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Continuous-flow Polymerase Chain Reaction Microfluidics by Using Spiral Capillary Channel Embedded on Copper

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Abstract: This paper presents a novel method for performing polymerase chain reaction (PCR) amplification by using spiral channel fabricated on copper where a transparent polytetrafluoroethylene (PTFE) capillary tube was embedded. The channel with 25 PCR cycles was gradually developed in a spiral manner from inner to outer. The durations of PCR mixture at the denaturation, annealing and extension zones were gradually lengthened at a given flow rate, which may benefit continuous-

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flow PCR amplification as the synthesis ability of the Taq polymerase enzyme usually weakens with PCR time. Successful continuous-flow amplification of DNA fragments has been demonstrated. The PCR products of 249, 500 and 982 bp fragments could be obviously observed when the flow rates of PCR mixture were 7.5, 7.5 and 3.0 mm s^{-1}, respectively, and the required amplification times were about 25, 25, and 62 min, respectively. Besides, the successful segmented-flow PCR of three samples (249, 500 and 982 bp) has also been reported, which demonstrates the present continuous-flow PCR microfluidics can be developed for high-throughput genetic analysis.

Keywords: Continuous-flow, polymerase chain reaction, microfluidics, spiral capillary channel

INTRODUCTION

Since it was first reported in 1986, the polymerase chain reaction (PCR) has become an indispensable tool in life science (Mullis et al. 1986). It can be applied for the exponential amplification of specific target sequences to the levels that are several orders of magnitude higher than the initial starting target molecules. When a PCR reaction is performed, the fast reporting of target sequence requires the ability to rapidly cycle the PCR mixture containing the target sequence between the three temperatures. Rapid reporting of PCR results is particularly important in fields such as the monitoring of related biowarfare agents (Belgrader et al. 2001, 2003) and the diagnosis of serious infectious disease such as SARS-coronavirus (Zhou et al. 2004).

The PCR speed is usually determined by instrument limits. For the conventional PCR device based on the metal block, a complete PCR amplification with 20–40 cycles usually requires 1–3 h. A large fraction of that time is used to heat and cool the PCR mixture due to the need for bringing the large metal block to the cycling equilibrium temperature and to transfer heat to the PCR mixture through the PP tubes. Thus, the PCR cycling rate is determined by the thermal mass of the metal block and the heat transfer through the PP tubes.

Therefore, increasing the PCR cycling speed depends mainly on the development of desirable PCR devices capable of acquiring fast temperature ramp and rapid heat transfer between the heating block and the PCR mixture. At present, focus is on the development of the miniaturized PCR devices. Compared with the conventional PCR devices, they can offer small thermal mass, require small sample volumes and low power consumption, possess the potential for automation, and can be easily integrated with other analytical steps. During the last decade, a number of research groups have investigated the micro-chamber PCR microfluidics, where the PCR mixture is thermally cycled in one or more stationary chambers (Northrup et al. 1993; Wilding et al. 1994; Woolley et al. 1996; Poser et al. 1997; Oda et al. 1998; Daniel et al. 1998; Lagally et al. 2000; Lee et al. 2000, 2003; Giordano et al. 2001; Trau et al. 2002; Yoon et al. 2002; Koh et al. 2003; Liao et al. 2005). From these studies, temperature

ramp rates of 2–10°C s^{-1} (Lee et al. 2000, 2003; Trau et al. 2002), 10–40°C s^{-1} (Woolley et al. 1996; Oda et al. 1998; Giordano et al. 2001; Yoon et al. 2002; Koh et al. 2003; Liao et al. 2005) or even 60–90°C s^{-1} (Poser et al. 1997; Daniel et al. 1998) for heating, and of 2–10°C s^{-1} (Woolley et al. 1996; Lee et al. 2000, 2003; Giordano et al. 2001; Trau et al. 2002; Koh et al. 2003; Liao et al. 2005), 20–40°C s^{-1} (Poser et al. 1997; Oda et al. 1998; Yoon et al. 2002) or even 70–80°C s^{-1} for cooling (Daniel et al. 1998) have been reported. In terms of heating and cooling rates, these micro-chamber PCR devices are superior to the conventional PCR device.

