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RESEARCH ARTICLE

Miniaturized continuous-flow polymerase chain reaction microfluidic chip system

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Abstract A miniaturized continuous-flow polymerase chain reaction (PCR) microfluidic chip system was developed to perform DNA amplification. This system consists of a 20-cycle continuous-flow PCR microfluidic chip, an electrical heating system and a miniature air pressure-vacuum pump. The chip was ablated with excimer laser direct-writing micromachining technique on a polymethyl methacrylate (PMMA) sheet. The ablated microchannel was inverse trapezoidal with a depth of 70 μm, top width of 200 μm and bottom width of 120 μm. Its surface roughness *Ra* was 1.42 μm after being treated with excimer laser polishing. The substrate sheet ablated with the microchannel was bonded with other cover sheets using hot-press bonding method to form a closed structure. The electrical heating system consisted of three groups of heating membranes, Pt100 sensors, copper blocks and PID temperature digital controllers. It could provide three distinct maintained temperature zones and a uniform temperature distribution in each zone. PCR amplification of a 170 base pair (bp) DNA fragment was carried out to validate the system's feasibility. The PCR temperatures were set as 94°C for denaturation, 55°C for primer annealing and 72°C for extension. The flow rate in the microchannel was 40 nL/s and the total time for the completion of a 20-cycle amplification of 20 μL reagent was 15 min.

Keywords continuous-flow, polymerase chain reaction (PCR), microfluidic chip, excimer laser, micromachining

1 Introduction

Polymerase chain reaction (PCR) is a valuable and well-established nucleotide amplification technique. The reaction is highly specific and capable of creating large amounts of copied DNA fragments from minute amounts of samples. In the last few years, much attention has been paid to the wide applications of PCR technique in clinical medicine, genetic disease diagnostics, forensic science and evolutionary biology [1–3].

PCR technique utilizes repeated temperature cycling involving three different temperatures to realize sample amplification. Conventional PCR process is performed in a thermal cycler device and most of the conventional thermal cyclers roughly require 2 hours to complete the whole amplification. Recently, miniaturized PCR devices have attracted great interest because of their many advantages over conventional PCR devices, such as portability and significantly reduced reagents consumption. To date, a variety of miniaturized PCR devices have been demonstrated and most of them can be classified into two types, static-chamber PCR chip system [4–6] and continuous-flow PCR chip system [7–9].

Static-chamber PCR chip system uses stationary thermal cycler to heat and cool a static volume of liquid in a microchamber. However, its microchamber is difficult to fabricate and its size restricts the sample volume. The temperature control system in this kind of device is large and heating/cooling rate is relatively slow.

Contrarily, the continuous-flow PCR chip system was first reported by Kopp [10], in which the reaction sample, instead of being stationary in a thermal cycling microchamber, is pumped continuously through the chip and flows repeatedly through the zones of different temperatures to ensure the denaturation, primer annealing and extension. This kind of device allows for a very rapid heat transfer and thermal cycling of minute fluidic element due to both high surface-to-volume ratio and short linear thermal diffusion distance in the microchannel. Therefore, the problems of slow heating/cooling rate and fixed sample volume could be resolved successfully. Moreover, it is suitable for high throughput analysis.

The fabrication of microfluidic chip mainly includes photolithography, wet-etching, soft lithography, LIGA, laser ablation and moulding techniques. Silicon, glass

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and polymer are commonly used as substrate material according to different applications and fabrication techniques. Compared to other fabrication techniques and materials, using excimer laser micromachining technique to ablate the microchannel on transparent polymer material is simple and flexible. The excimer laser has short wavelength and short pulse width of nanosecond grade. According to the action mechanism between laser and materials, excimer laser ablation on polymer is based on the photo-chemical etching mechanism. In this mechanism, the ground state of polymer molecules are resonantly excited to a higher state by absorbing ultraviolet photons, then the photo-chemical etching process takes place and the dissociation of bonds occurs when laser fluence reaches the ablation threshold of the polymer. During ablation, there is a little thermal effect. Therefore, the polymer can be ablated exactly by the excimer laser without obvious damage to the ambient material. Furthermore, the microfluidic chip based on polymer is disposable after a single use, thus contamination due to repeated uses is avoided.

In this work, a continuous-flow PCR microfluidic chip was fabricated on a PMMA sheet using excimer laser direct-writing micromachining technique, and excimer laser polishing was used to improve the surface roughness of the chip's microchannel.

