CHAPTER 7

PCR in Integrated Microfluidic Systems

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Abstract

Miniaturized integrated DNA analysis systems offer the potential to provide unprecedented advances in cost and speed relative to current benchtop-scale instrumentation by allowing rapid bioanalysis assays to be performed in a portable self contained device format that can be inexpensively mass-produced. The polymerase chain reaction (PCR) has been a natural focus of many of these miniaturization efforts, owing to its capability to efficiently replicate target regions of interest from small quantities template DNA. Scale-down of PCR has proven to be particularly challenging, however, due to an unfavorable combination of relatively severe temperature extremes (resulting in the need to repeatedly heat minute aqueous sample volumes to temperatures in the vicinity of 95°C with minimal evaporation) and high surface area to volume conditions imposed by nanoliter reactor geometries (often leading to inhibition of the reaction by nonspecific adsorption of reagents at the reactor walls). Despite these daunting challenges, considerable progress has been made in the development of microfluidic devices capable of performing increasingly sophisticated PCR-based bioassays. This chapter reviews the progress that has been made to date and assesses the outlook for future advances.

Introduction

Advances in genomic analysis technology continue to be made at a rapid pace and have contributed to the development of new instrumentation that is paving the way for high-throughput low-cost DNA assays to become commonplace. These technologies have the potential to impact an unprecedented array of fields including medical diagnostics, forensics, biosensing and genome-wide analysis.[1-4] Many of these methodologies rely on the ability to replicate selected sub-regions within a larger DNA template. The polymerase chain reaction (PCR) offers a straightforward and highly efficient means to perform this replication, thereby making it one of molecular biology's key enabling technologies.

The PCR process involves repeatedly cycling a reagent mixture containing template DNA, primers, dNTPs, a thermostable polymerase enzyme and other buffering additives, through thermal conditions corresponding to (1) denaturation of the double-stranded template (~ 95°C), (2) annealing of single-stranded oligonucleotide primers at complementary locations flanking the target region (~50-65°C) and (3) enzyme directed synthesis of the complementary strand (~72°C). The number of target DNA copies increases exponentially as this cycling process is repeated, doubling with each cycle under ideal conditions. Although the kinetics associated with each step in the PCR process are rapid when considered individually,[5,6] a typical 30-40 cycle replication still requires timescales of order 1-2 hours to complete. These prolonged reaction times are largely a reflection of the highly inefficient design of many conventional thermocycling instruments, where thermally massive hardware components (e.g., metal thermal blocks) and low-conductivity plastic reaction vessels (e.g., polypropylene tubes) combine to produce an unfavorable coupling between

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increased thermal energy requirements associated with rapid heating and cooling and the necessity to hold the temperature constant at each step for a sufficiently long time to ensure that the entire reagent volume attains thermal equilibrium. Progress has been made in the development of faster benchtop-scale thermocyclers, most notably by approaches that involve replacing plastic reaction tubes with thin glass micro-capillaries and using techniques ranging from forced air circulation to infrared heating for temperature control.[7-14] These configurations allow the reaction mixture to be distributed over a greater surface area for more efficient heat transfer, although overall throughput can still be limited by the capillary loading, sealing and unloading processes.

PCR in Microfluidic Systems

In addition to these benchtop-scale approaches, increasing interest has been focused on performing PCR in miniaturized systems where ultra-small reaction volumes (typically in the nL range) not only greatly reduce reagent consumption, but can also be rapidly heated and cooled while simultaneously lowering costs due to greatly reduced reagent consumption. Moreover, the use of photolithographic microfabrication offers the potential to produce hundreds or thousands of devices at once, bringing hardware costs to a level of $1 or less. These highly desirable characteristics have stimulated considerable interest in the area of microfabricated thermocycling systems. However the use of such small reaction volumes can pose significant challenges including adverse effects associated with evaporation and nonspecific absorption to the microchannel walls under high surface to volume conditions. Progress continues to be made toward addressing the challenges facing development of microfluidic thermocycling systems and the interested reader is referred to several recent reviews for more details.[6,15-24]

Despite its importance as a key enabling technology, PCR is only one step in a broader sequence of processes that comprise a complete molecular biology assay. Some combination of sample isolation and collection, pre and postreaction purification, subsequent biochemical reactions and product detection and analysis must also be performed. On the macroscale, these steps are typically carried out in a conventional laboratory setting, often requiring dedicated instruments and personnel to be employed at each stage along with the need to repeatedly prepare and dispense precise sample and reagent mixtures. These processes can not only be tedious and time consuming, they also introduce multiple opportunities for measurement errors and sample contamination. The ability to adapt a series of these operations to a miniaturized 'lab-on-a-chip' format has the potential to address these limitations, yielding significant reductions in analysis time and cost. Moreover, a fully self-contained portable design offers the potential to minimize tedious manual fluidic manipulations and sample contamination issues.

