Sample-Shunting Based PCR Microfluidic Device

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ABSTRACT

A new method to perform on-chip DNA amplification is presented in this abstract: we shunt the sample back and forth over temperature-controlled zones. Compared to existing systems, this approach offers, in addition to a greater flexibility in terms of cycling programme, an incomparable ease of parallelisation.
We report in this paper the first results obtained when using a sample-shunting approach to achieve on-chip PCR. An unoptimised chip displays an efficiency of 25% and is six times faster than a conventional thermo-cycler. To the extent of our knowledge no comparable results have been previously published.

Keywords: PCR, DNA, microfluidics, chip

1. INTRODUCTION

Since its discovery in 1983 by Kary Mullis et al., the polymerase chain reaction (PCR) has had a tremendous impact in the medical sciences and clinical applications. However, this technique suffers from critical drawbacks. It is for example prone to contamination and the cost associated with each reaction is not negligible. A lab-on-a-chip approach would address some of these issues. Indeed, as the sample is constantly enclosed in the micro-device, contamination risks are limited. In addition, the sample and reaction volumes are in the sub-microlitre range, thus reducing reactant consumption and costs.
To date, two approaches have been mainly studied to perform PCR on chip. The first one is based on a static-sample method and was first introduced in 1993 by Northrup et al. [1]. The device consisted of reaction chambers of 25 or 50 microliters, inside which the sample was subjected to temperature cycles by means of polysilicon heaters. An alternative approach based on a dynamic sample was proposed by Kopp and co-workers in 1998 [2]. In this continuous-flow system, the sample was pumped through a meandrous channel. Thermocycling was achieved by the PCR reactants passing several times over three fixed heating zones. Both methods have proved quite successful and several groups have since presented similar devices [3]. However, they also present some disadvantages. In the case of the static-sample approach for example, the devices present unnecessary inertia. In addition these designs take limited advantage of the flexibility of lithographic methods. Furthermore, with dynamic-sample based devices, it would be difficult to achieve a high throughput without a significant increase in the heater design complexity or in the microfluidic chip size.
A third approach was recently presented [4, 5]. It consists of shunting the sample back and forth over two/three heating zones in a single channel (see Figure 1). It elegantly associates the temperature cycling flexibility of the static-sample-chips to the quick temperature transition of the dynamic-sample microstructures. Indeed, the number of cycles can easily be modified by altering the pumping programme. Transition times from one temperature zone to another can also be optimised by adapting pump velocities. In addition, parallelisation is easily obtained due to the flexibility of lithographic methods. Finally, it is also possible to incorporate post-PCR applications (such as hybridization and/or optical based assays) at the end of each channel, providing unique capabilities to simultaneously apply different treatments to the same original sample. In this paper we present the first results obtained when using a sample-shunting approach to achieve on-chip PCR. In addition, the influence of the cycling conditions over the DNA amplification efficiency is studied.

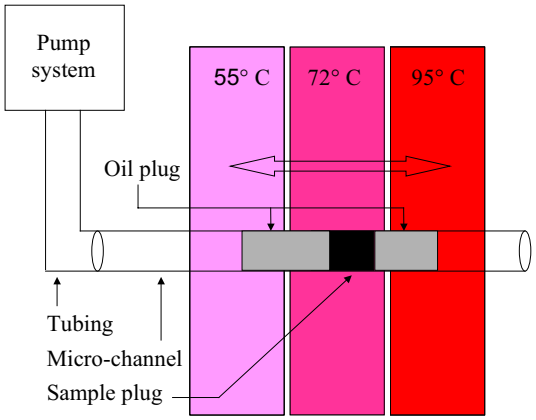

Figure 1. Schematic drawing of the shunting system. The thermocycling conditions are provided by shunting the sample back and forth over the temperature-controlled zones (the given temperatures are only examples).

2. APPARATUS

The device was designed using AutoCAD 2000. The length of the channels (4 cm) was determined by the heating system, whereas their width and depth (100 µm x 50 µm) were chosen to minimize risks of blockage from dust or PCR reagents (see Figure 2). The fabrication of the SU-8 chips was performed by Epigem Limited (Malmo Court, Kirkleatham Business Park, TS10 5SQ, Redcar, UK). It consisted in spin-coating and curing

a thin layer of SU-8 (10 μm) on a PMMA substrate. An additional layer of SU-8 was subsequently spin-coated, the thickness of which was customized according to the application (50μm in this case). This layer was lithographically patterned prior to curing. A second PMMA substrate was also coated with a 10 μm SU-8 layer. The bonding of the substrates was achieved by bringing in contact the two SU-8 layers and applying high pressure and high temperature.