Alternatively, PCR can be achieved in a continuous-flow fashion by driving a PCR mixture in a microchannel to flow repetitively through two or three different thermostable zones (Nakano et al. 1994; Kopp et al. 1998; Schneegaβ et al. 2001; Chiou et al. 2001; Zheng and Chen 2002; Chou et al. 2002; Liu et al. 2002; Zhang et al. 2002; West et al. 2002; Sun et al. 2002; Park et al. 2003; Obeid et al. 2003; Obeid and Christopoulos 2003; Bu et al. 2003; Curcio and Roeraade 2003; Fukuba et al. 2004; Hashimoto et al. 2004; Zhao and Zhang 2005; Chen et al. 2005; Dorfman et al. 2005; Yao et al. 2005; Wang et al. 2005; Kim et al. 2006; Pilarski et al. 2005; Jia et al. 2005). For continuous-flow PCR, the cycling rates do not depend on the thermal mass of the PCR device, but on the thermal mass of the PCR mixture and the flow speed of the PCR mixture through the microchannel. Consequently, the continuous-flow PCR can be performed at relatively high speeds as it is not necessary to thermally cycle the PCR device repeatedly. Up to now, the fastest PCR was performed with continuous-flow PCR microfluidics (Hashimoto et al. 2004).

In most of the literature on continuous-flow PCR, three thermostable zones are linearly arranged in the order of denaturation, extension, and annealing, which is good to establish a smooth temperature gradient on the chip (Kopp et al. 1998; Schneegaβ et al. 2001; Chou et al. 2002; Zhang et al. 2002; Obeid et al. 2003; Obeid and Christopoulos 2003; Bu et al. 2003; Fukuba et al. 2004; Yao et al. 2005; Wang et al. 2005; Kim et al. 2006), but this type of temperature arrangement could reduce the PCR efficiency (Wittwer and Garling 1991; Park et al. 2003; Jia et al. 2005). In order to overcome this problem, some researchers have made attempts to investigate the PCR device with a circular arrangement of temperature zones in the sequence of denaturation, annealing, and extension (Liu et al. 2002; West et al. 2002; Sun et al. 2002; Park et al. 2003; Curcio and Roeraade 2003; Hashimoto et al. 2004; Chen et al. 2005; Dorfman et al. 2005; Jia et al. 2005). However, most of the reported continuous-flow PCR chips have used the microchannel architecture with the constant cycling times at each cycle. Recently, great attention has been focused onto the spiral channel-based PCR chip with variable cycling times at each cycle (Hashimoto et al. 2004; Jia et al. 2005). Among the reported spiral channel based continuous-flow PCR methods, however, the sample inlet lies outside the chip and the product outlet lies inside, which couldn't benefit the

continuous-flow PCR (Liu et al. 2004). In addition, fabricating a spiral microchannel on a chip usually involves many microfabricating steps and bonding processes. Thus, it is time-consuming, high in cost, and complicated in technology that hampers the practical use of the chip-based continuous-flow PCR.

In this study, we present a simple and novel method for performing the spiral channel based continuous-flow PCR amplification. By the general fabricating technology, the spiral channel is first machined onto the three heating copper blocks and then the transparent capillary is well embedded onto the spiral channel on copper. The sample inlet lies inside the PCR microfluidics and the product outlet lies outside. Biochemical performance of the continuous-flow PCR microfluidics is experimentally evaluated by letting three different DNA fragments to be amplified at different flow rates. Besides, a segmented-flow PCR of three fragments has also been demonstrated.