This enhanced functionality, however, is accompanied by a corresponding increase in the level of design and operational complexity. A myriad of challenges must be addressed, including ensuring biochemical compatibility among successive steps in the analysis process, careful thermal design to ensure that the relatively high temperatures achieved during PCR thermocycling do not negatively impact activity on other parts of the chip, the ability to accurately dispense and transport nanoliter liquid volumes within a microchannel network and ensuring that reagents can be properly sealed to prevent evaporation during thermocycling. In this chapter, we review some of the remarkable progress that has been made toward developing sophisticated miniaturized devices that incorporate PCR as part of an integrated molecular biological assay system. The review focuses on developments reported in refereed journals, with the understanding that additional studies may be documented in conference proceedings and patent literature.

Integrated PCR and Gel Electrophoresis

Considerable progress has been made in the development of microdevices capable of combining PCR with postreaction product analysis performed by electrophoretic separation of the amplified DNA fragments (Table 1). The Mathies group was the first to demonstrate this approach using a hybrid design consisting of a 20 μL microfabricated silicon PCR reactor bonded on top of a glass electrophoresis microchip.[25] Unfavorable surface interactions at the reactor walls that inhibited PCR amplification were avoided through the use of disposable polypropylene liners, allowing targets

Table 1. Summary of microfluidic systems demonstrating integration of PCR with gel electrophoresis

Reference	Integrated Functions	Device Fabrication	Fluid Handling	Temperature Control	Templates	Targets
Wooley et al (1996) [25]	PCR, gel electrophoresis	Microfabricated Si reactor with polypropylene liner bonded to glass	Electrokinetic	Integrated polysilicon heaters	Plasmid DNA	268 bp
					Bacterial genomic (*Salmonella*)	159 bp
Lagally et al (2000) [26]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Pneumatic valve/ vent manifold	Thin film heaters and temperature sensors affixed to chip	136 bp fragment	136 bp
Lagally et al (2001) [27]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Pneumatic valve/ vent manifold	Thin film heaters and temperature sensors affixed to chip	136, 231 bp fragments, 2686 bp cloning vector	136, 231 bp
Lagally et al (2001) [28]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Pneumatic valve/ vent manifold	Integrated micro-fabricated heaters and temperature sensors	Human genomic	157, 200 bp
Legally et al (2004) [29]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Pneumatic valve/ vent manifold	Integrated micro-fabricated heaters and temperature sensors	Bacterial genomic (*E. coli*)	348, 625 bp
					Bacterial genomic (*S. aureus*)	219, 310 bp
Waters et al (1998) [32]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Benchtop thermocycler	Lambda DNA	500 bp
					Bacterial genomic (*E. coli*)	154, 264, 346 bp
					Plasmid DNA	410 bp

