Development of a Slug-Flow PCR Chip with Minimum Heating Cycle Times

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ABSTRACT

A microfluidic PCR device is presented capable of rapid temperature ramping and handling of sample volumes in the microliter and submicroliter range. The PCR chip comprises a straight micro channel in which a sample slug is periodically moved over three temperature zones. As part of the sample preparation a method for metering of the sample volume was developed. The PCR chip and the chips for sample preparation and fluidic actuation were fabricated by ultra-precision milling in polymer substrates. Computer simulations suggest that the sample slugs are heated or cooled on a time scale of some ten milliseconds when transported to a different temperature zone. The fluidic actuation based on a ferrofluid transducer is capable of positioning the sample volumes with a high accuracy after a large number of cycles. The design developed should be ideally suited for fast PCR of small sample amounts in a highly parallel manner.

Keywords: Microfluidics, PCR, heat transfer, slug flow.

1 INTRODUCTION

The polymerase chain reaction (PCR) is an enzyme catalyzed reaction for amplification of nucleic acids requiring three different temperature levels. At the highest temperature, a denaturation of double-stranded DNA takes place. The lowest temperature is required for annealing of primers being attached to the DNA strands, whereas at the intermediate temperature an extension step occurs using the existing strands as templates. In the past decades the PCR reaction has become one of the most important building blocks of nucleic acid analysis and has found a plethora of applications in such fields as medical diagnostics.

A number of systems allowing to perform the PCR on a microfluidic chip have been reported, among others the continuous flow chip developed by the group of Manz [1]. The solutions presented so far suffer from a number of disadvantages. As an example, a pressure-driven continuous flow device usually exhibits a considerable sample dispersion, thus preventing that all sample fractions can be processed at the same speed. Furthermore, many devices are not well suited for parallel, multiplexed PCR and require comparatively long heating cycle times, thus resulting in considerable time requirements for nucleic acid amplification. In addition, the processing of small samples in the micro- or submicroliter range is often not taken care of. The PCR system presented in this article was designed to eliminate some of these disadvantages.

2 DESIGN AND FABRICATION OF A TEST SYSTEM

In order to avoid cross-contamination of different samples, a PCR chip should ideally be designed as a disposable. Due to their low material and processing costs polymers are perfectly suited for corresponding Lab-ob-a-Chip applications. The feasibility of PCR amplification in polymer chips was proven earlier by Yang et al. [2]. The design concept which is studied in this article and was realized in polymers is sketched in Fig. 1.

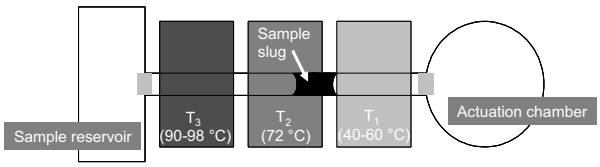

Fig. 1: Design principle of the slug-flow PCR chip.

A sample reservoir is connected to an actuation chamber via a micro channel in which a liquid slug is transported. The chip comprises three temperature zones corresponding to the temperatures required for the different steps of the PCR protocol. The sample slug is surrounded by air and stands in pneumatic contact with the actuation chamber. Through pneumatic transduction the slug is periodically transported between the temperature zones and is positioned in the center of each zone as appropriate. A similar principle based on a periodic motion of a sample slug between temperature zones has previously been reported [3,4]. However, in these studies no integrated microsystem was used and the slug was surrounded by an organic liquid phase.

For the experimental studies several test chips were realized in Poly (methyl methacrylate) (PMMA) and Cyclo olefin copolymer (COC) by ultra-precision milling

(Precitech Nanoform 350). In order to study different channel geometries, three straight channels with a depth of 200 µm and a width of 200, 600 and 1000 µm, respectively, were placed on each chip. At both ends of each channel ports for connection with a sample reservoir and an actuation chamber were provided. The polymer substrates were sealed with polymer sheets of 125 µm thickness by solvent bonding.

The PCR chip is coupled to a sample preparation chip and an actuation chip via fluidic interfaces. The complete setup of three chips in a row is placed on a metal rack which also provides the housing for three heating blocks. The heating blocks are connected with each other and comprise rectangular temperature zones on which the PCR chip is placed. In each of the blocks a heating cartridge and a thermocouple is inserted. A feedback control loop for the thermal management of the device was realized within the LabView (National Instruments) environment.

