RIGHT TRIANGULAR PRISM-SHAPED POLY(DIMETHYLSILOXANE) (PDMS) MICRODEVICE FOR MULTIPLEX PCR EMPLOYING A SINGLE HEATER

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ABSTRACT

We propose a right triangular prism-shaped microdevice with serpentine microchannels fabricated on its slanted plane, and apply the device for on-chip flow-through PCR employing a single heater. The inclined nature of the qiandu-shaped microdevice enables the formation of a surface temperature gradient along the slanted plane of the microchannel by the use of a single heater. A 409 bp lung cancer biomarker and a plasmid vector targeting a 230 bp gene fragment were simultaneously amplified in less than 30 min on a single microdevice, paving the way for a microscale, multiplex PCR on a single platform with a single heater.

KEYWORDS

Right triangular prism-shape, PDMS microdevice, flow-through PCR, single heater, thermal gradient, multiplex PCR

INTRODUCTION

Multiple temperature control in an on-chip continuous-flow PCR hinders device miniaturization and integration requiring multiple heating accessories, and leads to operational complexity. This paper proposes the fabrication of a right triangular prism-shaped poly(dimethylsiloxane) (PDMS) microdevice engraved with a serpentine microchannel on its inclined plane, and its potential application for performing an on-chip continuous-flow polymerase chain reaction (PCR), including a multiplex PCR, employing a single heater. The proposed microdevice combines the low thermal conductivity of the device material (PDMS) with a right triangular prism shape to create a height-dependent temperature gradient along its inclined surface, using a single heater. In this way, the number of heater is reduced to one, and the overall footprint of the microdevice is subsequently decreased, both of which are considered two main drawbacks when performing spatial PCRs on a miniaturized platform.

Similar concepts were previously demonstrated to create a linear thermal gradient on a glass surface for DNA melting curve analyses [1], or to create a linear thermal gradient on a glass surface for hold-less thermocycling for PCR [2]. The formation of the thermal gradient mentioned above, however, is based on the heat flow in two dimension between the heating and cooling elements aligned in parallel, where the cooling element is required for maintaining stable thermal gradient due to high thermal conductivity of the glass (1.1 $W \cdot K^{-1} \cdot m^{-1}$).

Compared to the previous works, the right triangular prism-shaped microdevice proposed in this work enables the formation of a temperature gradient along its inclined plane without utilizing a cooling unit. Instead, stable temperature gradient is established between the air and the microdevice fabricated using a material with relatively low thermal conductivity such as PDMS whose thermal conductivity is 0.16 ~ 0.2 $W \cdot K^{-1} \cdot m^{-1}$. In this regard, simply by controlling the inclination angle, wide ranges of temperatures, including three temperature zones – denaturation, annealing, and extension temperatures – necessary for performing a typical *in vitro* enzymatic amplification, the PCR, can be created on the inclined surface of the microdevice employing a single heater.

The proposed microdevice is an improved format compared to our previous study also demonstrating on-chip continuous-flow PCR employing a single heater [3] in that the newly proposed microdevice is specialized for its simplicity in design, fabrication, and operation for performing on-chip continuous-flow PCR, while still retaining to the use of a single heater. Most of all, the proposed microdevice is highly potential for performing a multiplex PCR. A 409 bp-long gene fragment effective as a marker for diagnosing lung cancer and a 230 bp-long gene fragment from a plasmid vector were simultaneously amplified successfully in less than 25 min on a single microdevice using a commercially available hot plate, paving the way for a microscale, multiplex PCR on a single device employing a single heater.

EXPERIMENT

Figure 1 illustrates a comparison of the temperature controls when employing a right triangular prism-shaped microdevice and a thermocycler.

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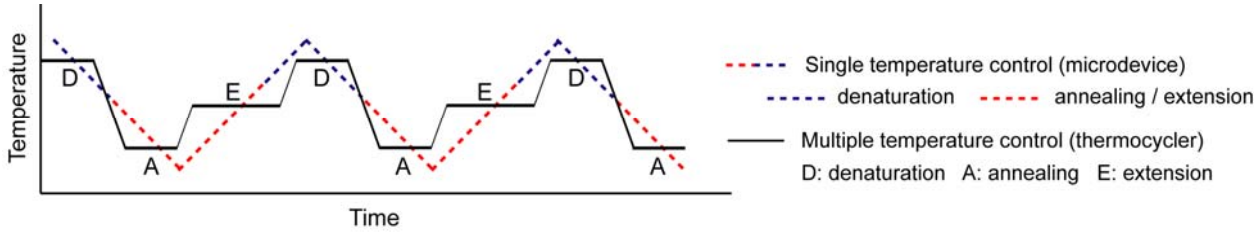

Figure 1. Schematics comparing temperature transition patterns when employing a thermal cycler (straight line) and a right triangular prism-shaped microdevice (dotted line).

