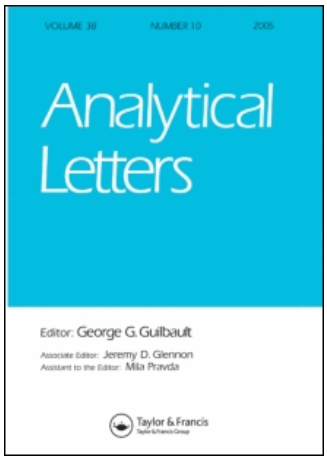

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Continuous-Flow PCR Microfluidics for Rapid DNA Amplification Using Thin Film Heater with Low Thermal Mass

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Abstract: We have described a compact capillary-based continuous-flow polymerase chain reaction (PCR) microfluidics device, which uses flexible thin film heaters with low thermal mass to construct three isothermal zones. Due to the decreased thermal mass of flexible thin film heater, the low power supply and rapid thermal response was obtained. The energy consumption of a 33-cycle continuous-flow PCR was less than 0.0088 kW h, which is much lower than that of the metal block or liquid bath based capillary continuous-flow PCR microfluidics. Special attention was also paid on the surface passivation of the capillary inner surface based on the competing bovine serum albumin (BSA), and the results showed the effect of dynamic passivation was superior to that of static passivation. With the help of the dynamic passivation, the 249 bp human β-actin gene fragment was amplified in 15 min, which is several times faster than that of the conventional PCR machine. In addition, the effect of initial DNA template concentrations on continuous-flow PCR was also investigated.

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The concentration limit of DNA template reached 0.18 ng μl^{-1}, which can satisfy the requirements from different application fields.

Keywords: Continuous-flow, polymerase chain reaction (PCR), microfluidics, DNA amplification, thin film heater

INTRODUCTION

The polymerase chain reaction (PCR) (Saiki et al. 1985) has become an important tool of nucleic acid amplification. It enables us to amplify and detect small amount of target DNA that is from all kinds of species. Nowadays, however, most of PCR amplifications are performed on a conventional PCR machine and the PCR system possesses larger volume and thermal mass, leading to a slower PCR amplification.

The way of increasing the PCR speed depends on the development of the PCR devices that can perform the quick temperature transition and heat exchange between the device and the PCR sample. Such a PCR device should not only heat the PCR solution rapidly, but also dissipate rapidly the heat to the environment. Recently, great attention has been paid on the development of the PCR microfluidics. The PCR microfluidics can be divided into two types: micro-chamber PCR and continuous-flow PCR. Northrup et al. first proposed the chip-based micro-chamber PCR in 1993 (Northrup et al. 1993). Since then, it has been replicated by many research groups (Zhang et al. 2006a), which differed with respect to the size, the amplification time, and the integration with other analytical units. The micro-chamber PCR is a miniaturized version of conventional PCR. It allows small volumes of PCR solution ranging from nanoliter to microliter, which are temperature cycled rapidly. In addition, it offers the expected integration with other analytical steps on a single format (Zhang et al. 2006a). However, the size of the micro-chamber specifies the limit on the sample volume. The amplification time and the power consumption for the microchamber PCR are mainly dependent on the thermal mass of the given PCR system. Thus it lacks the flexible adjustment for the thermal cycling rate. For a faster microchamber PCR, the precise optimization of the reagents and temperature cycling profile should be performed. Moreover, the PCR solution evaporation becomes an important issue when the chamber volume is decreased.

The continuous-flow PCR is an alternative approach to perform PCR and it was first proposed by Nakano et al. in 1994. In principle, the continuous-flow PCR is realized by a time-space conversion concept. In 1998, the first chip-based continuous-flow PCR was demonstrated (Kopp et al. 1998). Now, the continuous-flow PCR is mainly classified into two categories based on the configurations: chip-based continuous-flow and capillary-based continuous-flow. Many groups (Zhang et al. 2006a) performed studies on the former flow, where the micro-channel is etched in silicon, glass, quartz,

ceramic, or polymer chip by microfabricating technology. The etched chip is then bonded with another cover chip such as silicon, glass, quartz, or polymer to form the closed PCR system. The chip-type continuous-flow PCR has some obvious advantages including high-integration, small footprint, and good compatibility with other microfluidic chip technologies (Zhang et al. 2006a). However, fabricating the micro-channel structure on silicon, glass, quartz, or polymer chip involves many processes, leading to a long way to reach the practical use of the chip-based continuous-flow PCR. In addition, the cycle number of serpentine or spiral micro-channel is fixed once the chip design is finalized.

