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A microheater on polyimide substrate for hand-held realtime microfluidic polymerase chain reaction amplification

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Abstract

The development of a DNA microfluidic device with a high speed, low power, and low reagent volume is very critical for real-time genotyping and diagnosis in point-of-care applications. This paper reports a polymer-based thermal cyclers for a handheld and battery-powered polymerase chain reaction (PCR) system using a polyimide (PI) film-based micro-fabricated heater module and polymer film microfluidic chambers of 10 μ L, with a handheld and low power consumption, compared to state of the art. It took 21 min for 40 thermal cycling for DNA amplification and a maximum power consumption of 0.6 W. The microheater on PI film substrate fabricated and real-time quantification of deoxyribonucleic acid (DNA) using the heater in hand-held sizes experimentally shown here. The device would be applicable for on-site molecular diagnostics.

Keywords: Portable, Polymer-based thermal cyclers, Polymerase chain reaction, Molecular diagnostics, Point-of-care testing

Introduction

The design and fabrication of a DNA microfluidic devices with a high speed, low power, and low reagent volume is very important for real-time genotyping and point-of-care testing for disease diagnosis [1, 2]. The work builds on a microfabrication process of the polymer-based heating module reported before [3–7]. At first we had reported a thermal cyclers with a polymer chamber and a silicon-based MEMS heater. The device showed a very fast operation and very low power consumption, however, there are problems like fragility, high fabrication cost and low heat capacity of micro-heater [3, 4]. Next, we had developed a thermal cyclers with a silicon micro-chamber and a polymer-based heating module [5–7]. The device showed a reasonably fast operation, low power consumption, and robustness of heaters. Because of excellent thermal conductivity of silicon, the PCR has worked well. However, there are problems like high

fabrication cost and fragility of silicon micro-chambers [5–7]. This time, we have developed a polymer-based thermal cyclers, consisting of polymer micro-chambers and the polyimide (PI)- or flexible printed circuit board (FPCB)-based heating modules. Advantage for using PI as a substrate for microheaters is its compatibility with silicon micro fabrication processes with benefits of thermal and chemical endurances, cost effectiveness and clean room fabrication procedures with less instrument usage. Through proper device designing and including integration of double thin metal layers into the polymer heating module for good thermal uniformity, we have successfully implemented a polymer-based thermal cyclers [8]. Here, we reported on the design, microfabrication, and experimental results on the polymer-based thermal cyclers for a battery-based and real-time DNA amplification system. The method reported here differs from previous works [3,4] in the specific heater materials, [5–7] in the geometry of heating modules and their placement within the microfluidic device structures.

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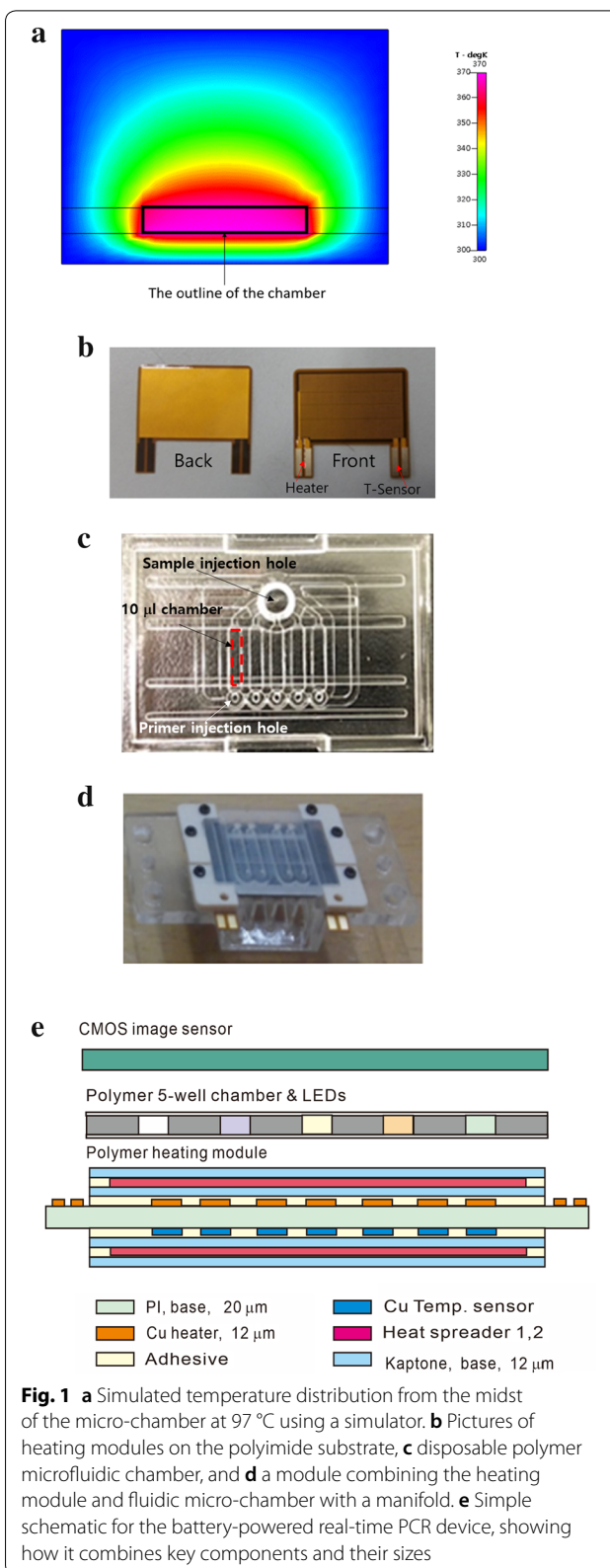
Design