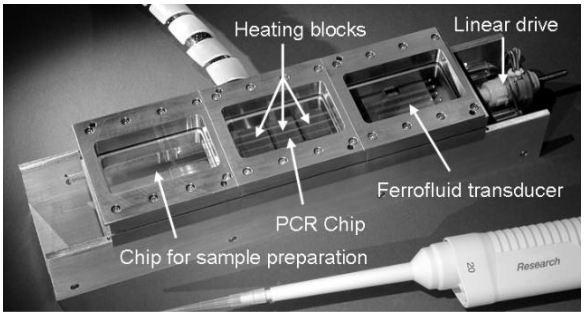

Fig. 2: Setup of a test system for on-chip PCR comprising a sample preparation and an actuation chip besides the actual PCR chip.

The complete test system comprising the three chips is shown in Fig. 2. One of the key components in this context is the actuation mechanism. The actuation chip contains a chamber partly filled with a ferrofluid (Ferrofluidics APG 047 n). Below this fluidic transducer a permanent magnet is moved by a linear drive. The ferrofluid follows the motion of the permanent magnet and displaces the sample slug in the PCR chip via pneumatic transduction. That way a periodic motion of the magnet is translated to a periodic motion of the slug which can be positioned within the temperature zones with a high accuracy.

3 SAMPLE PREPARATION

On of the basic steps performed in the sample preparation chip is the metering of a liquid slug from a larger volume. Typical sample volumes in which the PCR should be carried out are in the range of 1 µl. The series of steps allowing to create a sample slug of corresponding size is depicted in Fig. 3. The key element is a three way channel junction. In the channel extending to the right a ferrofluid volume is placed with the help of a permanent magnet. The sample liquid is introduced from above, being transported through the channel extending to the left while the flow through the right channel is blocked by the ferrofluid. By subsequently moving the ferrofluid volume to the right a well defined amount of sample liquid is sucked into the channel provided for sample transfer. The actual metering occurs when the residual liquid volume is blown out with air. In this manner a well defined sample slug remains which can be transferred to the PCR chip.

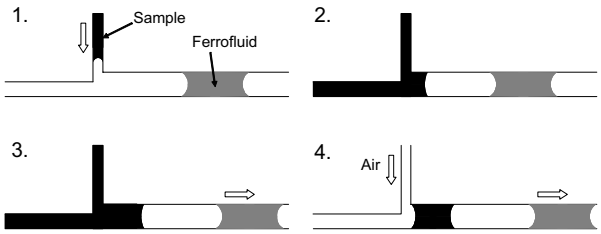

Fig. 3: Description of the steps employed for metering of the sample slug.

4 CFD SIMULATIONS

As noted by by Giordano et al. [5], among others, the time required for thermal ramping usually determines the time scale of a PCR cycle when the protocol is executed for DNA strands with a moderate number of base pairs. Under these conditions a reduction of the cycle times can only be achieved when the heating and cooling of the sample volume is speeded up. Typical heating and cooling rates of conventional thermocyclers are in the range of 2-10 K/s [6]. One of the drivers behind the development of the PCR system presented here is the reduction of the thermal ramping time scale.

As the temperature inside the sample slug is quite difficult to measure, simulations employing methods of computational fluid dynamics (CFD) were performed to study the flow and temperature distributions. In addition, such simulations allow for parameter studies which can not easily be conducted experimentally.

In order to study the heating of a liquid slug, the Navier-Stokes equation, the mass conservation equation and the enthalphy equation were solved on a structured hexahedral grid using the commercial finite-volume solver CFX5.6 (Ansys-CFX). For the free-surface flow problem the volume-of-fluid (VOF) method [7] was used assigning a "color" field to the different fluids, where a value of 1 indicates fluid 1 and a value of 0 fluid 2. Within the VOF method basically an advection equation for the color field is solved. A liquid slug with a length of 5 mm was initialized in a channel with a width of 1 mm and a depth of 200 µm (being the widest channel found on the test chip). The properties of water and air were chosen for the liquid and the gas phase, respectively. A value of 90° was assumed for the contact angle. In order to safe computational time, the problem was solved in the frame-of-reference co-moving

with the slug. In that manner the number of computational cells required was reduced considerably. The channel walls were assumed as isothermal, having a temperature of 72 °C. The initial temperature of the fluids was set to 52 °C.

