Rapid PCR amplification using a microfluidic device with integrated microwave heating and air impingement cooling†

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Received 6th January 2010, Accepted 6th April 2010
First published as an Advance Article on the web 23rd April 2010
DOI: 10.1039/c000357n

A microwave heating system is described for performing polymerase chain reaction (PCR) in a microfluidic device. The heating system, in combination with air impingement cooling, provided rapid thermal cycling with heating and cooling rates of up to 65 °C s^{-1} and minimal over- or under-shoot (±0.1 °C) when reaching target temperatures. In addition, once the required temperature was reached it could be maintained with an accuracy of ±0.1 °C. To demonstrate the functionality of the system, PCR was successfully performed for the amplification of the Amelogenin locus using heating rates and quantities an order of magnitude faster and smaller than current commercial instruments.

Introduction

PCR is a commonly used biochemical tool for the amplification of DNA and features thermal cycling between two or three distinct temperatures to achieve the denaturing of the DNA, primer annealing and DNA extension.

In order to increase sample processing and facilitate the integration of PCR with other techniques, the speed of processing is very important. In addition, a reduction in the volume required for PCR is advantageous as it not only increases speed but also reduces the amount of reagents required therefore reducing the cost of analysis. Since the early development of PCR in microfluidic systems, either as a stand-alone technique[1] or as an integrated process,[2] numerous examples of miniaturised PCR have been reported which can be found in substantial reviews on the subject.[3–6]

A wide variety of heating and cooling methods have been reported in the literature for achieving thermal cycling. Broadly speaking these methods can be divided into contact and non-contact forms. Commonly used contact heating methods include block heaters *e.g.* Peltier heaters, or the deposition of thin film resistive heaters *e.g.* platinum, on the exterior of the microfluidic device. While Peltier heaters are widely used to achieve thermal cycling for DNA amplification as they produce reliable heating, they suffer from relatively slow temperature ramp rates.[3]

Non-contact heating methods described for DNA amplification in microfluidic systems include the use of infrared[7] and halogen lamps.[8] Induction heating has been shown to provide a low power consumption method for performing thermal cycling with a temperature stability of ±0.2 °C.[9,10] An alternating electric current induced Joule heating method has also been described where platinum electrodes are used to transfer an electric current directly into the PCR solution, producing heating and cooling rates of 15 °C s^{-1}.[11]

The use of microwaves for dielectric heating of liquids within microfluidic devices has previously been shown to have the potential for thermal cycling but the technology has not been fully exploited to demonstrate actual DNA amplification.[12–14] Issadore *et al.*, for example, reported a system whereby droplets are flowed through a microwave heater, which relies on integrated metal electrodes that run parallel to the fluidic channel to deliver the microwave power.[12] The use of direct sample coupling of microwave heating has also been demonstrated for PCR, with the emphasis of this work being on the thermal cycling of relatively large volumes of liquid rather than miniaturisation. For example, Orrling *et al.* presented a microwave heating system capable of performing PCR in a 15 ml reaction volume for increased sample processing.[15] A total of 33 cycles were used to amplify a 53 bp fragment in 2 hours and 7 minutes. Despite successful amplification of the target product, this system was only capable of heating and not cooling therefore the sample required manual transfer into a thermally controlled block for each annealing step.

The work presented here demonstrates the successful use of a tuned microwave cavity for direct substrate heating, configured to perform both heating and air impingement cooling resulting in rapid thermal cycling, enabling DNA amplification on a microfluidic device.

Experimental

Microwave cavity and power source

A custom built re-entrant microwave cavity operating at 8 GHz was used to directly heat the glass microfluidic device (Fig. 1). The copper cavity was connected *via* a coaxial coupling loop to the microwave power source, a CPI VZM6991 series 8–18 GHz 20 W travelling wave tube amplifier [CPI, US] capable of

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† Electronic supplementary information (ESI) available: Details of temperature measurement technique used; description of the microwave cavity and power source developed. See DOI: 10.1039/c000357n