The capillary-based continuous-flow PCR is another important approach of performing the continuous-flow PCR (Chiou et al. 2001; Curcio and Roeraade 2003; Park et al. 2003; Dorfman et al. 2005; Zhang et al. 2006b). Among them, the inner channel of the capillary tube is utilized for the reaction system for continuous-flow PCR. Compared to the chip-based continuous-flow PCR, the benefits are summarized as follows: (1) The construction of the PCR device is simple and it only involves the wind-up of a capillary tube around the three temperature zones for the PCR amplification. (2) The cycle number can be changed. The number of revolutions that a capillary is crossing the three temperature zones in a loop ranges from 20 to 50, depending on the required PCR cycles. (3) The capillary of different inner diameters in a given thermal cycler can be used to meet the different demands such as the single molecular detection. Most importantly, in capillary-based continuous-flow PCR, the arrangement of three temperature zones is circular in the sequence of denaturation, annealing, and extension, with which the advantage associated is that the melted sample won't be subject to the extension temperature zone before reaching the annealing temperature zone, which can effectively avoid the likely formation of melted single-stranded DNA fragments into double-stranded DNA with template strands or their complementary strands (Park et al. 2003).

However, in the reported previously capillary-based continuous-flow PCR microfluidics, the heating elements with high thermal mass were used to maintain the three thermostable zones. Therefore, this PCR microfluidics consumes more energy, making them difficult to become portable. Curcio and Roeraade have used three thermostable water baths as the three temperature zones required for continuous-flow PCR amplification (Curcio and Roeraade 2003), where the magnetic agitation was required to keep the temperature homogeneous and water at high temperature might easily suffer fast evaporation, leading to the increased energy consumption. Importantly, the water bathes with high thermal mass will also cause energy consumption to increase when heated and required to maintain the temperature constant. Therefore, it would be quite unfeasible to realize a portable continuous-flow PCR device by using this water bath thermocycler. In order to overcome this shortcoming, several continuous-flow PCR microfluidics based on bulky metal blocks such as copper (Park et al. 2003; Dorfman et al. 2005;

Zhang et al. 2006b) or aluminum (Chiou et al. 2001) has been developed. Although these metal blocks can well define the temperature zones for continuous-flow PCR and can provide better temperature uniformity, as they possess the higher thermal mass and thus consume more energy when heated and maintained the temperature homogeneous.

In this work, we have developed a compact capillary-based continuous-flow PCR microfluidics, in which the three temperature zones are constructed by using the flexible thin film heaters with low thermal mass, thus resulting in the decreased energy consumption. These thin film heaters are evenly arranged on an insulating plastic core in an order of denaturation, annealing, and extension so as to form a thermal cycler with cylindrical structure. The presented PCR microfluidics can readily perform the continuous-flow PCR amplification by winding up a capillary around the cylindrical thermal cycler, therefore a series of biochemical performances on the presented continuous-flow PCR were evaluated experimentally.

EXPERIMENTAL

Chemical Reagents and Materials

Chemical reagents for PCR amplification were purchased from Tiangen Biotech Co., Ltd. (Beijing, China), and they include ten times Taq buffer (200 mM KCl, 200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$), thermostableTaq DNA polymerase (5 U μl^{-1}), deoxynucleotide triphosphates (dNTPs) (2.5 mM each of dATP, dGTP, dCTP, and dTTP), and double-deionized (dd) H_2O. The DNA markers, which consist of 100-, 250-, 500-, 750-, 1000-, and 2000-bp DNA fragments, were also from Tiangen Biotech Co., Ltd. The primer pair for 249 bp human β-actin gene fragment was ordered from Invitrogen (Shanghai): 5′-aaggc caacc gcgag aagat-3′ (upstream)/5′-tcggt gagga tcttc atgag-3′ (downstream). Target genomic DNA was rapidly extracted from blood samples from a patient volunteer in the presence of BioGene-Expuze^{TM} DNA Extration kit (Texas (Beijing) BioGene Inc.), whose concentrations were determined by the spectrophotometer (Ultrospecw 2100 pro, Amersham Pharmacia Biotech, Piscataway, NJ, USA) (15 ng μl^{-1}). Bovine serum albumin (BSA) (Fraction V, Purity $\geq$98%, Biotechnology Grade) was purchased from Roche (Guangzhou, China). GoldView^{TM} dye was obtained from SBS Genetech Co., Ltd. (Beijing, China). Agarose was from Biowest (Shanghai, China). A five times TBE buffer was prepared by dissolving 54 g Tris, 27.5 g boric acid, and 20 ml 0.5M EDTA (pH 8.0) in dd H_2O.