EXPERIMENTAL SECTION

Materials

PCR reagents, 10 × Taq buffer (200 mM KCl, 200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$), thermostable Taq polymerase (5 U μl^{-1}), deoxynucleotide triphosphates (dNTPs) (2.5 mM each of dATP, dGTP, dCTP, and dTTP), and double-deionized (dd) H_2O were purchased from Tiangen Biotech Co., Ltd. (Beijing, China). The DNA markers, which consist of 100-, 250-, 500-, 750-, 1000-, and 2000-bp DNA fragments, were also from Tiangen Biotech Co., Ltd. Three pairs of primes for human β-actin gene fragments 5′-aaggc caacc gcgag aagat-3′ (upstream)/5′-tcggt gagga tcttc atgag-3′ (downstream) (249 bp), 5′-tcacc aactg ggacg acatg-3′(upstream)/5′-cagct cgtag ctctt ctcca-3′ (downstream) (500 bp), and 5′-gaagg attcc tatgt gggcg-3′(upstream)/5′-ctaga agcat ttgcg gtgga-3′ (downstream) (982 bp) were from Invitrogen (Shanghai). Target genomic DNA was rapidly extracted from blood samples from a patient volunteer by using BioGene-Expuze™ DNA extraction kit {Texas (Beijing) BioGene, Inc.}, whose concentrations were determined by spectrophotometer (Ultrospec® 2100 pro, Amersham Pharmacia Biotech, Piscataway, NJ, USA) and subsequently diluted to the fixed concentration of 40 ng μl^{-1}. Bovine serum albumin (BSA) was purchased from Roche (Guangzhou, China). GoldView™ dye was from SBS Genetech Co., Ltd.(Beijing, China). Agarose was from Biowest (Shanghai, China). A 5 × TBE buffer was prepared by dissolving 54 g Tris, 27.5 g boric acid, and 20 ml 0.5M EDTA (pH8.0) in dd H_2O.

Polytetrafluoroethylene (PTFE) capillary (500-μm i.d. and 900-μm o.d.) was purchased from Wuxi Xiangjian Tetrafluoroethylene Product Co., Ltd. (China). K-type thermocouples (unsheathed, with a diameter of 0.0508 mm) were from Omega (Singapore). Disposable 3-ml syringes were from Jiangxi Lule Medical Instrument Co., Ltd. (China). The proportional-integral-derivative

(PID) temperature controller (model CD901FK02-M*AN-NN) was from RKC Instrument, Inc. (Japan). The Data Acquisition/Swich Unit (model 34970A) was from Agilent Technologies Co. Ltd. and two-channel PC-controlled precision syringe pump (model TS2-60) was bought from Baoding Longer Precision Pump Co., Ltd. (China).

Design of Spiral Channel-Based PCR Thermocycler

Figure 1 shows schematic presentation of the spiral channel on copper for the continuous-flow PCR microfluidics. It consists of a 25 × 15 × 1.8 cm copper block with spiral channel machined into three pieces corresponding to the denaturation, annealing, and extension regions. The extension regime is about double the size of the other two zones, thus occupying almost half of the block. Three temperature zones are isolated from one another by using the thermally insulating isinglass material. The pieces of the copper block can be firmly fixed by the metal clamp to provide structural stability. The thermally insulating isinglass material was also used between the copper blocks and the metal clamp in order to further avoid the thermal interaction between the pieces of the copper block. Two small holes (1 mm diameter and 20 mm deep) for the thermocouples and one larger central open hole (8 mm diameter) for the heater cartridge were drilled in the denaturation and annealing zones. Due to the bigger size, two large and five small holes of similar size were drilled in the extension zone.

The spiral channel of 1.1 mm width and 1.1 mm depth was fabricated by using the conventional mechanical machining technology and incorporated 25 cycles were incorporated having inlet and outlet portions, as shown in Fig. 1. The channel was designed to execute a residence time ratio of approximate 1:1:2 in each PCR cycle for denaturation, annealing and extension, respectively. The beginning of the denaturation regime and the end of the extension regime were elongated to complete the initial melting and final extension steps, respectively. The PTFE capillary with a 0.9 mm o.d. was embedded onto the open spiral channel on copper to form a spiral capillary channel. In order to ensure good thermal contact between the heating copper blocks and the outer wall of the capillary, the thermally conductive mineral oil was added within the spiral channel on copper. Each temperature zone was then covered with a thin layer of copper foil whose edges were in contact with the copper ribs between the spiral channels in each temperature zone. The copper foil in each zone could provide heating from above and improve temperature uniformity inside the PTFE capillary. In order to control the temperatures necessary for continuous-flow PCR, the heater cartridges and the miniature K-type thermocouples were mounted into each copper block and then the PID controller was used to control the necessary temperature profile. The temperature distribution in each zone depicting