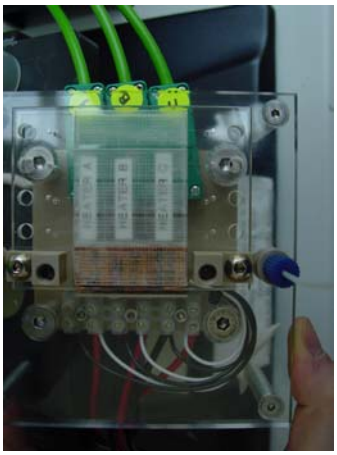

Figure 2. Top view of the PCR chip. The inlet is on the right. The three heating films are the dark-brown areas labeled A, B and C. The green wires link three thermo-couples recording the heater temperature to a monitor device.

The micro-fluidic inlet was linked to a Kloehn pump controlled by a home-written LabView programme. Three heating films were each connected to individual power supplies (RS, cat. #: 201-3446) and provided very stable temperatures (see Table 1). The test real-time PCR amplicon system was derived from Exon 2 of the protooncogene MYCN (forward primer: GCCGAGCTGC-TCCACGT; reverse primer: TCAAACTCGAGGTCTG-GGTTCT) and the PCR reagent kit was purchased from Roche Diagnostics (LightCycler DNA Master – SYBR Green I, cat. #: 2 015 099). The polymerase chain reaction was performed either with the micro-device previously described or with a Touchgene Gradient manufactured by Techne. The amplified samples were analysed using the Agilent 2100 Bioanalyzer with a 500 DNA kit (Agilent 500 DNA Array kit, cat. #: 5064-8284).

	Temp. A	Temp. B	Temp. C
average	95.91	72.18	60.33
stdev	0.11	0.09	0.12
% error	0.002	0.002	0.003

Table 1. Temperature stability study of the different heaters. Very stable temperatures were achieved giving percentage errors.

We performed two sets of experiments. The first one was conducted to assess the efficiency of a non-optimised system. The influence of the cycling programme over the PCR yield was estimated by the second set of experiments. To determine the effectiveness of the micro-device during PCR, a 1μL sample (template concentration: 179ng/μL) was first amplified using the microchip system. The amplified sample was then diluted 10 times and used as a 1μL-template for PCR using a conventional thermocycler. After increments of 5 cycles the amplified samples were analysed by gel electrophoresis using the Agilent 2100 Bioanalyzer. This set of samples was compared to a series directly amplified from the conventional thermocycler and using 17.9ng/μL concentrated DNA templates. The cycling programmes were: 40 cycles of 95°C for 15s and 60°C for 60s for the thermocycler amplifications; 40 cycles at 95°C for 8s and 60°C for 15s for the microchip amplifications.

Another set of experiments was performed in order to study the effect of the on-chip cycling programmes on the PCR efficiency. A template was first amplified with the micro-device following the procedure described above. A subsequent amplification was performed on a thermocycler in increments of 5 cycles. However, the on-chip thermocycling conditions differed. Three programmes, all performed for 40 cycles, were investigated: programme 1: 95°C for 8s, 60°C for 15s; programme 2: 95°C for 6s, 60°C for 6s; programme 3: 95°C for 2s, 60°C for 4s.

4. RESULTS

Table 2 presents the results of the efficiency assessment of the micro-fluidic PCR reactor. During this experiment a DNA sample was first amplified on-chip and was then used as a template for subsequent amplification on a conventional Techne thermocycler. The amplified product was then compared to a sample that had only undergone PCR on the Techne thermocycler (positive control). In this table, the correlation areas of the two series of amplified products are monitored after *n*-extra cycles on the thermocycler. As can be seen, the on-chip pre-amplified sample series displays a visible correlation area (amplified product) prior to the positive control series: amplified product from the micro-device series can be detected at 15 extra cycles, whereas the positive control series is still un-detected. This result leads to a first conclusion: the micro-reactor produces some DNA amplification. The effect of the micro-device can be further assessed. Indeed, the correlation area of the on-chip pre-amplified series at 25-extra cycles corresponds to the correlation area of the positive control series at 35-extra cycles (correlation areas of 22.05 and 22.18 respectively). Consequently, the 40-cycle on-chip amplification is the equivalent of a 10-cycle thermocycler amplification. It can therefore be concluded that the micro-device presents a 25% overall efficiency.