Polytetrafluoroethylene (PTFE) capillary (500 μm IDand 900 μm OD) was purchased from Wuxi Xiangjian Tetrafluoroethylene Product Co., Ltd. (Wuxi, China) Polyimide insulated flexible thin film heaters (7.5 cm $\times$ 2.5 cm $\times$ 0.2 mm, resistance 20 $\pm$ 0.5 Ω) were ordered by Beijing Hongyu Space Technology Corporation (Beijing, China). Double-sided

adhesive tape (100 μm thick), which can withstand temperatures of up to 260°C, was obtained from 3M (Guangzhou, China). K-type thermocouples (unsheathed, with a diameter of 0.002 inch) were from Omega (Singapore). Disposable 1 ml syringes were from Jiangxi Lule Medical Instrument Co., Ltd. (Guangzhou, China). Two-channel PC-controlled precision syringe pump (model TS2-60) was bought from Baoding Longer Precision Pump Co., Ltd.

Construction of Continuous-Flow PCR Microthermocycler

Figure 1A shows the schematic diagram of the continuous-flow PCR set-up, which consists of a cylindrical thermally insulating PTFE plastic core, three completely identical microheaters used for the denaturation, annealing, and extension regions, and a PTFE capillary. The three microheaters are evenly adhered onto the surface of the plastic core using double-sided adhesive tapes, leading to the approximately cylindrical surface of the PCR set-up (34.4 mm in diameter and 75 mm in height). The detailed schematic diagram of the microheater is shown in Fig. 1B. A micro-heater was constructed by holding two pieces of thin brass sheet, polyimide insulated thin film heater and plastic insulating tape together with three pieces of double-sided adhesive tapes. The brass sheet above the thin film heater is used to improve the temperature uniformity on the polyimide surface of the thin film heater, while the brass sheet below the thin heater can prevent the thin heater from destruction when peeled off from the double-sided tape so as to be reused. A PTFE capillary was readily wound up around the three microheaters from the beginning of the denaturation region with the help of the marker line (not shown in Fig. 1A) made on the inlet place of the capillary. It is noted that by this way the capillary can repeatedly pass through the three temperature regions according to the PCR cycle numbers to be required. In addition, one could select the capillary with different inner diameters to accommodate

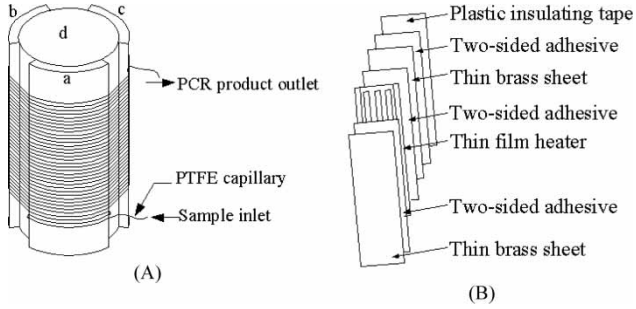

Figure 1. (A) Schematic diagram of the continuous-flow PCR device: the thin film heaters (a), (b), and (c) for denaturation (94°C), annealing (54°C), and extension (72°C), respectively, and (d) the thermally insulated plastic core. (B) Schematic diagram of laboratory-made micro-heater using flexible thin film heater.

different volume of continuous-flow PCR system. The length of the capillary wound on each temperature region is proportional to the desired time ratio of the three PCR steps. In order to control the temperatures necessary for continuous-flow PCR, a miniature bare K-type thermocouple is adhered onto the surface of each microheater to measure the temperature, and a proportional-integral-derivative (PID) controller (model CD901FK02-M*AN-NN, RKC Instrument Inc., Japan) is used to control the temperature profile necessary. The temperature distribution on the surface of each microheater is real-time collected and recorded with Data Acquisition/Switch Unit (model 34970A, Agilent Technologies Co. Ltd., Singapore), which is connected to a computer with a standard RS-232 serial port.

Continuous-Flow PCR Amplification and Positive-Control PCR Amplification

The 4.5 m PTFE capillary was passed 33 times through the three temperature regions, leading to 33 PCR cycles. The inlet length of the capillary was about 0.5 m and the outlet length 0.3 m. The capillary length per circle was about 108 mm, and the capillary length for denaturation, annealing and extension is 25, 25, and 25 mm, respectively, thus the capillary length between two micro-heaters next to each other is 11 mm, causing the 11 mm air gap long enough to avoid the thermal cross-talk between the temperature zones. To ensure the initial denaturation and the final extension complete, the 75 mm denaturation step for the first cycle and the 75 mm extension step for the last cycle were performed by capillary elongation on the corresponding temperature regions (Fig. 1A).