Table 1. Continued

Reference	Integrated Functions	Device Fabrication	Fluid Handling	Temperature Control	Templates	Targets
Waters et al (1998) [31]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Benchtop thermocycler	Lambda DNA	199, 500 bp
					Bacterial genomic (*E. coli*)	346 bp
					Plasmid DNA	410 bp
Dunn et al (2000) [30]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Benchtop thermocycler	Mouse genomic	107,114, 121, 149 bp
Khandurina et al (2000) [33]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Thermoelectric elements affixed to chip	Lambda DNA	199 bp
Zhou et al (2004) [34]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Thermoelectric elements affixed to chip	Reverse transcribed cDNA from SARS-CoV	240, 438 bp
Hong et al (2001) [35]	PCR, gel electrophoresis	Microfabricated chip (PDMS/glass)	Electrokinetic	Thermoelectric elements affixed to chip	Lambda DNA	500 bp
Rodriguez et al (2003) [36]	PCR, gel electrophoresis	Microfabricated chip (Si/glass)	Electrokinetic	Integrated micro-fabricated heaters and temperature sensors	Plasmid DNA	350 bp
Koh et al (2003) [37]	PCR, gel electrophoresis	Molded poly(cyclic olefin) chip	Electrokinetic, with polyacrylamide gel sealing valves	Integrated micro-fabricated heaters and temperature sensors	Bacterial genomic (*E. coli, Salmonella*)	232, 429, 559 bp
Ferrance et al (2003) [38]	PCR, gel electrophoresis	Microfabricated chip (glass/glass)	Electrokinetic	Non-contact infrared heating	Human genomic	380 bp
Easley et al (2000) [39]	PCR, gel electrophoresis	Multilayer microfabricated chip (PDMS/glass)	On-chip pneumatic PDMS valves	Non-contact infrared heating	Bacterial genomic (*Salmonella*)	278 bp

from both plasmid and bacterial genomic DNA templates to be successfully amplified. Following PCR, the products were electrokinetically injected into a separation channel filled with a sieving gel matrix containing a fluorescent intercalating dye and detected using a laser excited confocal imaging system. Total analysis times ranging from 20 to 45 minutes were reported.

Subsequent refinements to this basic design involved integrating both the PCR reactor and electrophoresis column into a single continuous glass microchannel network. In one adaptation reagents were loaded and sealed inside a 280 nL reaction chamber using a valve/vent manifold mounted on top of the chip and temperature control was provided by thin film heater and thermocouple elements affixed to the back side.[26] Upon completion of the reaction, the manifold was removed and platinum electrode wires were inserted into the access holes so that the products could be electrokinetically transported to the electrophoresis channel for analysis. This design was used to demonstrate amplification of targets from cloning vector and genomic control templates with sufficient sensitivity to permit single molecule detection.[27] A further improvement to the design involved integration of microfabricated heaters and temperature sensors to actuate thermocycling within a 200 nL reaction chamber.[28] This device was used to perform multiplex sex determination from human genomic DNA in under 15 minutes. A self-contained portable version was also constructed and used to perform multiplex PCR directly from whole bacterial cells in 30 minutes, with product detection accomplished using fluorescently labeled primers (Fig. 1A).[29]

Another notable family of glass microchips for performing integrated PCR and gel electrophoresis has been developed in the Ramsey group. Here, the basic design consisted of a 10-20 μL reaction reservoir fabricated by drilling a hole in one of the glass substrates. After loading, the PCR reagents were covered with mineral oil or wax to prevent evaporation and thermocycling was performed by placing the chip inside a conventional thermocycler.[30-32] After the reaction was completed, an intercalating dye was added to the sample reservoir and electrophoretic product analysis was performed with laser-induced fluorescence detection. Subsequent improvements included the use of a specially designed thermoelectric fixture with dual thermoelectric heating elements and incorporation of a porous membrane structure for injection of reaction products into the electrophoresis gel.[33] Successful amplification of targets from a variety of templates ranging from lambda DNA to bacterial and mouse genomic DNA have been reported using these devices, with total analysis times of 20 minutes or less.

Zhou and coworkers also demonstrated a glass microchip design integrating PCR and gel electrophoresis with an external thermoelectric thermocycling apparatus to perform a duplex PCR analysis of 240 and 438 base pair (bp) targets associated with the SARS coronavirus.[34] Other examples of integration include designs employing hybrid PDMS/glass microchips[35] and designs interfacing a silicon PCR microchip with integrated temperature control to a glass electrophoresis microchip.[36] A particularly novel design reported by Koh et al consisted of a plastic microfluidic chip with integrated temperature control that incorporated on-chip photopolymerized gel valves that not only sealed reagents inside the PCR reactor but also allowed the products to be electrokinetically extracted through the valve material and directed into an electrophoresis channel for separation and detection.[37] The device was used for successful analysis of targets from two different bacterial genomic templates with a detection limit on the order of six DNA copies.