The heat distribution in the microchamber was simulated based on buffer solution conditions using CFD-ACE+ simulator (Computational Fluid Dynamics Research Corporation (CFDRC), Alabama, US). In case of heating evenly on surface, we could see that the temperature sustained uniformly in the chamber, as shown in Fig. 1a. The utilized parameters for the simulation bring from the previous report [3]. The system consists of a disposable microfluidic component with a polymer film-based cartridge and a polymer FPCB micro-heating module, a sample-preparation kit, and a miniaturized fluorescence meter. A 100 μm -thick polymer FPCB film-based micro-heating modules, integrating a copper film heater, a temperature sensor, and two heat-spreading metal film layers, and the disposable component has five 10 μL microfluidic chambers in polycarbonate films, a module combining the heating module and fluidic micro-chamber with a manifold as shown in Fig. 1b–d respectively. The simple, facile, and embedded fluorescence meter using the LEDs and CMOS image sensors, has implemented for quantification of amplicon.

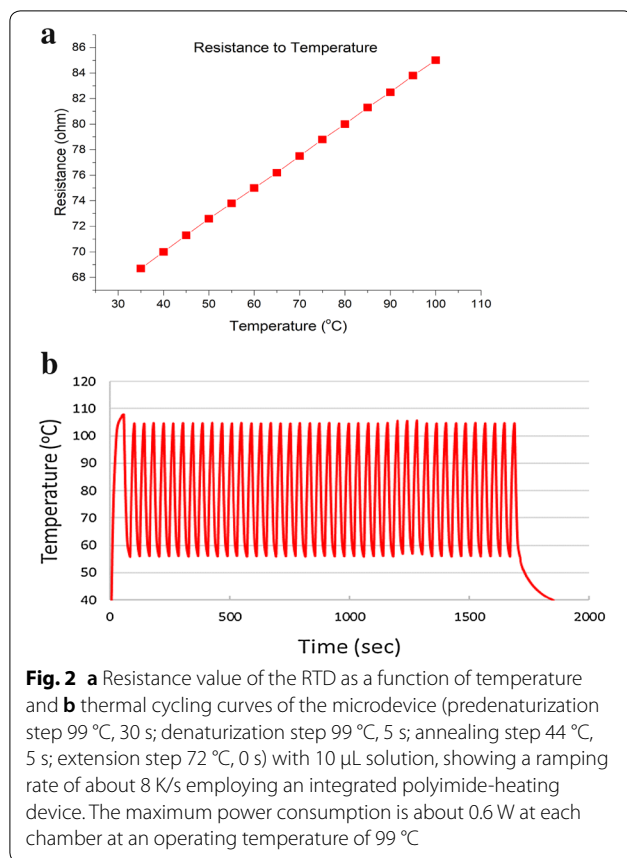
Results and discussion

A schematic view of the thermal cycler for polymer-based and miniaturized PCR system showed in Fig. 1e, with a close-up detail of the polymer heating modules and placement of all modules in the devices. The pictures of the micro-fabricated heater modules and the 10 μL -volumed polymer chamber cartridge showed in Fig. 1d. The thermal properties of the heating elements on the polyimide-based film component, including the temperature-consumption power and the time response curve, observed. Top copper resistive lines utilized as a heater and a temperature sensor, respectively. The copper resistance temperature device showed linear relationship between the temperature and resistance for 35–100 $^{\circ}\text{C}$ and an approximate temperature coefficient of resistance (TCR) of 4400 ppm/K was determined as shown in Fig. 2a. The initial resistance of heater is 34 Ω and the resistance of the resistance temperature detector (RTD) is 65 Ω in the room temperature. The polymer heating modules shows the heating and cooling rate of about 8 $^{\circ}\text{C}/\text{s}$ and the low power consumption of about 0.6 W at each chamber at maximum working temperature of 96 $^{\circ}\text{C}$ (Fig. 2).