Figure 2 shows the overall fabrication scheme of the microdevice proposed in this study. A serpentine microchannel whose width, depth, and total length were 200 μm, 75 μm, and 2.9 m, respectively, was fabricated. A total of 32 thermal cycles were designed for the amplification. Si master having photolithographically patterned SU-8 microchannels was placed inside a cuboid container, and the container was slanted to a predetermined angle in order to acquire the desired inclination (Figure 2a). A 10:1 (w/w) mixture of the PDMS prepolymer and curing agent was poured inside the container on the Si master (Figure 2b). The PDMS prepolymer was cured at 80°C for 30 min, and then peeled off the Si master (Figure 2c). The PDMS replica was flipped over, and the flat bottom of the PDMS was treated with oxygen plasma (90 W, 0.7 torr, 40 s) (Figure 2d). After thermally bonding with a plasma-treated glass substrate (Figure 2e), the microchannel-patterned side was also treated with oxygen plasma under the same conditions (Figure 2f). A thin and flat sheet of PDMS was then permanently bonded with the microchannel-patterned, slanted plane of the resulting right triangular prism-shaped PDMS (Figure 2g). Two through-holes (~ 1 mm) were punched into the thin PDMS sheet at the inlet and outlet positions of the serpentine microchannels patterned on the slanted plane, and silicone tubes (i.d. 1 mm, o.d. 2 mm) were then inserted into the ports for the introduction of fluids (Figure 2h).

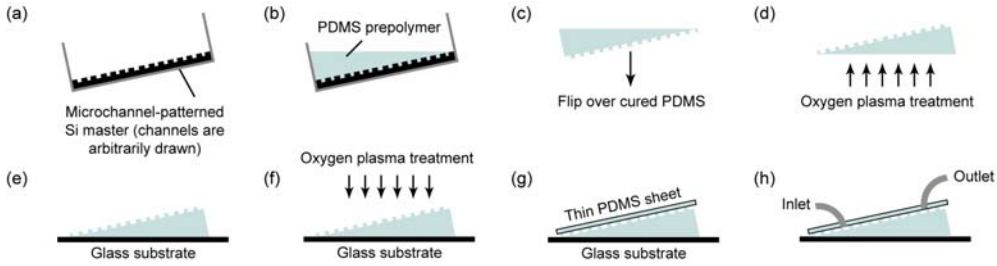

Figure 2. Schematic illustration for the fabrication of a right triangular prism-shaped microdevice.

Figure 3 shows the captured IR camera images with a temperature gradient generated on the slanted plane of the right triangular prism-shaped microdevice whose inclination angle was 50°, and placed on the center of a hot plate. As shown in Figure 4a, the temperature gradient was divided into ten regimes based on similarities in temperature, and each color band represents 8 – 10°C variations in temperature. Height-dependent temperature gradient was well established regardless of the plane – slanted or vertically straight – of the microdevice as shown in Figure 4b. The average temperatures of the highest (slanted bottom) and lowest (slanted top) regions were measured to be 94.8 ± 0.6°C and 53.1 ± 0.4°C, respectively. A temperature of 71.1 ± 0.6°C was measured at the center region of one specific color band. This demonstrates the potential of performing a multiplex PCR for amplifying 230 bp (T_m = 72.0°C) and 409 bp (T_m = 60.0°C) gene fragments on the same microdevice. The temperature gradient was stably maintained on the entire surface of the slanted plane over a 1 h time period without fluctuation.

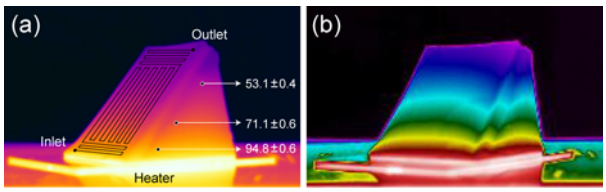

Figure 3. IR camera images showing temperature gradient created on the inclined surface of the microdevice with a 50° inclination angle.