The 25 μl PCR mixture used in the continuous-flow PCR amplification consists of 20 mM Tris-HCl (pH 8.4), 20 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, the primer pair (0.5 μM each), $0.018 \sim 9$ ng μl^{-1} human genomic DNA as the PCR template (except for the negative control and Taq DNA polymerase adsorption experiments), $0.025 \sim 0.3$ U μl^{-1} Taq DNA polymerase, BSA of a certain concentration, and corresponding volumes of ddH_2O. The prepared PCR mixture was introduced into the capillary from the inlet and then the filled air in the 1 ml disposable syringe was driven by the precision syringe pump so as to compel the PCR mixture to flow continuously through the capillary micro-channel at a certain flow rate. Meanwhile, a 0.2 mL thin-wall polypropylene tube was utilized to collect the amplified PCR products for further analysis. For all of the continuous-flow PCRs, the temperature for each temperature region was set as follows: denaturation at 94°C, annealing at 54°C, and extension at 72°C.

Positive-control PCR was performed on a commercial PCR machine (model iCyler, Bio-Rad, California, USA) using the corresponding PCR mixture. PCR conditions were set as follows: 5 min at 94°C for initial denaturation, subsequent 33 cycles with 30 s at 94°C, 45 s at 54°C, and 45 s at 72°C,

5 min for final extension. The program was concluded by cooling the PCR products to 4°C. The time of completing 33 PCR cycles was about 120 min.

Capillary Inner Surface Passivation

In order to decrease the adsorption of bio-molecules, especially Taq DNA polymerase and DNA template, onto the inner surface of the PTFE capillary, two surface passivation techniques, namely static and dynamic passivation, have been applied in the continuous-flow PCR microfluidics. For static surface inactivation, a 50-μl 1 × PCR buffer containing BSA ranging from 0 to 0.4% (w/v) in concentration was driven to flow through the capillary microchannel at a rate of 4.24 mm s^{-1} before starting a real PCR chemistry. For dynamic surface passivation, BSA was added to the PCR mixture during the practical operation of PCR microfluidics, whose concentrations in the PCR mixture ranged from 0 to 0.075% (w/v), and then the corresponding 25 μl PCR mixture containing BSA was driven through the capillary microchannel and the continuous-flow PCR amplification was carried out.

PCR at Various Flow Velocities

In order to test the ability of rapid continuous-flow PCR, PCR of the 249 bp human β-actin gene fragments was performed when the 25 μl PCR solution flowed at different flow rates through the capillary. The flow rates were well controlled by the precision syringe pump, ranging from 1.5 to 7.5 mm s^{-1}. Corresponding positive-control PCR was also carried out in a commercial PCR machine using the same PCR mixture, while corresponding negative-control experiment (PCR mixture with no DNA template) was accomplished in the continuous-flow PCR microfluidics at a flow rate of 2.0 mm s^{-1}.

PCR at Various Initial Concentrations of DNA Template

In some applications, it may be crucial to acquire a detectable amount of PCR products from a low concentration DNA template sample. Therefore, a study was performed to establish the minimal detectable concentration of 249 bp human β-actin gene templates when amplified through 33 cycles in the case of 2.0 mm s^{-1} flow rate. The concentrations of DNA template varied from 0.018 to 9.0 ng μl^{-1}, with 0.025% (w/v) BSA included in the PCR mixture as an effective dynamic passivation of capillary inner surface.

Analysis of PCR Products

The 2.5% (w/v) agarose gel electrophoresis using GoldView™ as fluorescence dye was used to analyze the PCR products. GoldView™ is a kind

of new-style nucleic acid dye with the promise of replacing ethidium bromide (EtBr) as a commonly used dye because it has not been found to have any cancer risk up to now. Thin agarose gels (<5 mm thickness) were prepared in order to obtain the high detection sensitivity. 8 μl of each PCR product was loaded onto the loading holes of agarose gels in a gel chamber (MINI-SUB® CELL GT, BIO-RAD) and then electrophoresed in 0.5 × TBE buffer with a 8 V/cm electric field supplied by the electrophoresis power supply (EPS 601). After electrophoresis of about 45 min, the gels were visualized on a gel documentation and analysis system (model Gene Genius). The DNA markers were used as standards for the evaluation of the gels.