In Figure 3, the effects of on-chip thermocycling programmes over the PCR efficiency are reported. Three programmes, all performed for 40 cycles, were investigated: programme 1: 95°C

for 8s, 60°C for 15s; programme 2: 95°C for 6s, 60°C for 6s; programme 3: 95°C for 2s, 60°C for 4s. Although the three curves are comparable, the thermo-cycling programme 95°C for 6s, 60°C for 6s presents several advantages. First, it displays the highest amount of specifically amplified product. Furthermore, the efficiency/time-to-execute ratio for this programme is also excellent. The time to perform the polymerase chain reaction is then reduced by 84% when compared to a conventional thermocycler.

Extra cycles in the thermocycler	Corr. Area Microchip	Corr. Area Thermocycler
15	0.6	0
20	9.93	0.17
25	22.05	4.59
30	33.36	16.3
35	42.25	22.18
40	47.29	28.55

Table 2. Correlation areas of the specifically amplified product detected in the case of the microchip series and the thermocycler series after *n*-extra cycles in the thermocycler. The correlation areas underlined in the two series are comparable, which leads to an estimated 25% overall efficiency for the un-optimized microchip.

5. CONCLUSION

A sample-shunting based PCR microfluidic device has been successfully developed. An un-optimised chip presented a 25% overall efficiency and performed six times faster than a conventional thermocycler. Further optimisation is in progress.

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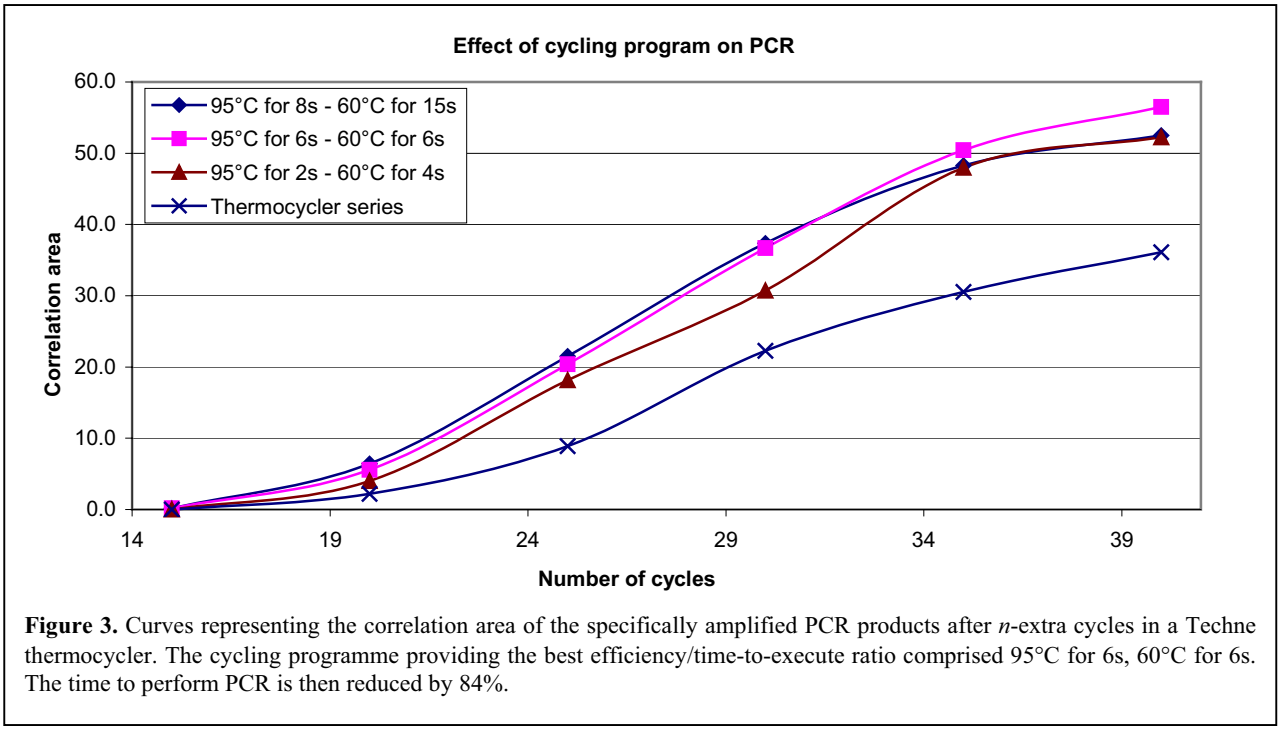

Figure 3. Curves representing the correlation area of the specifically amplified PCR products after *n*-extra cycles in a Techne thermocycler. The cycling programme providing the best efficiency/time-to-execute ratio comprised 95°C for 6s, 60°C for 6s. The time to perform PCR is then reduced by 84%.