Simulation results for the temperature field as a function of time based on 46,000 grid cells are shown in Fig. 4. In these simulations a slug velocity of 2.5 cm/s was chosen, a realistic value easily achievable with the ferrofluid transducer. The figure shows the temperature field over a cut through the center of the channel for different times (in ms) indicated on the left. Each of the patches shown has a height of 200 µm corresponding to the channel height. The temperature range extends from 52 °C (white) to 72 °C (black).

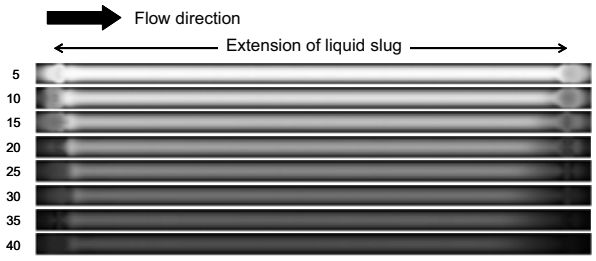

Fig. 4: Temperature field in the PCR channel as obtained from a CFD simulation.

As apparent from the figure, the temperature field is quite uniform over the slug, with gradients mainly occurring in the vertical direction. The figure also indicates heating times of some ten milliseconds. In order to quantify the heating time more precisely, the average temperature over a patch cutting through the center of the channel was determined by integration. This provides an upper bound to the heating time since liquid volumes located closer to the channel walls are disregarded in that way.

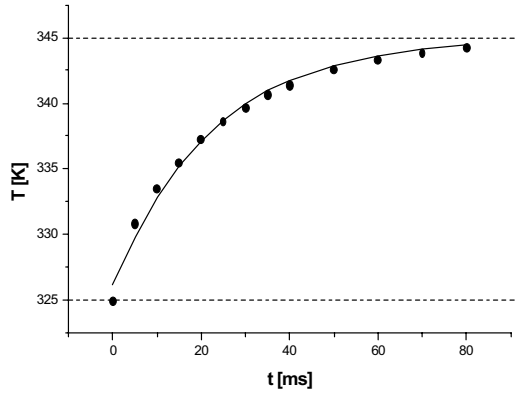

Fig. 5: Average temperature over a central plane cutting through the sample slug as a function of time.

In Fig. 5 the average temperature obtained in such a way is displayed as a function of time. The data points represent the CFD results and the solid line was obtained from a fit to the data using an exponential approach to the wall temperature. A temperature differing by less than 1 K from the wall temperature is reached after only 80 ms. The rapid heat transfer from the channel walls to the slug is due to two different effects. On the one hand, the small thermal diffusion path allows a rapid heating by heat conduction. On the other hand, the recirculation flow within the slug speeds up heat transfer additionally by convection.

5 EXPERIMENTAL TESTS

In a first experiment the temperature distribution on the PCR chip was measured using a infrared camera (FLIR ThermaCAM SC 500). The temperature along a PCR channel is displayed in Fig. 6. The three temperature zones are clearly visible as plateaus. Superposed to the temperature curve are three bands indicating the spatial extension of a typical sample slug. A worst-case estimate for the temperature variation over the slug can be obtained by evaluating the temperature span covered by the bands. Corresponding ΔT values are indicated in the figure. The real temperature variation over a sample slug is smaller than that, since owing to the higher thermal conductivity of water compared to the polymer material of the chip, a thermal equalization occurs.

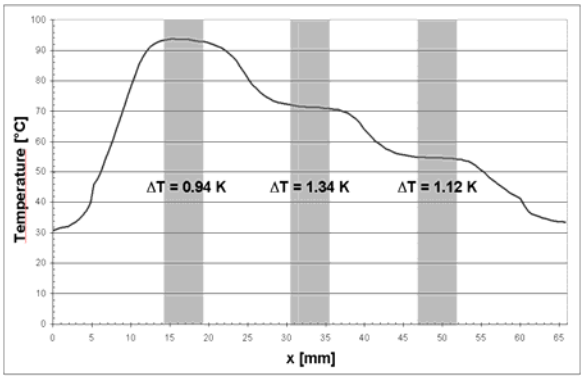

Fig. 6: Temperature distribution along a PCR channel.

