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Development of a continuous-flow polymerase chain reaction device utilizing a polymer disk with a spiral microchannel of gradually varying width

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ABSTRACT

This paper describes a continuous-flow polymerase chain reaction (CF-PCR) device utilizing a polymer disk, which is equipped with a long spiral microchannel. In the literature, some CF-PCR devices employing a spiral microchannel have been presented to overcome an inherent deployment problem of heaters in CF-PCR of a serpentine microchannel, however, the spiral arrangement also made a problem of non-constant PCR cycle time, which would reduce the PCR efficiency. As a novel solution in this paper, we made the width of the spiral channel decrease gradually in the radial direction in order to keep the cycle time of one round same independent of radial positions and the PCR speed. In the spiral channel of 5.4-m long, through which the PCR reactant is pumped at a constant flow rate, a cycling zone made of thirty spiral rounds corresponding to 30 PCR cycles was placed between a pre-denaturation zone and a post-extension zone, and each zone was designed to have a fixed flow residence time ratio against to the total PCR time. We developed a fast thermal bonding technique minimizing the destruction of the microchannel in the wide polymer disk of 66-mm diameter. A compact heating apparatus was fabricated in order to impose different temperatures at three heating sectors deployed circumferentially in the disk. As a novel trial, the disk is sandwiched by isolated metal plates of constant temperature for stable thermal maintenance. We conducted numerical simulations for the heat transfer to PCR mixture depending on the PCR speed, and discussed on its effect on PCR result. A successful amplification of a human-genome DNA was obtained in less than 10 min. The unique architecture used in this CF-PCR device is understood to be well applied to a field-applicable fast PCR.

1. Introduction

Polymerase chain reaction (PCR) is a general tool for the detection of tiny amount of DNA molecules without the use of an ultra-highly sensitive sensor. Recently, miniaturized PCR devices using a microchip have attracted much interest due to their various advantages over the conventional PCR thermocyclers, in terms of speed, efficiency, throughput, integration, reproducibility, miniaturization, sample consumption, and cost.

A number of miniaturized PCR devices made of various chip materials, such as silicon, glass, and polymers, have been developed [1–4], and they can be classified into two categories on the basis of the handling of PCR sample mixtures during PCR cycling, i.e., "stationary (or static) type PCR" and "continuous-flow (or flow-through) type PCR" [4,5]. In the stationary type PCR, the PCR mixture is stationary during the PCR process in a confined space, such as a microchamber, which is subjected to external temperature variations in a repetitive manner for each PCR cycle. On the contrary, in the continuous-flow type PCR (CF-PCR), the PCR reactant moves consecutively along a few regions maintained at different temperatures for the thermal cycling of PCR. Generally, the CF-PCR devices allow for more rapid heating and cooling of the PCR reactant as well as more uniform temperature distribution inside the PCR sample mixture. Moreover, energy consumption is reduced since the thermal mass of the PCR mixture is very small relative to that of the preheated apparatus. Thus, they are suitable for the fast PCR using an instrument operated with low power.

Some research groups have proposed CF-PCR devices using a capillary tube. A tube, through which the sample is pumped, was exposed to oil or water baths of different temperatures [6,7]. Heated cylindrical metal blocks, around which a tube was wound, were utilized [8–10]. Immiscible transport liquids were used to separate the different sample plugs and to prevent cross-contamination among multiple samples [9,10]. A glass capillary tube was covered with the

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transparent resistive film for the real-time fluorescent monitoring of the PCR [11]. Also, bidirectional pumps and an in situ optical position sensors were equipped for a microcapillary thermocycler [12].

A microchip-based CF-PCR was proposed by Kopp et al. [13]. The PCR mixture was pumped through a serpentine channel in a glass chip, and the channel passed through three heated copper blocks with constant temperatures. Based on this novel trial, some improvements have been reported with the variations in the device configurations, the chip material, the surface passivation and the PCR conditions [14–16]. However, because the serpentine channel is placed on a two-dimensional chip, the consecutive heating among three temperature regions is inherently not possible without one quick movement of the sample mixture through an auxiliary narrow channel [5,17].

In order to overcome an abovementioned problem in CF-PCR utilizing a serpentine microchannel, some groups presented CF-PCR devices employing a spiral microchannel [18–21]. The spiral setup is suitable for fast temperature transition because there is no intermediate region in the serpentine one. Liu et al. [18] demonstrated a rotary PCR device utilizing a microfluidic chip with integrated heaters and plumbing which allowed adjusting the number of cycles. Hashimoto et al. [19] fabricated a CF-PCR device including a 20-cycle spiral microchannel in a rectangular polycarbonate plate and characterized its amplification performance at a range of flow velocities. Wu et al. [20,21] proposed a CF-PCR device employing pressurized syringes for semi-automated sample injection and a single heater for PCR amplification for system simplification.

As mentioned above, many versions of CF-PCR devices have been presented, and they have continuously improved the PCR performance in speed, throughput, sample consumption, cross-contamination, cost, and so on. However, one common problem preventing them to be used commercially is that their PCR efficiency and reproducibility are relatively low compared with commercial thermocyclers.