2 Experimental results

2.1 Fabrication of continuous-flow PCR microfluidic chip

The structure of continuous-flow PCR microfluidic chip should be designed according to the requirement of PCR amplification, as shown in Fig. 1. The extension zone is arranged in the middle of the chip with the denaturation and annealing zone at the opposite sides. Three zones are used for performing DNA denaturation, primer annealing and extension steps of PCR at a length ratio of 4:4:9. The PCR sample is pumped by a miniature air pressure-vacuum pump. After being pumped in the inlet, the sample first crosses the denaturation zone to complete the initial denaturation of the DNA template and then to have a normal temperature cycle. After the final cycle, the sample will flow through an additional extension zone which is incorporated to complete the synthesis of amplification products. Capillary tubes are connected to the access holes (0.5 mm diameter) as inlet and outlet in order to facilitate the introduction of samples as well as the collection of amplification products. The chip is designed for 20 cycles and its overall dimension is 64 mm × 44 mm × 3 mm. The length for each PCR cycle is 82.4 mm and total length of the serpentine microchannel is 1720 mm.

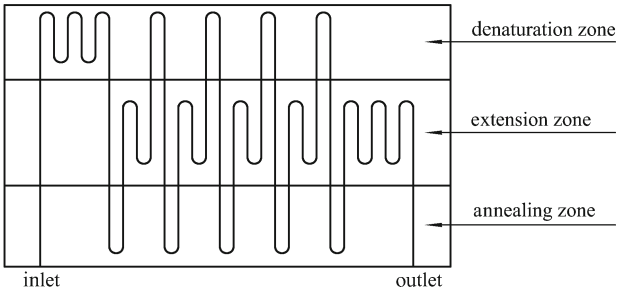

Fig. 1 Schematic sketch of continuous-flow PCR microfluidic chip (schematically 5 cycles)

The continuous-flow PCR microfluidic chip was fabricated using excimer laser direct-writing micromachining technique. Figure 2 illustrates the fabrication process. The position of the excimer laser beam is fixed and a PMMA substrate sheet is placed on the moveable working platform. The computer controls the move of the working platform according to the given structure in CAD format to ablate the serpentine microchannel.

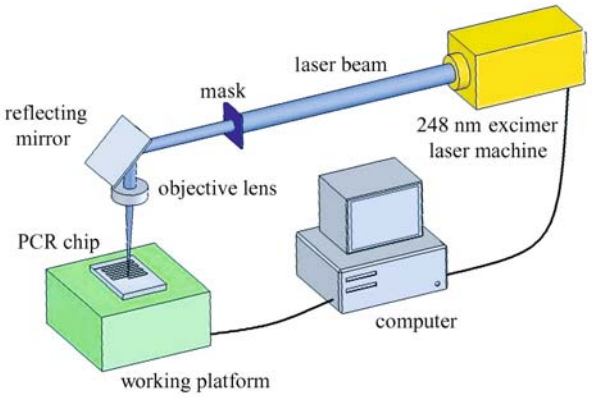

Fig. 2 Schematic view of self-developed excimer laser micromachining direct-writing system

An excimer laser machine (LPX305iF, Lambda Physik) was used in this work. Its emission wavelength is 248 nm, the maximum energy is 1200 mJ per pulse and the maximum repetition frequency is 50 Hz with pulse duration of 30 ns. The translational speed of the working platform can be adjusted between 1.5 to 60 mm/min. The reduction ratio of the objective lens is 10:1. During fabrication, the ablation area is continuously blown with compressed air of 0.2 MPa to blow away the generated impurity in the microchannel.

The influence of the working parameters on micromachining quality (microchannel's depth and surface roughness) has been analyzed in a previous paper [11]. In this work, the working parameters were set as 7.02 J/cm^2 excimer laser fluence, 20 Hz repetition frequency and 8 mm/min platform translational speed. The mask size was 2 mm × 2 mm, thus the size of the laser beam

projected on the surface of the PMMA substrate sheet was 200 μm × 200 μm.

After ablation of the microchannel, the PMMA substrate sheet was irradiated by excimer laser with lower fluence of 0.18 J/cm^2 for 30 times to make the excimer laser polishing process to further improve the surface roughness of the microchannel's bottom.

The PMMA substrate sheet ablated with a serpentine microchannel of 20 cycles was bonded with other plain PMMA cover sheets to form a closed structure by means of hot-press bonding technique.

2.2 Electrical heating system

An electrical heating system was improvised. It consisted of three groups of heating membranes, Pt100 sensors, copper blocks and PID temperature digital controllers. Pt100 sensors were attached to each heating membrane with silica gel as temperature monitors. A high thermal conductivity copper block was placed between the heating membranes and the PMMA based continuous-flow PCR microfluidic chip to provide a high thermal transfer rate and uniform temperature distribution. Two thermal insulation grooves were fabricated to form three distinct temperature zones.