Figure 4 shows the time-dependent surface temperature stabilization tendencies measured over 1 h time course using the IR camera. Although the temperature gradient on the slanted surface can be affected by the conductive heat transfer within the device as well as the natural convective heat transfer between the device surface and the surrounding atmosphere, it took approximately 10 ~ 15 min to establish a stable temperature gradient on the slanted surface of the microdevice, and the established temperature was stable over 1 h as shown in Figure 4.

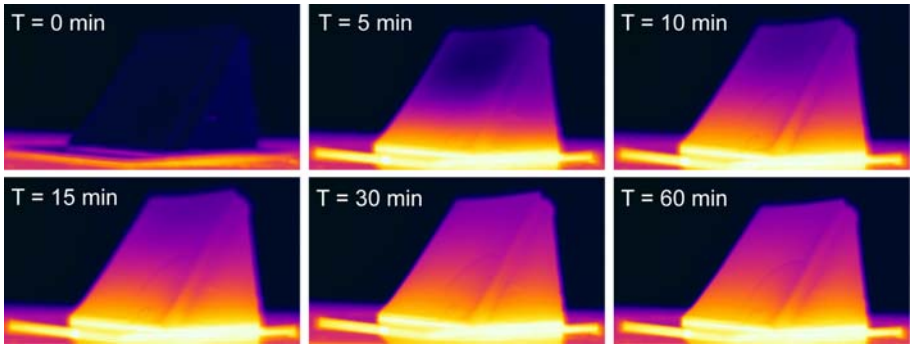

Figure 4. Time-dependent surface temperature stabilization tendencies measured on the slanted surface of the right triangular prism-shaped microdevice over 1 h.

Figure 5 shows the results of preliminary experiments performed using two right triangular prism-shaped microdevices with varying inclination angles (a ~ c) and the actual result of multiplex PCR (d). Figure 5a shows the results of a 230 bp gene fragment amplified from a pGEM-3Zf(+) plasmid vector using a PDMS microdevice with a 12° inclination angle (lanes 1 and 2) and a thermocycler (lane 3). Figure 5b shows the results of a 230 bp gene fragment amplified from a pGEM-3Zf(+) plasmid vector using a thermocycler (lane 1) and a PDMS microdevice with a 50° inclination angle (lane 2). Figure 5c shows the results of 409 bp gene fragments amplified on the PDMS microdevice with a 50° inclination angle (lane 1) and using a thermocycler (lane 2). Figure 5d shows the result of a multiplex PCR performed using a microdevice with a 50° inclination angle. Lanes 1 and 2 in Figure 5d show target amplicons obtained using a thermocycler and a PDMS microdevice, respectively. As were demonstrated in Figure 5d, two templates with significantly varying optimal annealing temperatures for their primers were successfully amplified via multiplex PCR on a single microdevice, demonstrating the versatile applicability of the right triangular prism-shaped microdevice proposed in this study.

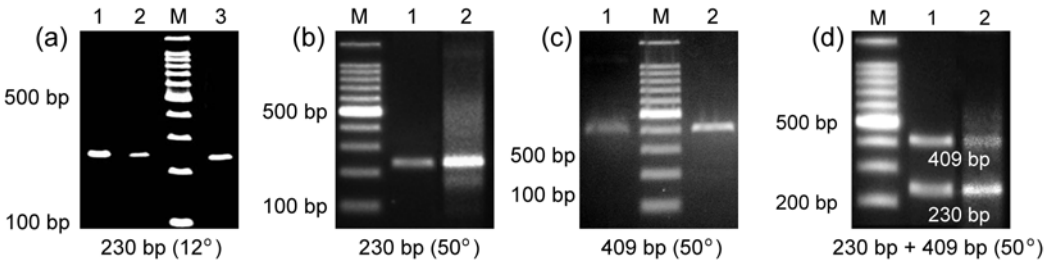

Figure 5. DNA amplification results. (a ~ b) 230 bp target amplicons obtained using microdevices with 12° and 50° inclination angles. (c) 409 bp target amplicon obtained using a 50° inclination angle. (d) Result of a multiplex PCR using a microdevice with a 50° inclination angle.

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