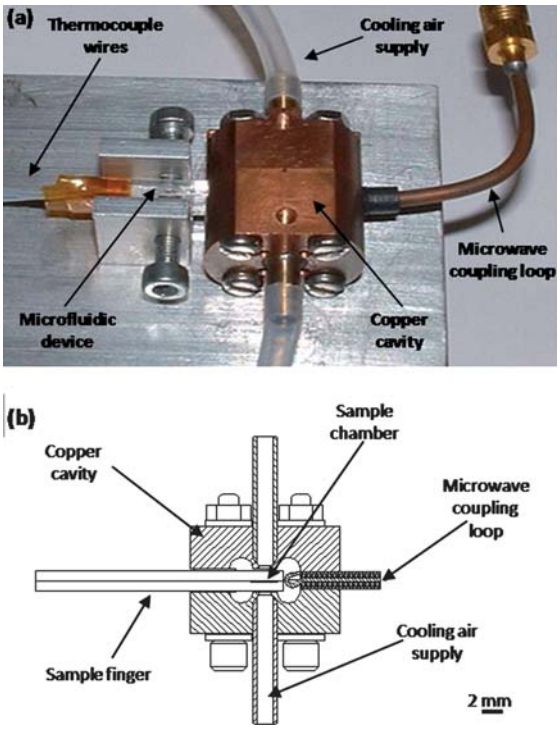

Fig. 1 Photograph (a) and schematic cross-section (b) of the re-entrant cylindrical microwave cavity.

delivering power levels up to 10 W and driven by a HP3850B microwave signal generator [Hewlett Packard, US].

After inserting a microfluidic device into the cavity the resonant frequency was determined at low power using a HP8719D network analyser [Hewlett Packard, US] by observing the resonance dip in the reflected power signal. The frequency was noted and transferred to the HP3850B signal generator. Coupling into the cavity was adjusted for a minimum of 10 dB return loss by varying the insertion depth of the coupling loop. The mean power consumption of the cavity during a typical heating cycle was found to be under 500 mW (for further details see ESI†).

Air impingement cooling was provided from a compressed air cylinder and controlled by the actuation of a solenoid valve which delivered short pulses of air to aid the transition between the denaturation and annealing temperatures.

Temperature measurement

A 75 μm type-k (Chromel-Alumel) junction thermocouple was inserted through the etched channels leading to the PCR chamber. By positioning the thermocouple junction in the centre of the PCR chamber, true sample measurement could be ensured. A digital display on the control system allows the temperature to be monitored in real-time, while a PicoScope 2200 [Pico® Technology Ltd., UK] was used to record the thermal cycling (for further details see ESI†). To ensure direct heating of the thermocouple was not occurring in the ac electric field associated with the microwaves, the microwave generator was (temporarily) 100% square wave amplitude modulated and the low level dc signal (around 40 μV °C^{-1}) of the thermocouple was monitored. The resulting oscilloscope temperature trace showed only the expected triangular heating and cooling waveform, with no step discontinuity occurring at the modulation switching points, thus confirming the adequacy of the screening and filtering used.

In order to check the accuracy of the thermocouple measurement a microfluidic device with a thermocouple in position was placed into an isothermal block with a glass thermometer and found to give complete agreement within the tolerances of the thermometer used, ±0.5 °C. Furthermore, the calculated error for the thermocouple electronics was ±0.4 °C, assuming that the thermocouples, the Pt resistance thermometer and all the resistors used were in tolerance.

Thermocycler

The thermocycler was designed for rapid throughput with maximum flexibility and portability; it is therefore self-contained and functions without an external computer. Hold times at the DNA denaturation, primer annealing and DNA extension temperatures are adjustable from 1 to 99 seconds, and from 1 to 999 seconds at initial and final hold temperatures if required. The target temperatures are all adjustable and displayed numerically to a resolution and an accuracy of ±0.1 °C. Time and temperature settings can be adjusted during cycling if necessary (for further details see ESI†).

Production of microfluidic device

Borosilicate glass microfluidic devices of cross-section 5 mm × 2 mm were produced using standard photolithography and wet etching techniques.[16] The DNA amplification chamber (3 mm diameter, 100 μm deep), which had a volume of 0.7 μl, was connected to inlet and outlet ports *via* etched channels (200 μm wide and 100 μm deep), which also served to allow access for the thermocouple (Fig. 2). To create a sealed device the etched glass plate was thermally bonded to a top plate which had 360 μm holes drilled in it to act as reagent and sample inlets. The thermocouple was threaded into the microfluidic device by hand from the open channel end after fabrication.