In this paper, we present a CF-PCR device utilizing a polymer disk equipped with a novel spiral microchannel. For more-efficient PCR cycling, the spiral microchannel was carefully designed to make the reaction time of each cycle same regardless of the cycle numbers at constant pumping rate, which was not considered in the previous papers employing the spiral channel arrangement. Moreover, for the first time, a pre-denaturation zone and a post-extension zone was installed in the disk before and after a cycling zone to increase the PCR efficiency. The residence flow times at each zones were designed to have constant ratio regardless of the total PCR time. For commercial availability, a fast fabrication technique for the disposable hard-plastic disk will be disclosed. Construction of a compact heating apparatus with sandwiched heaters in insulating materials for stable thermal maintenance will be explained. The transport characteristics in the CF-PCR device will be discussed based on the theoretical considerations and the numerical simulations of the flow and heat transfer to confirm the fast and efficient PCR of the device. For the performance verification of the CF-PCR device, a few PCR experiments using the human-genome DNA sequence will be shown.

2. Experimental

2.1. Device concept

A schematic diagram and photographic image of the present CF-PCR device are shown in Fig. 1. The PCR device consists of a syringe pump, a temperature controller, a heating apparatus, and a disposable polymer disk. The polymer disk was inserted into the heating apparatus, which was designed for the local heating of the disk. Six temperature controller (TZ4SP, Autonics Co., Korea) maintains the temperatures of the metal plates at different constant values. The syringe pump (KDS 260, KD Scientific, Holliston, MA) continuously pushes a transport liquid (DI water) in a glass syringe (Hamilton Co., Reno, NV) at a fixed flow rate through a Teflon capillary tube (AWG-24, 0.6 mm i.d., 1.1 mm o.d., Banseok Co., Ltd., Korea) connected to the inlet of the disk. A plug of PCR reactant of 5 μl (~18 mm in tube length) is introduced to the flow of the transport liquid through a T-channel connector (12-mm-o.d. Teflon rod with drilled 1.0-mm-i.d. inner channels), while the reactant plug is separated with the transport liquid by air gaps (of 5 mm in tube length). In order to stabilize the liquid flow and to prohibit the air bubble generation near the denaturation region, a syringe (1000 μl, 4.61 mm i.d., GASTIGHT #1001, Hamilton Co.) filled with DI water is connected at the outlet through a Teflon tube (length 0.3 m, AWG-24), which gives a pressurizing effect to the flow due to additional shear resistance. The total PCR reaction time can be modulated through the flow rate of the syringe pump. The reactant plug moves from the inlet located at the center of the polymer disk and continuously flow at a constant flow rate through a long spiral microchannel to the outlet located at the outer region of the polymer disk. The DNA amplification is accomplished by changing the temperature of the PCR reactant in the spiral microchannel consecutively to the values for the three steps of PCR cycling (denaturation, annealing, and extension). The PCR sample mixture goes through one three-step PCR cycle when it moves one round in the spiral microchannel. The consecutive variation of the reactant's temperature is done by placing the metal heating plates with different temperatures circumferentially on the top and bottom surfaces of the polymer disk. The disk is tightly sandwiched by the six metal plates on its two sides, which allows rapid and uniform heat transfer from the metal plates to the PCR reactant in the disk.

2.2. Heating apparatus

Fig. 2 shows the structure of the heating apparatus. A schematic diagram of its cross-sectional view and an exploded view are shown in Fig. 2a. The apparatus consists of fluidic connectors, metal plates, film heaters, and structures for thermal insulation and mechanical clamping. The fluidic connection between the disk and the heating apparatus is automatically established when the top and bottom sides of the heating apparatus are clamped. O-rings (with a hole of 0.8 mm), metal needles (BPN-23G, 420 μm i.d., 720 μm o.d., Banseok Co., Ltd., Korea), and sculptured fixing screws were placed at the top of the apparatus for this purpose. The needles are fixed onto the PEEK blocks by the fixing screw and are extruded about 0.3 mm from the metal plates to align them with the inlet and outlet holes (1-mm-diameter) of the polymer disk. The metal plates (aluminum, 2-mm thick) are heated by the flexible film heaters (3–7 W/24 V, 250 μm thick, polyimide Kapton film) attached to their backside. The shape of each film heater, in which a resistor line is wound, is the same as that of the corresponding metal plate (Fig. 2b). The metal plates were mounted on poly-ether-ether-ketone (PEEK, 11-mm thick, 95-mm diameter) blocks that were used for thermal insulation and mechanical clamping. On the PEEK blocks, trenches of 3-mm depth were carved under the metal plates in order to ensure thermal isolation between the plates. The temperature distribution inside each metal plate was made highly uniform by virtue of high thermal conductivity of the plates. A K-type thermocouple (5TC-TT-K-36-36, Omega Eng., Inc.) was inserted into each metal plate to measure and adjust its temperature. Fig. 2c shows an open view of the fabricated heating apparatus. The metal heating plates were distributed circumferentially and were aligned to the corresponding regions of the polymer disk. The ratio of the reaction times at the denaturation, annealing, and extension regions was set at 1:1:2 and did not change in all the