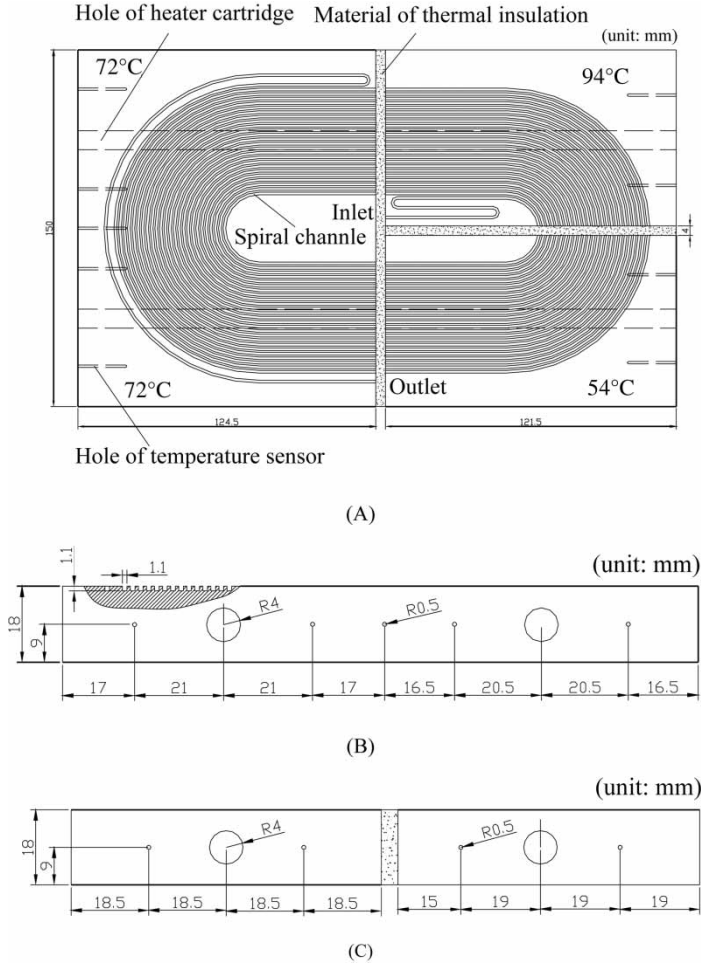

Figure 1. Schematic presentation of the spiral channel on copper for the continuous-flow PCR microfluidics. (A) plan view, (B) left view with a cutaway view of partial spiral channel, (C) right view. The capillary for continuous-flow PCR is not included in the figure.

real-time was recorded with a Data Acquisition/Switch Unit, which is connected to a computer with a standard RS-232 serial port.

Continuous-Flow and Positive-Control PCR Amplification

The 25 μl PCR mixture used in the continuous-flow PCR consisted of 1 × Taq buffer, 200 μM of each dNTP, the primer pair (0.5 μM each), 0.01–10 ng μl^{-1} human genomic DNA as the PCR template (except for the negative control PCR

experiments), 0.1 U μl^{-1} Taq DNA polymerase and corresponding volumes of dd H_2O. BSA of 0.025% (w/v) was included into all of the continuous-flow PCR mixture solutions to stabilize the Taq DNA polymerase and to reduce undesired adsorption of the polymerase onto the capillary inner surface. The prepared PCR mixture was introduced into the capillary from the inlet and then the precision syringe pump with a disposable 3-ml syringe was used to drive the PCR mixture to flow through the spiral capillary microchannel. Meanwhile, a 0.2-ml thin-wall PP tube was used to collect the amplified PCR product for further analysis. For all of continuous-flow PCR, the temperature in each zone was set as denaturation at 94°C, annealing at 54°C, and extension at 72°C.