The temperature distribution displayed in Fig. 6 suggests that for a rapid DNA amplification the sample slug needs to be positioned in the middle of the temperature plateaus with a sufficient accuracy. In order to study the sample transport, experiments with the ferrofluid drive were carried out for sample volumes ranging from 0.1-1.5 µl. A series of snapshots of such an experiment in the largest of the three micro channels is displayed in Fig. 7. A PCR cycle consists of successively positioning the slug in the middle of one of the temperature zones and moving it to the next temperature zone according to the protocol.

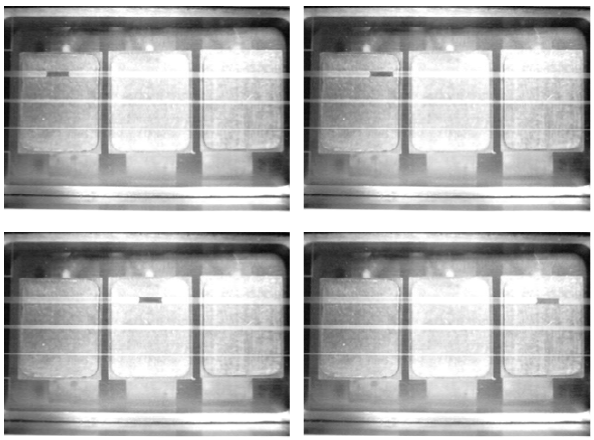

Fig. 7: Motion of a sample slug driven by pneumatic coupling to a ferrofluid transducer.

The experiments were carried out with the heating blocks brought to their appropriate temperature, so that possible problems with sample evaporation could be investigated. Besides evaporation, the focus of the experiments was on the positioning accuracy achievable with the ferrofluid transducer. It was found that problems due to evaporation mainly occurred in the smaller channels, whereas in the channel with a width of 1 mm evaporation is suppressed to a degree that enables a sufficiently large number of PCR cycles. More specifically, after 35 cycles the sample slug stayed intact, with no satellite droplets being observed, although some volume reduction due to evaporation was detected. In these experiments the position where a slug is deposited in the last cycle deviates from the corresponding position in the first cycle by less than 100 µm. Given a characteristic slug length of a few millimeters it can be concluded that the pumping mechanism by ferrofluidic transduction is suitable to carry out the PCR protocol by periodic slug motion. The simulation results presented in the previous section suggest that the sample virtually reaches its processing temperature already on the way to its "parking position".

In a second series of experiments the interaction of DNA strands contained in the sample slug with the walls of the micro channel was studied. Compared to conventional thermocyclers, the channels of microfluidic PCR chips are characterized by a significantly larger surface-to-volume ratio. For this reason, adsorption of DNA strands to the channel walls might drastically reduce the observed amplification efficiency. The corresponding samples were obtained from Evotec Technologies (Düsseldorf, Germany). Sample slugs with fluorescence-labelled human DNA strands of a molecular weight of 370 base pairs were moved through the PCR channel of 1 mm width over 30 cycles. The sample was then removed from the channel, analyzed by gel electrophoresis and compared with an unprocessed sample. The fluorescence intensity of the former was found to be only slightly reduced compared to the latter. Hence it may be concluded that adsorption to the walls of the polymer channels should not pose a major problem for on-chip PCR.

6 CONCLUSIONS AND OUTLOOK

In conclusion it may be stated that a microfluidic PCR system has been established and its fluidic and thermal performance has been studied by experimental and simulation methods. The device allows for rapid temperature ramping on a time scale of some ten milliseconds and is able to process samples volumes in the micro- or submicroliter range. Important thermal and fluidic requirements such as comparatively small temperature variations over the sample volume and a reproducible and accurate positioning of the sample slug within the temperature zones are met. In addition, a sufficiently small wall adsorption of DNA strands was proven.

Experiments focusing at carrying out the PCR protocol inside the system are currently under way. The goal of these experiments is to maximize the observed amplification efficiency. First results indicate that nucleic acid amplification proceeds inside the micro channel. If necessary, the system bears a considerable potential for further optimization. As an example, the temperature profile shown in Fig. 6 could be further improved using a modified chip design. Owing to the principle of periodic slug motion and the very simple channel design derived from that, the system is ideally suited for parallelization: It is easily conceivable that the sample reservoir and the actuation chamber shown in Fig. 1 are not only connected by one channel, but rather many parallel reactions could be carried out in a multitude of channels.

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