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All-plastic, low-power, disposable, continuous-flow PCR chip with integrated microheaters for rapid DNA amplification

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ABSTRACT

The design, fabrication and evaluation of a low-cost and low-power, continuous-flow microfluidic device for DNA amplification by polymerase chain reaction (PCR) with integrated heating elements, on a commercially available thin polymeric substrate (Pyralux® Polyimide), is presented. The small thermal mass of the chip, in combination with the low thermal diffusivity of the polymeric substrate on which the heating elements reside, yields a low power consumption PCR chip with fast amplification rates. A flow-through μPCR device is designed and fabricated using flexible printed circuit (FPC) technology on a foot-print area of 8 cm × 6 cm with a meandering microchannel realized at a very small distance (50 μm) above 3 independently operating resistive (copper) serpentine microheaters, each one defining one of the three PCR temperature zones. The 145 cm-long microchannel is appropriately designed to cross the alternating temperature zones as many times as necessary for the DNA sample to perform 30 PCR cycles. Numerical computations lead the design so that there is no thermal crosstalk between the 3 zones of our chip and indicate excellent temperature uniformity in each zone. In addition, the total power consumption during the chip operation is calculated to be in the order of a few Watts, verified experimentally by means of thermal characterization of our heaters. Thermal camera measurements also verified the excellent temperature uniformity in the three thermal zones. An external, home-made temperature control system was utilized to maintain the heater temperatures in the designated values (±0.2 °C). The PCR chip was validated by a successful amplification of a 90 base-pairs DNA template of the mouse GAPDH housekeeping gene within 5 min.

1. Introduction

Micro-total analysis systems has been a research field of increasing interest in the past years, enabling biochemical analysis for point-of-care (POC) applications, as it favorably combines advantages such as low reagent consumption and short analysis time, in devices of small footprint, and thus of increased portability and low fabrication cost [1,2]. Miniaturized polymerase chain reaction (μPCR) devices are expected to become a central part of most lab-on-a-chip (LOC) or POC systems intended for molecular or clinical diagnostics [3], and food safety monitoring [4,5]. PCR, a widespread biochemical process for amplifying trace amounts of DNA in a sample to generate copies sufficient for detection, is based on repeated thermal cycling of the DNA sample through three temperature steps (denaturation, annealing, and extension). After each cycle, the number of the DNA copies can be doubled, and thus 20–30 cycles can lead to millions of DNA copies. PCRs performed in microfluidic devices provide much faster processing, in the order of minutes rather than hours, compared to conventional thermocyclers [6], while allowing the production of highly integrated devices [7].

Two basic types of designs have been followed by researchers for the realization of μPCR devices: stationary chambers with cycling temperature, or continuous-flow devices typically with three zones maintained at constant temperatures and only the sample changing temperature as it flows through the zones. μPCRs with static chambers were historically the first to be realized [8], however continuous-flow devices have been proven faster [9] than the static ones. The choice of the type of μPCR design drives in many cases the

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choice of the material to be used for the fabrication of μPCR devices, in order to best exploit the advantages of each material for improving the device operation. For example, Silicon (Si) [8] has preferably been used for static devices, where the high thermal conductivity of the material favors fast heating or cooling rates, however to maintain high temperature uniformity within the sample, thermal isolation of the device is necessary. On the other hand, materials with low thermal conductivity, such as glass [9] or plastics [10], are preferable for continuous-flow devices, where temperature cycling of the entire device (large thermal mass) is avoided, while the temperature uniformity and low power consumption is ensured, under conditions, by the good thermal isolation provided by these materials. Plastics, such as polydimethylsiloxane (PDMS) [11], polymethylmethacrylate (PMMA) [12], polyimide (PI) [13–15], and printed-circuit-boards (PCB) [16] have a cost advantage over Si and glass, as well as ease of processing [17]. The lower fabrication cost of plastic μPCR devices combined with the capability for mass production of such devices makes plastics the material of choice for the fabrication and commercialization of μPCRs as well as their capability to be integrated into larger more complicated LOC systems [3,7,18].

Another important feature of μPCRs is the heating system required for performing PCR. Although external systems such as IR lamps [13], heating blocks or Peltier elements [11,19] have been mostly used in μPCRs, integration of microheaters and temperature sensors on the device becomes increasingly popular, due to the advantage it offers for a more compact and thus of increased portability system. Thin film microheaters are usually deposited on glass [20], and thus most of the μPCRs integrated with microheaters are hybrid devices [10,21,22] combining Si or plastic and glass, therefore hindering the mass production of such devices.