RESULTS AND DISCUSSION

Performance of Temperature Control for Continuous-Flow PCR

PCR is a typical temperature-controlled and enzyme-catalyzed biochemical reaction system. Since PCR is temperature-sensitive, a minute temperature difference will significantly affect the PCR efficiency, especially in PCR microfluidics. Therefore, it is important to accurately evaluate the performance of temperature control before the practical PCR. In the presented PCR microfluidics, in order to improve the temperature uniformity on the surface of the thin brass wafer and to increase the thermal contacting area between the capillary outer-surface and the thin brass wafer, a thin layer of thermally conductive oil (Sigma M5904, Guangzhou, China) was covered on the surface of the thin brass wafer at the cost of the extra energy consumption. In addition, for success of PCR amplification, the temperature in each temperature zone should be maintained at a constant level. The surface temperature on the brass wafer was measured with a miniature K-type thermocouple, and the measured temperature (T_{measure}) was compared with the set temperature that was shown on the display of the temperature controller (T_{set}). T_{measure} and T_{set} were well correlated with an R^2 value of 0.9995 and the standard derivation was less than 0.5°C.

For PCR microfluidics, energy consumption is becoming an important issue as the device size decreases. In our presented PCR microfluidics, the thin film heaters with low thermal mass were utilized to heat the three temperature zones. When the voltage of 6–10 V was applied onto the thin film heaters, the temperature in each zone was well maintained. The rate of temperature ramping is sensitive to the applied voltage. The heating rate is slow when the applied voltage is low, and the heating rate is fast when the high voltage is applied. Figure 2 showed the temperature changes on the brass surface and constant-temperature controls during the heating process with the input voltage of 7.5 V in the denaturation region. As seen from Fig. 2, it took about 10 min to heat the denaturation zone from room temperature (about 26°C) to 94°C. If the input voltage was 10 V, only 1–2 min was

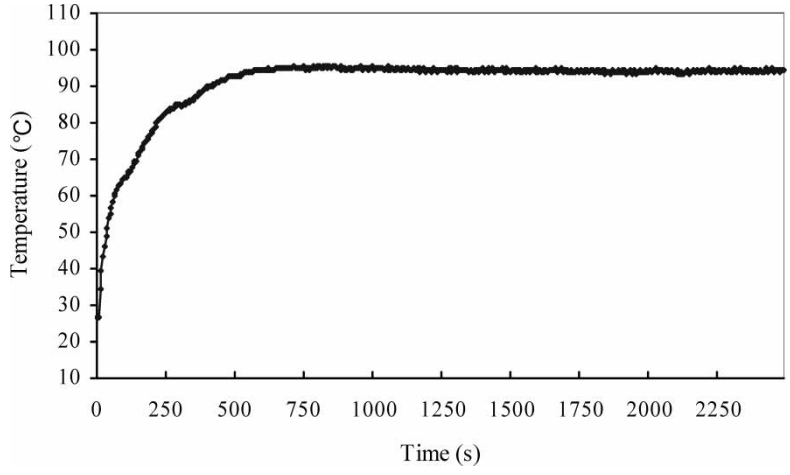

Figure 2. Temperature changes of the surface of thin brass wafer and constant-temperature controls during the heating process with input voltage of ~7.5 V in the denaturation region.

required, but the temperature overshoot was too large to meet the requirements (data not shown). Importantly, the electrical power consumption is small in the presented PCR microfluidics. If we consumed the average time 750 s for heating the three zones to the desired temperatures and the time 3000 s to perform PCR amplification, the energy consumption of a 33-cycle PCR was less than 0.0088 kW h, which is much lower than that of the previously reported capillary continuous-flow PCR microfluidics. We attribute this to the use of the thin film heaters with low thermal mass. Therefore, our presented PCR microfluidics could be integrated into a battery-supplied, portable analysis system.

Surface Passivation of the PTFE Capillary Inner Surface for Continuous-Flow PCR

The SVR (8.0 mm^{-1}) of capillary channel in the presented PCR microfluidics is several times higher than that of commercial PCR reaction tubes (~1.5 mm^{-1}), which will lead to the increased non-specific adsorption of the PCR reactants, especially Taq polymerase onto the capillary inner surface. Sometimes, more polymerase enzymes were used to compensate the surface adsorption as a result of large SVR so as to produce more 249 bp PCR products (Fig. 3). In Fig. 3, the amount of PCR products tended to increase with the concentration of Taq DNA polymerase (0.025~0.30 U μl^{-1} in the 25 μl PCR mixture). However, although the concentration of Taq DNA polymerase was increased up to 0.30 U μl^{-1}, the