The internal glass surfaces of the microfluidic device were silanised to prevent DNA polymerase adsorption.[17] A solution of 290 μl of trichloro(1*H*,1*H*,2*H*,2*H*-perfluorooctyl) silane in 5 ml of 2,2,4-trimethylpentane was flowed through the microfluidic device for 10 minutes at 5 μl min^{-1}.[18] Following this solutions of 2,2,4-trimethylpentane, acetone and distilled water were sequentially used to wash the microfluidic device.

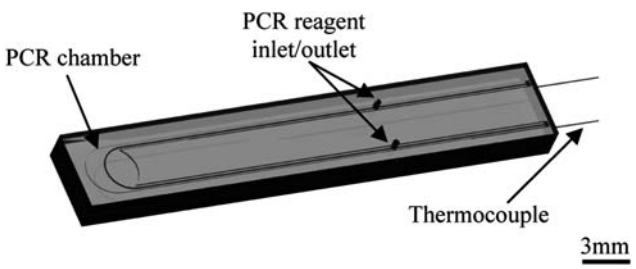

Fig. 2 Schematic showing the microfluidic device design, showing a type-k junction thermocouple which has been threaded along the channel length into the PCR chamber.

DNA amplification

Saliva was collected, from male and female volunteers, using a 0.9% (w/v) saline solution mouthwash which was swilled around the mouth. A 1 ml aliquot of the mouthwash was placed in a 1.5 ml microcentrifuge tube and centrifuged at 14 000 rpm for 3 minutes. The supernatant was removed and the pellet subjected to DNA extraction using a QIAamp® DNA Micro Kit [Qiagen, UK] following standard protocols.

The extracted DNA (1 ng) was added to a PCR reagent solution composed of the following: 1× reaction buffer [Promega, UK], 2 mM $MgCl_2$ [Promega, UK], 200 μM each dNTPs [Bioline, UK], 0.5 μM forward primer, 0.5 μM reverse primer, 10 mg ml^{-1} bovine serum albumin [NEB, UK], 0.01% (w/v) poly(vinylpyrrolidine) [Sigma-Aldrich, UK], 0.1% (v/v) Tween-20 [Sigma-Aldrich, UK] and 1 U GoTaq® DNA polymerase [Promega, UK] made up in purified water. Forward and reverse primers were custom-made for the Amelogenin locus (Forward: 5′-JOE-CCCTGGGCTCTGTAAAGAA-3′, Reverse: 5′-ATC-AGAGCTTAAACTGGGAAGCTG-3′) [Eurofins MWG Operon, Germany].[19] PCR control samples were run in parallel on a TC-312 thermal cycler [Techne, UK].

Analysis of PCR products

PCR products were analysed by capillary electrophoresis using an ABi Prism 310 Genetic Analyser [Applied Biosystems, UK]. The samples were collected from the DNA amplification chamber and added to 12 μl of Hi-Di™ formamide [Applied Biosystems, UK] and 0.5 μl GeneScan™ 500 ROX™ DNA size standard [Applied Biosystems, UK]. The solutions were heated to 95 °C for 5 minutes to denature the DNA and then snap-cooled on ice prior to loading on the ABi Prism 310 Genetic Analyser. Standard protocols for DNA fragment analysis were followed, using POP-4 [Applied Biosystems, UK] as the separation matrix.

Results and discussion

Microwave cavity and power source

The microwave heating system was designed to have an impedance mismatch between the substrate and sample in order to heat the glass of the microfluidic device and not the sample itself. This was achieved by combining the device geometry, electrical characteristics and very low sample volume and ionic strength together with the radio frequency (RF) field. For example as the sample solution was not simply water but a mixture of buffer and PCR reagents its ionic strength would make the solution sufficiently conducting to effectively act as a short circuit in its axial direction so supporting the preferential match of the transverse impedance of the glass with the impedance of the microwave cavity. Evidence that it was indeed direct heating of the glass substrate as opposed to heat conduction from the sample was established by the fact that heating rates were the same with or without the sample being present.

Whilst indirect microwave heating of the sample *via* the glass may seem to offer little advantage over simpler methods such as surface contact resistive heating, the uniformity of microwave heating throughout the volume of the glass rather than *via* the surface removes the delay in the thermal control loop, thus allowing far more rapid yet stable temperature control. The cooling process of two impinging air jets is, however, susceptible to the thermal delay of the glass, as it is its outer surface and not its bulk volume that is cooled. The air cooling was therefore excluded from the temperature control system by cooling to just below the target temperature then maintaining that temperature by the feedback controlled microwave heating. This also eliminated any need for proportional control of the cooling air flow, thus allowing a simple on–off solenoid valve to be used.