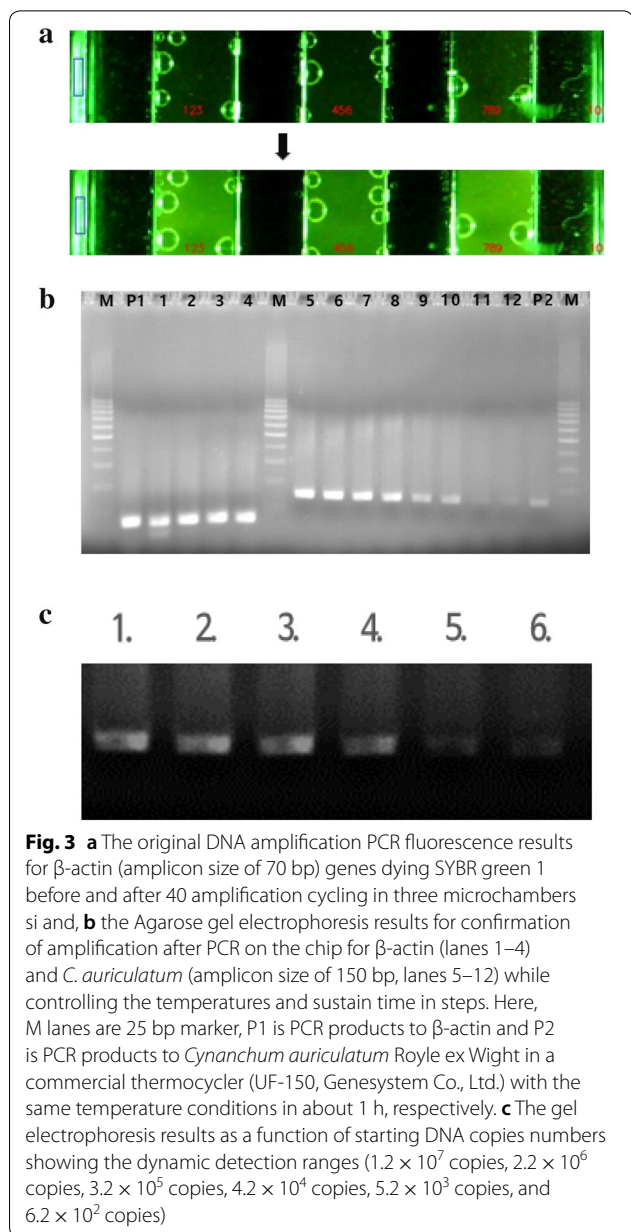
To investigate DNA amplification performances of the thermal cycler, two kinds of DNA sequence, β -actin, 70 bp, one of six different actin isoforms which have been identified in humans; and *Cynanchum auriculatum* Royle ex Wight (*C. auriculatum*), 150 bp, a species of climbing vine swallowworts) have been utilized. For extraction of DNA, we utilized a laboratory-made kit DNA extraction



kit. It took about 26 min totally to prepare, amplify and analyze DNA templates consisting of the sample preparation, and amplification and analysis of the DNA



amplicon. It took about 21 min for 40 thermal cyclings and a pre-denaturation as shown in Fig. 2b. In order to confirm DNA amplification using the microdevice, the original DNA amplification PCR fluorescence results for β -actin (amplicon size of 70 base pairs (bp)) genes dying SYBR green 1 before and after 40 amplification cycling were shown in Fig. 3a. We did control the three key temperatures (denaturation, annealing, and extension), sustaining times, and cycling numbers to get optimal working conditions by observing the intensities of Agarose gel electrophoresis band, as shown in Fig. 3b. As results, we determine to be 40 thermal cyclings of 99 °C for 5 s, 44 °C for 5 s with a predenaturation step of 99 °C for 30 s in three microchambers simultaneously, resulting in getting an excellent DNA amplification results. Furthermore, we have confirmed analysis performance with a high sensitivity ($\sim 5 \times 10^4$ /mL) and a low detection limit ($\sim 5 \times 10^2$ copies), as shown in Fig. 3c. It also showed a very high specificity ($\sim 99\%$) (not shown here).



Conclusion

In this study, we demonstrated that the designed and microfabricated microheater on PI film substrate showed the reliable thermal responses throughout forty thermal cyclings. We also demonstrated real-time quantification of DNA of both β -actin and *C. auriculatum* using the microheater, successfully through the analysis of the fluorescence and gel electrophoresis results. This device would be applicable to a molecular diagnostics and analysis tool in point-of-care applications.

Abbreviations

DNA: deoxyribonucleic acid; PCR: polymerase chain reaction; PI: polyimide; FPCB: flexible printed circuit board; LEDs: light-emitting diodes; CMOS: complementary metal oxide semiconductor; TCR: temperature coefficient of resistance; CFDRC: Computational Fluid Dynamics Research Corporation; RTD: resistance temperature detector; bp: base pairs; *C. auriculatum*: *Cynanchum auriculatum* Royle ex Wight.

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Authors' contributions

DS performed the device fabrication, experiments, analyzed the data and wrote the manuscript. OK carried out device characterization. DS and YJ supervised the research and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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