Positive-control PCR was performed on a conventional PCR machine (model iCyler, Bio-Rad, California, USA) using the corresponding PCR mixture. PCR thermal cycling programs were set as follows: pre-denaturing at 94°C for 5 min, followed by 25 cycles with denaturing at 94°C for 30 s, annealing at 54°C for 45 s, and extension at 72°C for 45 s, final extension for 5 min (249 and 500 bp); pre-denaturing at 94°C for 5 min, followed by 25 cycles with denaturing at 94°C for 60 s, annealing at 54°C for 60 s, and extension at 72°C for 90 s, final extension for 10 min (982 bp). These programs were concluded by cooling the PCR products to 4°C. It should be noted that the positive-control PCR herein didn't use the same residence time ratio of 1:1:2 as the continuous-flow PCR did, but used the optimized residence time ratio in the conventional PCR as the reference.

PCR at Various Flow Rates

In order to test the ability of rapid PCR in the spiral channel-based PCR microfluidics, PCR of the 249 bp fragment was first performed at different flow rates of the corresponding PCR mixture through the thermal-cycling capillary. The flow rates were well controlled by the precision syringe pump, ranging from 3.0 to 17.5 mm s^{-1}. Secondly, in order to investigate the ability of rapid PCR further for the longer DNA template fragments, the 500 bp and 982 bp fragments were selected to perform the PCR at various flow rates. Corresponding positive-control PCR was also carried out in a conventional PCR machine using the same PCR mixture.

PCR at Various Initial Template Concentrations

In some applications, it is crucial to acquire a detectable amount of PCR products quickly from a low concentration DNA template sample. Therefore, a study was performed to establish the minimal template concentration of 249 bp fragment, which was continuously amplified to 25 cycles at a flow rate of 5.0 mm s^{-1} in the presented spiral channel-based PCR microfluidics. The concentrations of DNA template varied from 0.01 to 10 ng μl^{-1}.

Segmented-Flow PCR of Different DNA Samples

25 μl PCR mixtures containing three different DNA templates for amplifying 249, 500 and 982 bp fragments are sequentially introduced into the capillary with about μl air interval between the neighbouring samples, and then sample segments were driven to flow through the thermal-cycling capillary at the flow rate of 3.0 mm s^{-1}. Between each PCR mixture introduced, we interposed about 10 μl 1 × PCR buffer containing 1 × bromophenol blue buffer and small air gaps (<15 mm) on both sides of the buffer segment. This sequence of introductions was repeated so that 25 μl of each PCR product could be collected separately for further analysis.

Analysis of Amplification Products

The 2.5% (w/v) agarose gel electrophoresis using GoldView™ as fluorescence dye was used to analyze the PCR products. Thin agarose gels (<5 mm thickness) were prepared in order to obtain the high detection sensitivity. Eight μl of the product was loaded onto the loading holes of agarose gels in a gel chamber (MINI-SUB® CELL GT, BIO-RAD, USA) and then electrophoresed in 0.5 × TBE buffer with a 8 V cm^{-1} electric field supplied by the electrophoresis power supply (EPS 601, USA). 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

The thermal cycling for the continuous-flow PCR was realized in this work by embedding the capillary onto the spiral channel on copper fabricated by the commonly used conventional mechanical machining method. The three copper blocks act not only as the thermal sources necessary for PCR thermal cycling but also as the substrate material of a spiral channel, thus a simple approach compared to complex microfabricated structures. However, some limiting factors need to be considered (Curcio and Roeraade 2003); the temperature change of the PCR mixture, which flows from one region to the next, is not instantaneous but requires some time to reach new temperature level and the time needed is dependent on the flow rate, the thermal conductivity of the transport tube and the PCR reaction liquid. In other words, the cycling rate of continuous-flow PCR thermocycler depends, to some extent, on the flow rate of the PCR mixture, the substrate material and the size of the flow channel. Therefore, it is very necessary to test and evaluate the amplification performance of the newly developed continuous-flow PCR as a function of flow rate. It is expected that a greater amount of the PCR