Thermal cycling profile

Using feedback controlled microwave heating coupled with air impingement cooling, the system showed minimal thermal overshoot or undershoot at any of the three set temperatures. Once the microwave system had reached a set temperature the variation was less than ±0.1 °C. The ramp rates for heating and cooling exceeded 65 °C s^{-1}, allowing very fast transitions between temperatures (Fig. 3). Note that the cooling rate is slower than the heating rate.

The thermocycler of the microwave system was designed to include an initial denaturing step, essential when using Hot-Start Taq DNA polymerases, and a final extension step to ensure complete adenylation of the PCR products. The time and temperature of these steps were adjustable in the same way as for the three amplification steps.

DNA amplification

DNA amplification of the Amelogenin locus was carried out using the microwave heating system described. Amelogenin is used to distinguish between male (XY) and female (XX), yielding PCR products of 106 bp (X) and 112 bp (Y). For PCR experiments, a 2 minute initial heating step at 95 °C was carried out to ensure complete denaturation of the DNA and to activate the Hot-Start GoTaq DNA polymerase. Thermal cycling between DNA denaturation (94 °C), primer annealing (59 °C) and DNA extension (72 °C) temperatures was performed for 28 cycles.

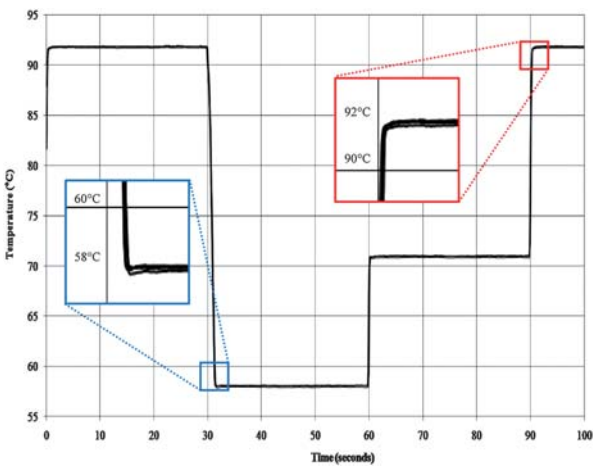

Fig. 3 Graph showing the thermal cycling profile of the microwave heating system based on selected temperatures of 92 °C, 58 °C and 71 °C. The image comprises 5 overlaid thermal cycles demonstrating the reproducibility of the system.

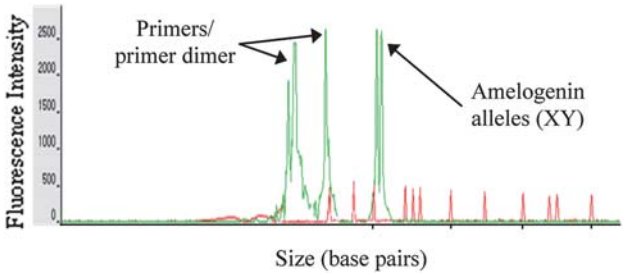

Fig. 4 Electropherogram showing PCR products from amplification of the Amelogenin locus, using DNA extracted from a male volunteer, on a microfluidic device using microwave heating. A DNA size standard was also included to enable accurate sizing of the PCR products.

Samples were then analysed using capillary electrophoresis. Successful PCR amplification, of DNA from both male and female donors, was achieved using the microwave system (Fig. 4).

Conclusions

The microwave heating system developed was found to have a response speed orders of magnitude faster than that of current commercial systems. This fast thermal transition capability enabled 28 cycles to be performed in 42 minutes. This represents a considerable time saving on previously reported microwave PCR systems where 33 cycles took 127 minutes.[13] Further work aims to reduce the hold times at each temperature to achieve DNA amplification which will be no longer thermally limited but restricted for example by the processivity of the DNA polymerase, where an average DNA polymerase enzyme adds 60–100 nucleotides per second.

Acknowledgements

The authors acknowledge Exxel Amplifiers Ltd. for providing the microwave heating system, Dr Steve Clark for microfluidic device manufacture, Mark Tarn for production of microfluidic device graphics and the EPSRC for funding EP/D040930.

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