amount of PCR product wasn't as enough as expected. In general, the amount of Taq DNA polymerase in the PCR mixture cannot be increased without limit. On one hand, Taq polymerase is the most expensive reagent used in PCR by far. On the other hand, varying its concentration in significant amounts requires the experimental titration of Mg^{2+} ions that, at high concentrations, would affect the reaction pH, thus leading to a cascade of experimental titrations for other PCR reagents (e.g., KCl) (Erill et al. 2003). Therefore, in order to successfully perform PCR in the capillary microchannel in the case of the normal amount of polymerase enzymes, special attention must be paid to the conditions of the capillary inner surface. At the present time, the surface passivation techniques applied in PCR microfluidics are mainly classified into two categories: static passivation and dynamic passivation (Zhang et al. 2006a). Moreover, the competing protein BSA is one of the most frequently used passivation agents (Zhang et al. 2006a). BSA can be used to stabilize the polymerase enzymes and to reduce undesired adsorption of protein onto the inner surfaces of the reaction system due to its competition with Taq polymerase for active adsorption sites on the inner surface. In the present study, we first investigated the static passivation effect of a certain concentration of BSA solution (Fig. 4A). Figure 4A showed that the static passivation of BSA solution with concentrations ranging from 0.05 to 0.4% (w/v) could effectively promote the continuous-flow PCR amplification and that the amount of 249 bp PCR products seemed to increase with the concentration of BAS in 1 × PCR buffer. However, the operation appeared somewhat difficult when the BSA solutions with higher concentrations such as more than 0.8% (w/v) were used to statically passivate the capillary inner

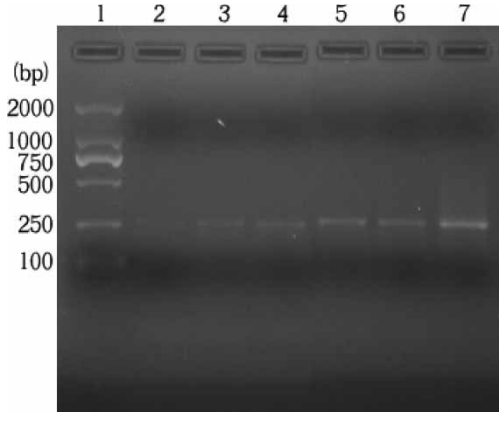

Figure 3. Effect of concentration of Taq DNA polymerase on amounts of continuous-flow PCR products. Lane 1: the DNA markers. Lane 2–7: 0.025, 0.05, 0.10, 0.15, 0.20, and 0.30 U μl^{-1} Taq DNA polymerase in a 25 μl PCR mixture, and the PCR mixture was delivered through the capillary microchannel at a flow rate of 2.0 mm s^{-1} in the case of no passivation measures.

surface at the temperatures required for PCR, and sometimes the capillary micro-channel was even plugged (data not shown). When BSA as a dynamic passivation agent was added into the PCR mixture, ranging from 0.01 to 0.075% (w/v) in concentration, the amount of 249 bp PCR products would obviously increase several times than that without BSA, as shown in Fig. 4B. It should be noted that higher concentrations of BSA in the PCR mixture probably will inhibit the PCR reaction (not within the scope of this study). Although we could not determine the amounts of Taq polymerase that were still adsorbed, we assume that the adsorption problem had been substantially overcome by adding the BSA to the PCR mixture. As seen from Fig. 4A and 4B, the effect of dynamic passivation of the capillary inner surface using BSA may be superior to that of static passivation. In the following experiments, 0.025% (w/v) BSA has always been included in the PCR mixture.

