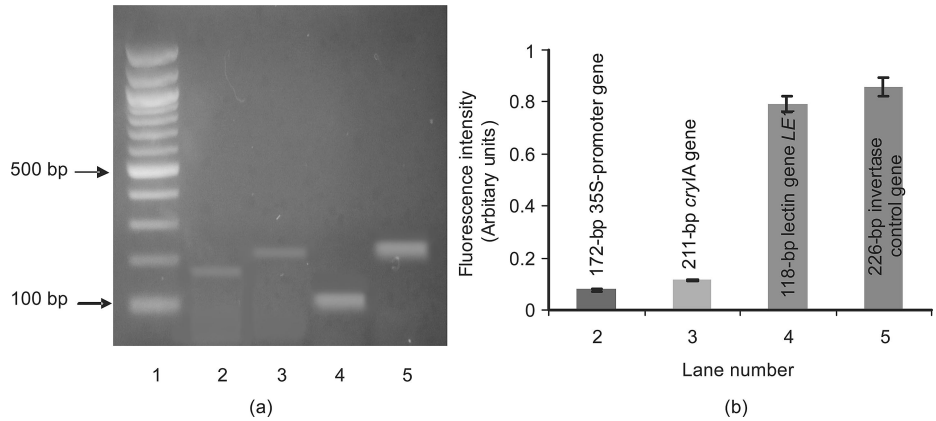

Figure 3. (a) UV image of amplified soybean and maize samples. The magnet speed was kept constant at 0.23 rad/s, resulting in a cycle time of 27 s. Triplicate amplifications were done with relative standard derivation less than 4%. Lane 1: 100 bp DNA ladder. Lanes 2−5: PCR products from the outermost loop (lane 2) to the innermost loop (lane 5). Lane 2: 172-bp 35S-promoter gene for genetic modified soybeans. Lane 3: 211-bp *cryI*A gene for transgenic maize samples. Lane 4: 118-bp lectin gene *LE*1, detectable in transgenic as well as in conventional soybeans. Lane 5: 226-bp invertase control gene, detectable in transgenic as well as in conventional maize. (b) Corresponding band intensities normalized with respect to the 500 bp fragment of the 100 bp ladder.

was induced by a cylindrical neodymium magnet (10 mm in diameter, 5 mm in height, coercitive field $H_c = 900$ kA/m and magnetic remanence $B_T = 1200$ mT). The magnet was attached to the rotor of a small stepper motor and was mounted on top of the microchip (Figure 1a). The oil-based ferrofluid (APG S10n, Ferrotec, CA) used in our experiment has a viscosity of $\eta = 0.406$ kg/m s. The calculated maximum allowable velocity of ferrofluid in the microchannels was 7 mm/s, corresponding to an angular velocity of 0.7 rad/s.

When the ferrofluid plugs were drawn around the microchannels, PCR samples were propelled to flow through the three temperature zones continuously. A full PCR cycle was completed when the magnet turned one round. The total cycle number was determined by the number of rounds the magnet has completed. The velocities of the magnet ranging from 0.14 to 0.7 rad/s result in cycle times ranging from 8 to 45 s. Thus, in this parallel PCR system, the parameters such as extension time, temperatures, and number of cycles can be fully controlled and adjusted for a specific template−primer combination. Since the magnet covers a width up to 10 mm, synchronized actuation of the ferrofluid plugs inside all the microchannels could be easily attained. Though the four loops had different diameters, samples inside the different loops had the same angular velocity and, consequently, the same residence time at each temperature zone. Therefore, the difference in diameter only resulted in slight variations in PCR product volume and had no influence on the PCR efficiency.

Initial experiments with the device demonstrated the proof of principle. We verified the concept of parallel magnetic actuation by amplifying 500 bp fragments of bacteriophage lambda DNA. PCR reagents were described before.[12] The amplified samples were analyzed using a gel electrophoresis setup (Sigma Chemical Co, MO). Parallel PCR was performed at a velocity of 0.23 rad/s, and 25 cycles were completed within 13 min. Subsequent to each run, the microchip was washed by DI water, and a negative control of PCR was done to make sure that there was no carryover. The simplicity of the system should make it possible to use the chip as a disposable, thus the washing step is not necessary. Triplicate amplifications were done to test the reproducibility. The same volume of each PCR product was taken for gel electrophoresis. Fragments of 500 bp were successfully detected from all of the four microchannels (Figure 2a). The standard deviations between the four channels in the same run and that between three consecutive runs in the same channels were 2% and 4%, respectively (Figure 2b). This demonstrates that the closed-loop ferrofluid-driven microchip is a reliable tool for efficient PCR and provides parallel amplification in a highly miniaturized format.

We also utilized the device to amplify genetically modified organism (GMO) samples. The use of GMOs as food and in food products is becoming more and more widespread. PCR is widely used for detection of GMOs in food products in order to verify the compliance with labeling requirements.[13] The multichannel PCR microchip was employed to amplify specific soya and maize sequences from plant DNAs. Freeze-dried powders with 5% GMO/non-GMO (w/w) Roundup Ready soybeans and Bt-176 maize materials were purchased from Sigma (MO). DNA was extracted from 100 mg of each sample using Wizard genetic DNA purification kit (Promega, WI). Four genes, the 172-bp 35S-promoter sequence that is specific for the detection of genetic modifications in soybeans, the soybean control 118-bp lectin gene *LE*1 that is detectable in both transgenic and conventional soybeans, the 211-bp *cryI*A gene specific for the transgenic maize, and the 226-bp invertase maize control gene were amplified simultaneously in the four different channels of the PCR microchip. The details of the primers were described elsewhere.[14] The magnet speed was kept constant at 0.23 rad/s, and 25 cycles were completed within 13 min. The time was greatly reduced compared to almost 2 h when using the conventional thermal cycler.

The four different fragments were simultaneously amplified (Figure 3a). As each loop was isolated in the same run, the products with different lengths were clearly distinguished in the gel from each other and no amplification attributed to cross-contaminations was detected. As the 172 bp and 211 bp amplicons appeared only in the 5% transgenic soybean and maize samples,

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fluorescent intensities of these two bands were much less than those for the 118 bp and 226 bp amplicons that were detectable in both transgenic and conventional samples (Figure 3b). The results show that PCR products obtained by our parallel ferrofluid-driven PCR microchip faithfully reflected the initial number of genome copies.

In conclusion, we demonstrated a parallel close-loop magnetically actuated microchip for high-throughput PCR reaction. Multiple loops were easily fabricated by the three-layer lamination technique, and the risk of cross-contamination was completely eliminated. Simultaneous actuation of PCR mixtures was realized by a single button-sized external magnet through ferrofluid plugs inside the microchannels, so that multiple sample handling became much simpler. As the cycle number and cycle time in this system are fully adjustable by controlling the magnet, optimum PCR conditions can be chosen for various DNA fragments of different concentrations. High reproducibility for different channels in the same run and for the same channels in consecutive runs show that this method is a reliable scheme for high throughput analysis with fast processing speed. Containing the samples in closed loops greatly reduces the footprint of the microchip when compared to other CF PCR devices. By placement of the loops closer to each other, more loops can be integrated to amplify even more samples simultaneously. The small footprint, easy liquid handling, and absence of cross-contamination would enable the microchip to be well suited for forensic, clinical, and biotechnological applications.

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