In this work, an all-plastic continuous-flow μPCR device is designed and fabricated with integrated heating elements, on a commercially available, biocompatible [23,24], very thin (100 μm) PI substrate (Pyralux®), utilizing FCB-compatible technology, amenable to mass production. To the best of our knowledge, polyimides have been used so far as substrates either for the realization of static μPCR chambers [13], or for the fabrication of resistive heating elements bonded to glass-based static chambers [14]. In spite of the undisputed advantages of continuous-flow μPCR devices, such devices have not yet been demonstrated on PI, and more specifically on Copper(Cu)-clad PI that allows the integration of microfluidic channels and resistive heating elements on the same substrate [25], without requiring an additional metal deposition step. Furthermore, the use of a very thin, thermally isolated PI substrate is expected to promote further the capability for even lower power consumption required for the device thermal operation, increasing the possibility of making a battery-operated and portable μPCR. Numerical calculations are performed to (a) demonstrate the advantages of a thin plastic substrate for μPCR, in terms of power consumption, thermal isolation between the PCR zones and temperature uniformity of the zones and (b) suggest suitable respective operating parameters. Finally, the fabricated μPCR is validated for successful and rapid DNA amplification.

2. Materials and methods

2.1. Chip design

A schematic of the continuous flow μPCR device comprising both a microfluidic circuit and resistive microheaters is illustrated in Fig. 1. This device, which is an improved version of a design presented previously [25], implements 30 thermal cycles in total for efficient amplification of DNA. Each cycle features three thermal steps, hence three discrete thermal zones are designed, each controlled by an individual resistive microheater.

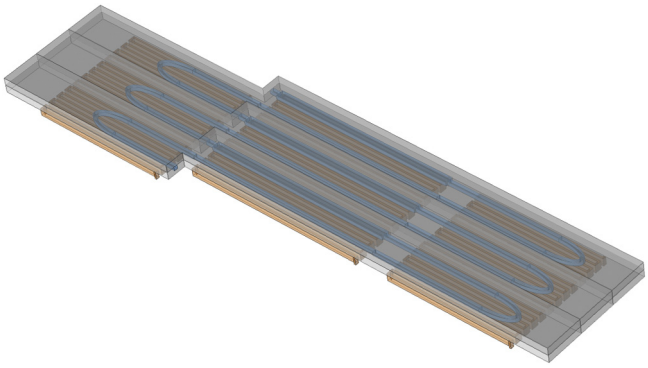

Fig. 1. A 3d schematic of the proposed flexible μPCR chip comprising a meandering microchannel (shown in blue) and resistive microheaters (shown in brown). Only three unit cells, each one implementing a thermal cycle, are shown.

The meandering microchannel, of a total length of 1.45 m, where the DNA sample flows continuously, is designed to cross the three alternating PCR temperature zones, so that the arclength along the channel axis, l, lying above each one of the zones is the same ($l_{de} = l_{an} = l_{ex} = 1.2$ cm). Since the device is designed to accommodate PCR protocols with relative time ratios of 1:1:2 for denaturation:annealing:extension, the channel width at the extension zone is designed twice as much of that at the denaturation and annealing zones, so that the DNA sample residence time in the three zones follows the same ratio, i.e., 1:1:2. Thus, a width of 400 μm was chosen for the extension zone microchannel, and 200 μm for the denaturation and annealing microchannels. The choice of doubling the extension microchannel width as well as using a microchannel minimum feature size at 200 μm further optimized our previous design [25], facilitating the chip fabrication and achieving lower pressure drop across the channel, thus increasing the device sealing durability.

The integrated resistive microheaters are designed to be fabricated at the bottom Cu layer of the commercially available Cu-clad PI substrate (Fig. 1). The resistors are also meandering, so as to provide the largest possible electrical resistance in the space available for each heating zone, thus ensuring sensitive temperature control. The Cu lines are 100 μm in width, 2 m in length for denaturation, 2.1 m for extension, and 1.5 m for the annealing microheater. The separation between the heating zones is determined through simulation for minimal thermal cross-talk. According to the geometrical characteristics of the Cu lines, their electrical resistance is calculated to be 23 Ω for denaturation/extension, and 30 Ω for the annealing microheater.

2.2. Heat transfer and fluid flow calculations in the device

The design of the μPCR device was assisted by simulations aiming at calculating (a) the temperature uniformity in each one of the μPCR zones, (b) the pressure drop in the microfluidic channel, and (c) the power requirements for heating the device. Regarding the first aim of the simulation, the requirement for a high performance μPCR device is uniform temperature of the fluid (DNA sample) within the zones: The fluid temperature variation should be less than 3 K [26] (±1.5 K) in the denaturation, the annealing, and the extension zone. Given that the fluid conveys heat from one zone to the other, the simulation suggests the suitable operating conditions to inhibit thermal "cross-talk" between the zones. The advantage of using polymeric layers instead of SiO_2 or Si layers as the device substrate was demonstrated in a previous work [27]; the temperature uniformity was much better in the device with