The heating membranes and Pt100 sensors were packaged and connected to PID temperature digital controllers (JCS-33A, Shinko Technos Co, Ltd) with a printed circuit board. Figure 3 shows the package sketch of the heating membranes and Pt100 sensors. According to the requirement of PCR amplification, the temperatures of three heating membranes were respectively set and controlled.

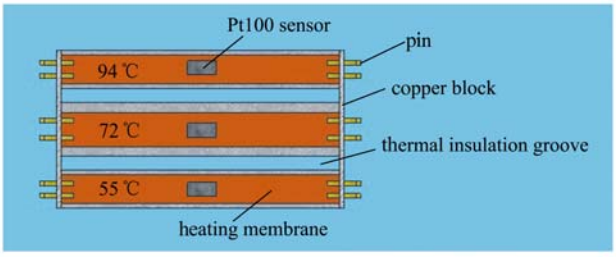

Fig. 3 Package sketch of heating membranes and Pt100 sensors (bottom view)

2.3 Continuous-flow PCR amplification

PCR amplification was carried out to validate the feasibility of the continuous-flow PCR microfluidic chip system. The DNA template was a 170 bp fragment of plasmid DNA (PGEM-T Vector). The PCR mixture with a final volume of 20 μL contained 5 × PCR buffer, 0.2 mM for each dNTPs, 0.5 unit/μL thermostable *Taq* DNA polymerase, 10 pM for each upstream and downstream primers, and 30 ng DNA templates.

The PCR temperatures were set as 94°C for denaturation, 55°C for primer annealing and 72°C for extension. An improvised miniature air pressure-vacuum pump (Patent application number: 200620012053.6) was employed for continuous delivery of samples. The flow rate for PCR was set as 40 nL/s in the microchannel, which represented a total flow-through time of roughly 15 min. After the completion of the 20-cycle PCR amplification, the product was collected in a microtube that was positioned on the top of the output capillary tube, and analyzed by agarose gel electrophoresis (2%) stained with ethidium bromide. A UV-fluorescence analyzer equipped with a CCD camera was also used to record the DNA product bands.

3 Results and discussion

3.1 Micromachining quality of the continuous-flow PCR microfluidic chip

Before hot-press bonding, the microchannel quality of the PMMA substrate sheet was viewed and measured with a non-contact 3D surface profiler (Wyko NT1100, Veeco Instrument Inc), and the data were analyzed using WYKO Vision32 software (Wyko Corp). The microchannel was inverse trapezoidal with a depth of 70 μm, top width of 200 μm and bottom width of 120 μm. Thus, the volume for each PCR cycle and the total volume were 0.92 and 19.26 μL, respectively. Excimer laser polishing has no marked damage to the profile of the microchannel, but the surface roughness *Ra* was reduced from 3.23 to 1.42 μm.

These results illustrate that excimer laser direct-writing micromachining is a predominant technique to fabricate microfluidic chips with an excellent profile of the microchannel on PMMA material. Moreover, excimer laser polishing could be used to improve the surface roughness of the microchannel to satisfy specific technical requirement in micro fluidics. The 3D simulation image (enlargement factor 10.3X) of the microchannel's cross-section treated by excimer laser polishing is shown in Fig. 4. Figure 5 shows the practical image of the 20-cycle continuous-flow PCR microfluidic chip.

The bonding performance of the PCR chip was also tested. The chip can endure a sampling pressure of over 0.1 MPa under 100°C. This indicates that this hot-press bonding method is suitable to bond PMMA sheets and the bonded chip can satisfy the sampling pressure requirement in PCR amplification (about 0.06 MPa).

3.2 Temperature condition of PCR microfluidic chip

Temperature condition is one of the most critical factors in PCR amplification. Temperature distribution between