product will be obtained with a decrease in the flow rate because a longer time for extension will result in complete polymerization. Figure 2A shows the gel electrophoresis results of the 249 bp PCR products obtained at various flow rates ranging from 17.5–3.0 mm s^{-1}. The corresponding time for the PCR mixture flowing through the PTFE capillary ranges from about 11–62 min. The experimental results clearly show that the amount of the PCR product decreases as the flow rate of the PCR mixture increases. At a flow rate of 3.0 mm s^{-1} (lane 3 in Fig. 2A), the amount of PCR product is about 75% of the positive-control PCR. However, the PCR product cannot be obviously observed when the flow rate increases to 10.0 mm s^{-1} (lane 6 in Fig. 2A) or higher. In case of 7.5 mm s^{-1} flow rate, the product band of the 249 bp fragment can still be observed (Fig. 2A), which demonstrates that the 249 bp fragment can be successfully amplified within a short period of 25 min compared to the conventional PCR machine.

In order to further investigate the amplification ability of longer DNA fragments in the presented continuous-flow PCR, we chose 500 bp and 982 bp human β-actin gene fragments as target DNA templates. Fig. 2B exhibits the results of the two longer DNA fragment products amplified at various flow rates. The 500 bp product could be obtained at flow rates of 3.0, 5.0, 7.5, and 10.0 mm s^{-1}, respectively and the product band was

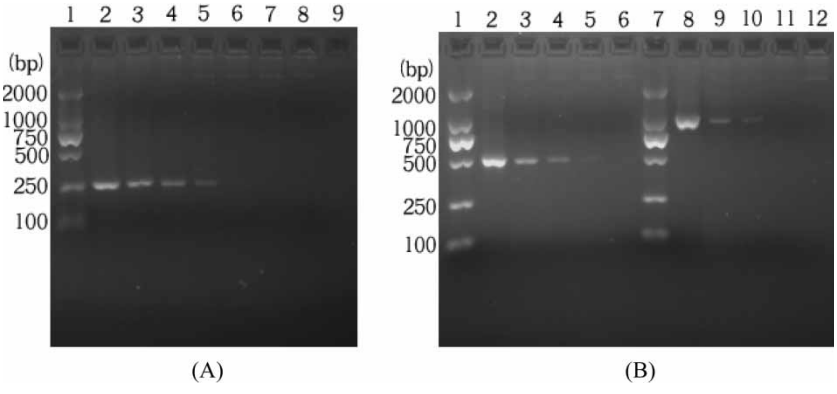

Figure 2. Effect of the flow rates on the continuous-flow PCR yield of the 249 bp (A), 500 bp and 983 bp (B) fragments. (A) Lane 1: the DNA markers. Lane 2: the positive-control PCR product from the conventional PCR machine. Lanes 3–8: continuous-flow PCR products at various flow rates of 3.0, 5.0, 7.5, 10.0, 12.5 and 17.5 mm s^{-1}, respectively. Lane 9: negative-control PCR, PCR mixture solution with no DNA template run at a flow rate of 5.0 mm s^{-1} (as in lane 4). (B) Lanes 1 and 7: the DNA markers. Lane 2 and 8: the positive-control PCR products for 500 bp and 982 bp fragments, respectively. Lanes 3–6: continuous-flow PCR products at flow rates of 3.0, 5.0, 7.5, 10.0 mm s^{-1}, respectively. Lanes 9–12: continuous-flow PCR products at flow rates of 2.0, 3.0, 5.0, and 7.5 mm s^{-1}, respectively. The identical concentration of input DNA molecules was used for all continuous-flow PCR results (4 ng μl^{-1}).

detectable up to a flow rate of about 7.5 mm s^{-1}, which allowed completion of 25 PCR cycles in about 25 min. For the 982 bp fragment, the maximum flow rate that can be used to produce a detectable product band is generally less than 3.0 mm s^{-1}, which indicates that the successful amplification of the 982 bp fragment in the continuous-flow PCR thermocycler usually takes about 62 min, which is still shorter than that in a conventional PCR machine. Strictly speaking, by using an appropriate flow rate even longer target DNA fragments, for example 2000–3000 bp DNA fragments, could also be amplified in our present continuous-flow PCR. However, when the flow rate is decreased to less than 1.0 mm s^{-1}, the Taq polymerase enzyme must be subjected to the extended stay at high temperature of 94°C in the denaturation zone, which will inactivate the polymerase enzyme and result in lower and even no amplification yields. Furthermore, the lower flow rate will probably offer the greater opportunity of bubble formation in the denaturation zone of 94°C, which will adversely affect the PCR amplification (Schneegaβ et al. 2001).