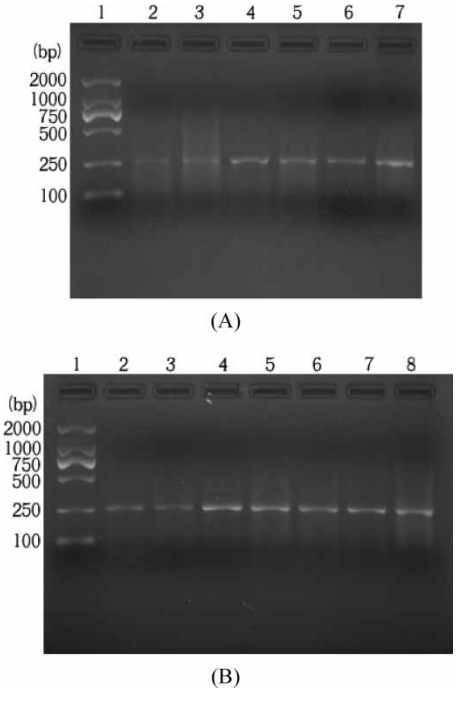

Figure 4. Static passivation (A) and dynamic passivation (B) of the inner surface of PTFE capillary. (A) Lane 1: the DNA markers. Lane 2–6: 0, 0.05, 0.1, 0.2, and 0.4% (w/v) BSA in a 50 μl 1 × PCR, which was driven to flow through the capillary micro-channel at a rate of 50 μl min^{-1} before starting a real PCR chemistry. Lane 7: the positive-control PCR. (B) Lane 1: the DNA markers. Lane 2–7: 0, 0.005, 0.01, 0.025, 0.05, and 0.075% (w/v) BSA in the 25 μl PCR mixture, which flowed through the PCR micro-channel at a flow rate of 2.0 mm s^{-1}. Lane 8: the positive-control PCR.

Effect of Various Flow Rates on Amount of PCR Products

The amount of PCR products in continuous-flow PCR depends no longer on the temperature to time protocol required for most of the commercial PCR machine, but only on a temperature to channel length protocol (Schneegaβ et al. 2001). The flow rate through the micro-channel determines the residence time of a PCR mixture in a special temperature zone. In order to determine the minimal time for the whole amplification process for 249 bp human β-actin gene fragment, the influence of different flow rates on the amplification yield was investigated (Fig. 5). Figure 5 showed that the amount of 249 bp PCR products decreased with the flow rate. We attribute this to the decreased stay of the PCR mixture at 72°C temperature in the extension zone. Since the extension rate of Taq polymerase is 60–100 nucleotides s^{-1} at 72°C in conventional PCR, an extension time of 5 s is considered sufficient for the 249 bp PCR products. Therefore, when the flow rate was 5.0 mm s^{-1} (the corresponding extension time was 5 s) (lane 6 in Fig. 5), the signal intensity of 249 bp PCR products could still be inspected by agarose gel electrophoresis although it was not as strong as those in the case of flow rates of 1.5, 2.0, and 3.5 mm s^{-1}. That is to say, the minimal amplification time of 249 bp human β-actin gene fragments was about 15 min, which is several times faster than that in the commercial PCR machine.

Effect of Initial Concentrations of DNA Template

For some fields such as archaeology and forensic analysis, it may be important to obtain a detectable level of PCR product from small amount of DNA

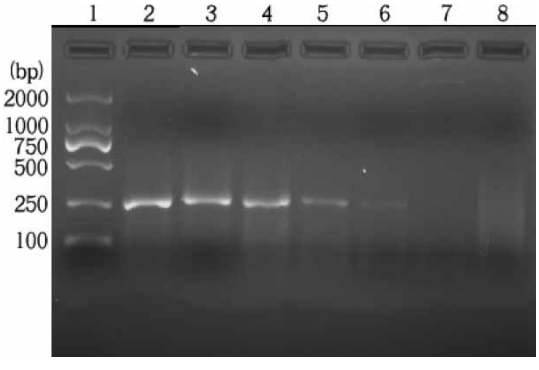

Figure 5. A photograph of agarose gel electrophoresis of PCR products of the 249 bp fragments at various flow rates. Lane 1: the DNA markers. Lane 2: positive-control PCR products obtained from the commercial PCR machine. Lane 3–7: continuous-flow PCR products at various linear flow rates, 1.5, 2.0, 3.5, 5.0, and 7.5 mm s^{-1}, respectively. Lane 8: negative control, PCR mixture with no DNA template run at a linear flow rate of 2.0 mm s^{-1} (as in lane 4).

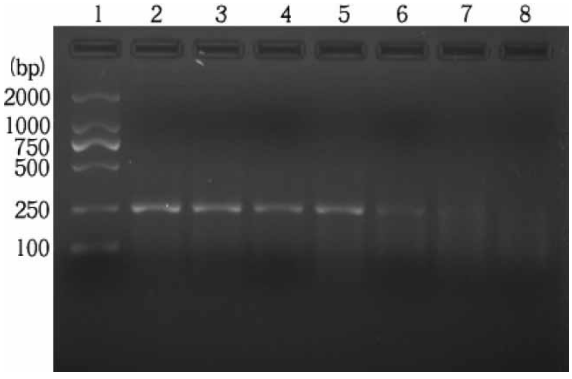

Figure 6. Fluorescence intensity of the PCR products as a function of the input DNa template molecules. A linear flow rate of 2.0 mm s^{-1} was used. Lane 1: the DNA markers. Lane 2–8: continuous-flow PCR products from various concentrations of the input DNA template molecules, 9.0, 3.6, 1.8, 0.9, 0.36, 0.18, and 0.018 ng μl^{-1}, respectively.

