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Continuous-Flow Polymerase Chain Reaction Chip by Water Cooling*

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Abstract

A novel continuous-flow polymerase chain reaction (PCR) chip has been analyzed. It operates by cycling a prepared sample within three temperature zones. Two temperature zones are controlled by two thermal controllers and the other is controlled by the flow rate of the fluid inside a cooling channel under a glass chip. The chip system consists of the reaction PDMS chip, a cover glass chip, a cooling channel, and is equipped with cartridge heaters and thermocouples for temperature control. Commercial software is utilized to determine the chip materials that are responsible for creating the denaturation, annealing and extension temperature zones within the chip. Therefore we utilize this chip to perform PCR experiments. Results show that DNA templates are amplified successfully.

[Keywords] PCR, microchannel, continuous-flow, microfluidics

I Introduction

Since polymerase chain reaction (PCR) was invented, PCR has become one of the most important bio-technologies for generic identification during the last few decades. PCR is a procedure to amplify the number of copies of a specific DNA template exponentially. The PCR process includes melting of the double-stranded DNA (denaturation step), binding of the specific primers to the targets (annealing step), and extending of the primers with the thermostable DNA polymerase (extension step). Each step is performed by heating or cooling the sample to the characteristic temperature for a certain time period. Nowadays the PCR process is mostly performed in a thermocycler. The PCR amplification efficiency is not only related to the sample pretreatment, but also to the temperature distribution of the sample as the sample temperature is varied.

With the progression of micro-fabrication technology, the applications of microfluidics have gradually moved from the sub-systems of the micro total analysis system (micro-TAS) into the crucial components of Micro Electro-Mechanical Systems (MEMS). For micro PCR systems, one of the significant issues lies in the thermal interference between the different thermal heaters integrated within the microfluidic device (Yang *et al.*, 2005). The thermal interference effect means that a temperature change in one section is caused by heat generation in the other. Researchers have shown that by including insulation features in the fabricated devices, thermal separation between the temperature zones is greatly improved. Shih *et al*. (2006) utilized a parylene-cross-linking structure to achieve on-chip air-gap thermal isolation for a continuous flow PCR (CFPCR) chip. Results showed that a 10-cycle PCR chip was prepared on an 8 mm × 8 mm silicon chip area. Chen *et al*. (2008) designed a polycarbonate CFPCR device fabricated with LIGA technology. LIGA is a German acronym for *Lithographie*, *Galvanoformung*, *Abformung* (Lithography, Electroplating, and Molding). They made grooves between temperature zones to increase the resistance to conduction.

Some studies utilized highly thermal conductive materials attached to the chips to increase temperature uniformity in the working regions. Hsieh *et al*. (2008) used array-type microheaters with active compensation heaters in the PCR chip. A layer of gold was deposited on the thin film heater to enhance the thermal uniformity. Li *et al*. (2011) designed a capillary-based CFPCR device, which used flexible thin film heaters to construct three temperature zones. A brass sheet above the thin film heater was utilized.

In the present work, a fully integrated microsystem for continuous flow PCR has been developed. This polydimethylsiloxane (PDMS) chip is fabricated through a photolithography technique and used to obtain the 30-cycle microchannl structures with a 150 μm width and a 50 μm depth. The annealing zone is arranged in the chip center; the denaturation and the extension zones at the opposite sides. The annealing temperature is controlled by the flow rate of the fluid inside a cooling channel under the glass chip. We investigate the influences of various chip materials in the water cooling channel of the system on the temperature uniformity of the chip surface.

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II Thermal Modeling

The continuous-flow PCR chip is based on a single meandering channel passing repetitively through the three stationary temperature zones. The overall dimension of this chip is 76 mm long × 26 mm wide × 3 mm high. The device consists of the cooling channel, the reaction PDMS channel chip and a cover chip made of glass. The three temperature zones are separated by grooves of 1 mm width to reduce heat conduction from higher to lower temperature zones. The annealing temperature can be controlled by the flow rate of the fluid inside a cooling channel under the glass chip.

The governing equation for a three-dimensional steady energy transfer problem can be written as the following

$$\nabla^2 T = 0 \quad (1)$$

where T is the temperature (K).