Compared with the earlier studies, the thermal cycling rates in the presented spiral channel based continuous-flow PCR seem to be somewhat slower than those reported earlier (Hashimoto et al. 2004; Liu et al. 2004). We attribute this to the size of the PTFE capillary used herein. At present, we have used the PTFE capillary with inner diameter of 500 μm and wall thickness of 200 μm. The use of thick-walled PTFE capillary with low thermal conductivity of about 0.2 $W \cdot m^{-1} \cdot K^{-1}$ is disadvantageous in the continuous-flow PCR, compared with chip-based structures in glass (Liu et al. 2004) or even plastic PC (Hashimoto et al. 2004). The thick wall will increase the time required to transfer the heat from the outer to the inner of the capillary. In addition, the larger diameter of the capillary channel, which is much lager than that in the previous studies (Hashimoto et al. 2004; Liu et al. 2004), will reduce the speed of thermal equilibration of the PCR reaction mixture in the capillary channel. Fortunately, the most attractive feature of the continuous-flow PCR thermocycler is that it can adjust the thermal cycling rate within the wider range of flow rate and can accommodate the various volumes of the PCR mixture onto a continuous-flow channel.

In some fields such as archaeology and forensic analysis, it is very important to obtain a detectable level of PCR product quickly from very small amount of DNA template. In this study, we have taken a 249 bp fragment as an example and investigated the effect of the amount of input DNA template on continuous-flow PCR amplification, as shown in Fig. 3. The concentrations of DNA template in the PCR mixture ranged from 10.0–0.01 ng μl^{-1} and the continuous-flow PCR was performed for 25 cycles at a constant flow rate of 5.0 mm s^{-1}. It can be seen from Fig. 3 that the PCR products decrease with decreasing input concentration of the DNA template and that the minimal template concentration that could be obviously detected by agarose gel electrophoresis was up to 0.1 ng μl^{-1}.

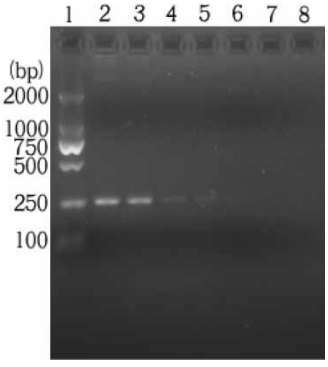

Figure 3. Fluorescence intensity of the 249 bp continuous-flow PCR product as a function of the input DNA molecules. A flow rate of 5.0 mm s^{-1} was used. Lane 1: the DNA markers. Lanes 2–7: continuous-flow PCR products from various concentrations (10, 4, 1, 0.4, 0.1, 0.01 ng μl^{-1}) of the input DNA molecules. Lane 8: negative-control PCR, PCR mixture solution with no DNA template.

When the DNA template concentration was decreased to be 0.01 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 that can be used in our present continuous-flow PCR thermocycler is 0.1 ng μl^{-1}. It is, however, obvious that nonspecific PCR products, either due to mispriming or due to primer dimmers, were not observed at a high concentration of DNA template such as 10.0 and 4.0 ng μl^{-1} (lanes 2 and 3 in Fig. 3).