template. Therefore, the effect of the amount of input DNA template was studied by performing PCRs in the 25 μl PCR mixture containing DNA template ranging from 9.0 to 0.018 ng μl^{-1}, where continuous-flow PCR was carried out for 33 cycles at a flow rate of 2.0 mm s^{-1} (Fig. 6). As was seen from Fig. 6, the minimal template concentration that could be obviously detected by agarose gel electrophoresis was 0.18 ng μl^{-1}. When the DNA template concentration was decreased to be 0.018 ng μl^{-1}, no visible PCR product band was obtained or at least the amount of PCR product under this concentration condition was smaller than the detection limit of the fluorescence scanner associated with the gel imaging system. In other words, the limit concentration of DNA template used in the present continuous-flow PCR thermocycler was 0.18 ng μl^{-1}. It should be noted, additionally, that nonspecific PCR products due either to mispriming or to primer dimmers were not observed at high concentration of DNA templates such as 9.0 and 3.6 ng μl^{-1} (lanes 2 and 3 in Fig. 6).

CONCLUSIONS

A capillary-based continuous-flow PCR microfluidics based on flexible thin film heaters with low thermal mass has been successfully developed in this work. Due to the use of the thin film heaters, the energy consumption of a 33 continuous-flow PCR cycles was less than 0.0088 kW h, which presented the possibility toward a battery-powered portable continuous-flow PCR system. To our knowledge, it was not reported previously that this type of flexible thin film heater is applied onto the capillary-based

continuous-flow PCR microfluidics. With the help of BSA dynamic passivation, the amplification of 249 bp human β-actin gene fragment was effective and specific, and the amplification time required was only 15 min, which is several times shorter than that in the conventional PCR machine. In addition, the limit concentration of DNA template in the presented continuous-flow PCR microfluidics was 0.18 ng μl^{-1}, which can meet the requirements from different application fields.

REFERENCES

Chiou, J., Matsudaira, P., Sonin, A., and Ehrlich, D. 2001. A closed-cycle capillary polymerase chain reaction machine. *Anal. Chem.*, 73 (9): 2018–2021.

Curcio, M. and Roeraade, J. 2003. Continuous segmented-flow polymerase chain reaction for high-throughput miniaturized DNA amplification. *Anal. Chem.*, 75 (1): 1–7.

Dorfman, K.D., Chabert, M., Codarbox, J.H., Rousseau, G., de Cremoux, P., and Viovy, J.-L. 2005. Contamination-free continuous flow microfluidic polymerase chain reaction for quantitative and clinical applications. *Anal. Chem.*, 77: 3700–3704.

Erill, I., Campoy, S., Erill, N., Barbé, J., and Aguiló, J. 2003. Biochemical analysis and optimization of inhibition and adsorption phenomena in glass-silicon PCR chips. *Sens. Actuators B*, 96: 685–692.

Kopp, M.U., de Mello, A.J., and Manz, A. 1998. Chemical amplification: Continuous-flow PCR on a chip. *Science*, 280: 1046–1048.

Nakano, H., Matsuda, K., Yohda, M., Nagamune, T., Endo, I., and Yamane, T. 1994. High-speed polymerase chain-reaction in constant flow. *Biosci. Biotechnol. Biochem.*, 58: 349–352.

Northrup, M.A., Ching, M.T., White, R.M., and Watson, R.T. 1993. DNA amplification in a microfabricated reaction chamber. In *Tranducer'93*, *Seventh International Conference on Solid State Sensors and Actuators*; Yokohama, Japan, ISBN 4-9900247-2-9, pp. 924–926.

Park, N., Kim, S., and Hahn, J.H. 2003. Cylindrical compact thermal-cycling device for continuous-flow polymerase chain reaction. *Anal. Chem.*, 75 (21): 6029–6033.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, 230: 1350–1354.

Schneegaβ, I., Bräutigam, R., and Köhler, J.M. 2001. Miniaturized flow-through PCR with different template types in a silicon chip thermocycler. *Lab Chip.*, 1: 42–49.

Zhang, C.S., Xu, J.L., Ma, W.L., and Zheng, W.L. 2006a. PCR microfluidic devices for DNA amplification. *Biotechnol. Adv.*, 24 (3): 243–284.

Zhang, C.S., Xu, J.L., Wang, J.Q., and Wang, H.P. 2007. Continuous-flow polymerase chain reaction microfluidics by using spiral capillary channel embedded on copper. *Anal. Lett.*, 40: 497–511.