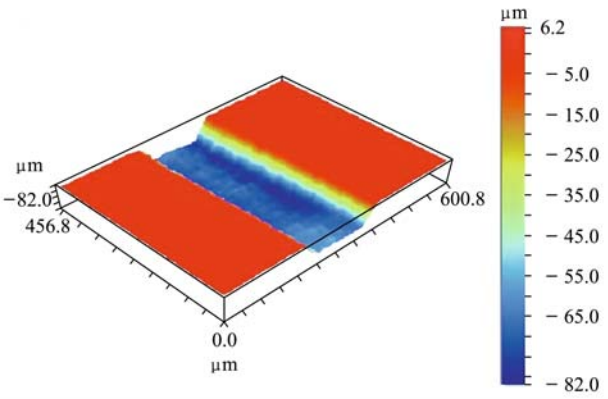

Fig. 4 3D simulation image of the microchannel's cross-section

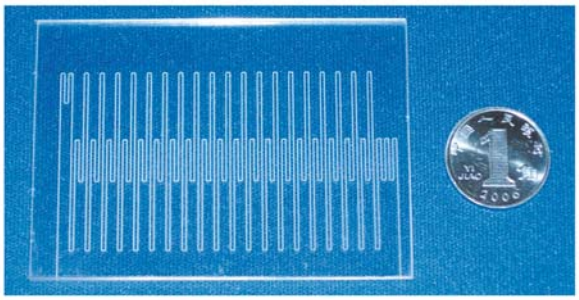

Fig. 5 Top-view of the continuous-flow PCR microfluidic chip

three temperature zones should be very distinct and maintained at a constant level in each of the zones. An infrared thermal imaging camera (ThermoView Ti30, Raytek Corp) was used to evaluate the performance of the integrated electrical heating system. The temperature distribution of the PCR microfluidic chip's surface is shown in Fig. 6. It shows that two thermal insulation grooves were effective in generating three distinct temperature zones and the temperature distribution in each temperature zone was almost uniform and met the temperature requirement of PCR amplification.

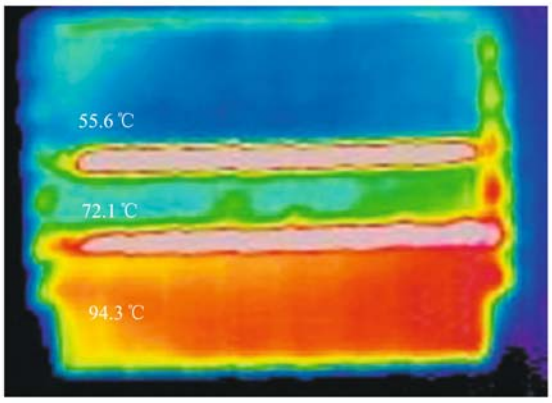

Fig. 6 Temperature distribution of the PCR microfluidic chip's surface (infrared image)

3.3 PCR performance

The collected sample after PCR amplification with the miniaturized continuous-flow PCR microfluidic chip system was analyzed in 2% agarose gel electrophoresis stained with ethidium bromide. Fluorescence was imaged by a CCD camera, as shown in Fig. 7. For reference, the PCR sample was run on a normal commercial thermal cycler (iCycler, Bio-Rad Laboratories, Inc) for 20 cycles, with cycling parameters of 94°C for 30s, 55°C for 30s and 72°C for 40s. The DNA maker has fragment sizes of 2000, 1000, 750, 500, 250 and 100 bp. It was used as a standard for the construction of calibration. From Fig. 7, it is shown that the 170 bp PCR product amplified with miniaturized continuous-flow PCR microfluidic chip system was clearly observed with a little smearing.

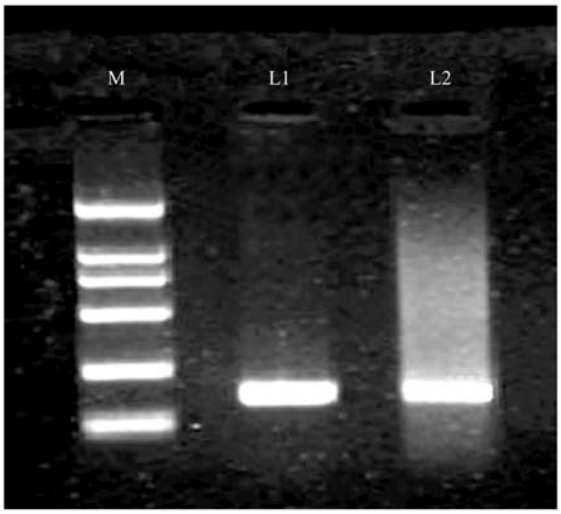

Fig. 7 Agarose gel (2%) electrophoresis of the amplification product (M: standard DNA marker; L1: reference PCR amplified in commercial thermal cycler; L2: PCR product amplified in miniaturized continuous-flow PCR microfluidic chip system)

4 Conclusions

A 20-cycle continuous-flow PCR microfluidic chip was fabricated by excimer laser direct-writing micromachining technique and hot-press bonding method on PMMA sheets. Excimer laser polishing was used to improve the surface roughness of the microchannel.

An electrical heating system consisting of three groups of heating membranes, Pt100 sensors, copper blocks and PID temperature digital controllers was developed. It could provide three distinct maintained temperature

zones on the chip surface and make the temperature distribution in each zone constant, which has been proved by infrared thermal imaging.

PCR amplification and agarose gel electrophoresis (2%) of a 170 bp DNA fragment were utilized to validate the feasibility of the miniaturized continuous-flow PCR chip system successfully.

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