For continuous-flow PCR, one important advantage is that it can be very easily modified to perform sequential DNA amplification by using a continuous segmented flow of different PCR mixtures containing different template types. In the past few years, some researchers have investigated the sequential amplification of different target sequences and the strategy of contamination-free amplification in a continuous-flow format (Schneegaβ et al. 2001; Park et al. 2003; Obeid et al. 2003; Curcio and Roeraade 2003; Dorfman et al. 2005). In our experiment of investigating the high-throughput capability of the spiral channel based continuous-flow PCR thermocycler, we used the method similar to the Park et al. (2003) work to eliminate the cross-contamination from sequential PCR amplification of three target sequences with different sizes. Figure 4A demonstrates the segmented-flow mode for PCR amplification of three different DNA fragments, 982, 500, and 249 bp. First, three different PCR mixtures for amplifying different DNA fragments were introduced into the thermal cycling capillary in series in the sequence of 982, 500, and then 249 bp. Figure 4B shows that three different DNA fragments were successfully amplified sequentially at a constant flow rate of 3.0 mm s^{-1} in our continuous-flow PCR thermocycler, without any

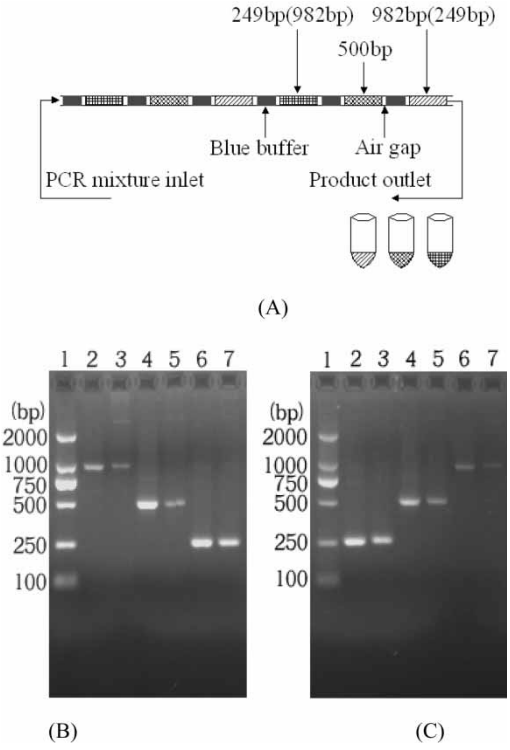

Figure 4. (A) Segmented-flow mode of continuous PCR amplification of three different DNA fragments, 982 (249), 500 and 249 bp (982 bp). (B) An electrophoretogram of separately collected PCR products when the sampling sequence of PCR mixture was for 982, 500 and 249 bp, respectively. Lane 1: the DNA markers. Lanes 2, 4 and 6: the positive-control PCR products of 982, 500 and 249 bp DNA fragments, respectively. Lanes 3, 5 and 7: continuous-flow PCR products of 982, 500 and 249 bp DNA fragments, respectively. (C) An electrophoretogram of separately collected PCR products with the sampling sequence of PCR mixture as 249, 500 and 982 bp, respectively. Lane 1: the DNA markers. Lanes 2, 4 and 6: the positive-control PCR products of 249, 500 and 982 bp DNA fragments, respectively. Lanes 3, 5 and 7: continuous-flow PCR products of 249, 500 and 982 bp DNA fragments, respectively.

evidence of cross-contamination. In order to investigate whether the introduction sequence of target DNA fragments have an effect of sequential segmented-flow PCR amplification, we introduced three different PCR mixtures for amplifying different DNA fragments in the sequence of 249, 500, and then 982 bp. As expected, the three sample fragments were amplified independently and no cross-contamination was observed, as shown in Fig. 4C. The results presented in Fig. 4B and 4C imply that our continuous-flow PCR thermocycler has the ability to perform the high-throughput PCR amplification within a short time.

CONCLUSION

A new continuous-flow PCR microfluidics has been successfully realized by embedding a capillary onto spiral channel on copper, and its performance has been successfully demonstrated. The experimental results have shown that the continuous-flow PCR products of 249, 500, and 982 bp human gene fragments could be obviously observed within 25, 25, and 62 min, respectively. The sequential segmented-flow PCR for three different fragments of 249, 500 and 982 bp could also be well achieved within a short time. Besides, the limit concentration of human genomic DNA template in the present continuous-flow PCR microfluidics may reach to 0.1 ng μl^{-1}, which can meet the requirements of different applications. The ease of fabrication and the low cost associated with the present PCR microfluidics should make it attractive in basic research related to life science, clinical diagnosis of diseases, and forensic science.

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