The boundary conditions for the energy equation are thus as follows. The temperature zones in the device supported by the separate aluminum blocks supply a uniform temperature input, and the isothermal boundary condition is utilized.

$$T = T_{w,i} \quad (2)$$

where $T_{w,i}$ is the temperature of the heating block i, for i = 1 and 2. Two isothermal regions are a high-temperature region (denaturation) and a medium-temperature region (extension). The convective boundary condition is used on all external surfaces except the bottom surface on each aluminum block.

$$-k\frac{\partial T}{\partial n} = h(T - T_\infty) \quad (3)$$

where k is the thermal conductivity (W/mK), h is the convective heat transfer coefficient (W/(m^2 K)) and T_∞ is the ambient temperature (K). For the cooling channel, a fixed velocity condition and ambient temperature condition are set at the inlet; the boundary condition at the outlet is a fixed pressure. The finite volume method is used to solve the energy field.

III Experimental Design

The continuous-flow PCR chip is based on a single channel passing repetitively through the three temperature zones, and shown in Fig. 1. Two commercial cartridge heaters and thermocouples have been integrated onto the glass substrate, and the denaturation and the extension temperatures are controlled by two commercial proportional / integral / derivative (PID) controllers. All mixtures are pumped by hydrostatic pressure. To reduce adsorption of enzymes and DNA into the PDMS surface, the hydrophobic surface of the PDMS channel is modified by filling it with 20 % Tween 20 solution, kept still for 1 hr, and then 2.5 % Tween 20 solution is added into the PCR mixture. The PCR mixture is also run on a commercial thermal cycler. The PCR product is collected in a vial and analyzed by a polyacrylamide gel stained with ethidium bromide to confirm the PCR accuracy.

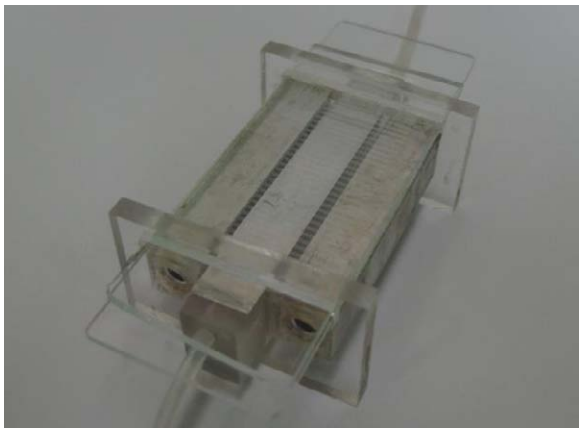

Fig. 1 Experimental setup of the water cooling CFPCR chip

The chip is designed for different temperature zones to perform the DNA denaturing, primer annealing and enzymatic extension steps of PCR. Two separate aluminum blocks, measuring 40 mm long × 8 mm wide × 8 mm high, are integrated onto the chip and utilized as the heating stages. Each heating block below each temperature zone is equipped with one bore (about 3.2 mm diameter) and provides the necessary energy input for carrying out the PCR process. The bore houses the resistance cartridge heater (3.175 mm diameter, 3.8 mm length, 10 W) (C1J-9412, Watlow, USA), and a K-type thermocouple (K30-2-506, Watlow, USA). The temperature difference of the block surface at three measured points is about 1 K. The thermocouple which is adhered onto the surface of the heater is connected to a thermal control system (93 Control Box, Watlow, USA). The control box receives the temperature signal and determines the power input to the heater using a PID controller. The temperature controllers can be used repeatedly.

The water cooling channel is fabricated by cutting work with a numerical control machine (EGX-400, Roland, Japan), which is automatically operated using the VISI - Series Release program. The poly (methyl methacrylate) (PMMA) cooling channel with the thin aluminum cover is utilized to enhance the temperature uniformity. The length, width and height of the cooling channel are 36 mm, 8 mm, and 11 mm respectively, and the channel wall thickness is 1 mm. The thickness of the thin aluminum cover is 1 mm. The aluminum heating blocks and the water cooling channel are mounted on a PMMA frame to thermally insulate each zone and ensure good positional contact. This allows easy replacement of the fluidic chip without requiring mounting of the heating and