Recent work by the Landers group has yielded further reductions in analysis time through the use of an innovative noncontact infrared heating technique to actuate thermocycling. This concept was successfully employed in an integrated device capable of amplifying a 380 bp β-globin target in a 600 nL reactor followed by electrokinetic injection and electrophoretic separation.[38] In addition, a separate microchip was used to perform solid phase extraction of DNA from whole blood prior to PCR. Subsequent work has resulted in further optimizations to this design by a reduction in reactor volume to 280 nL and through development of a fluid handling system based on a multilayer hybrid glass/PDMS assembly that provides an addressable array of pneumatic valves capable of performing sealing, pumping and injection into the electrophoresis gel matrix (Fig. 1B).[39] This design offers an impressive capacity for analysis speed, as demonstrated by the ability to perform integrated PCR and electrophoresis of bacterial targets with total analysis times of about 12 minutes.

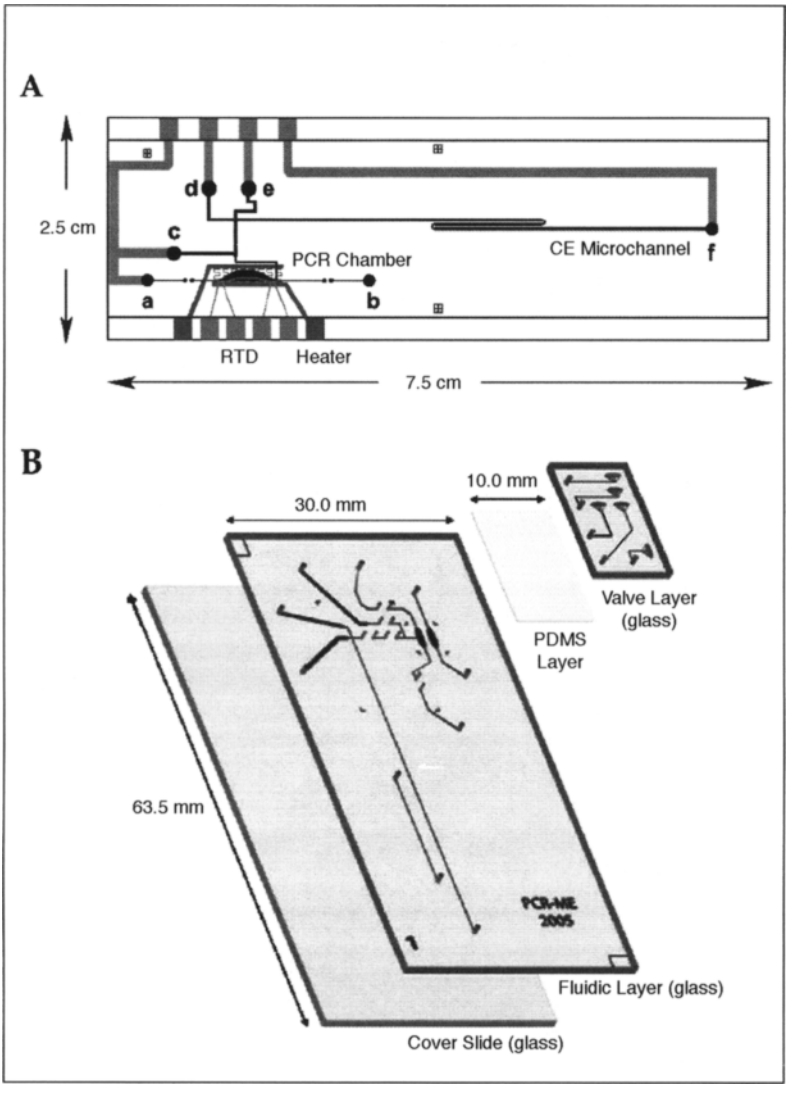

Figure 1. Microdevice designs capable of performing integrating sequential PCR and gel electrophoresis. A) Schematic layout of a device incorporating an etched glass PCR reactor and electrophoresis channel network (indicated in black) bonded to a second glass wafer patterned with electrodes and temperature sensors (indicated in green) along with a heating element (indicated in red)[29] (reproduced with permission, copyright 2004 American Chemical Society). B) Four-layer microdevice design incorporating integrated pneumatically actuated PDMS valves for liquid handling.[39] Thermocycling is actuated using a noncontact infrared heating process (reproduced with permission, copyright 2006 Royal Society of Chemistry).

Integrated PCR and Sample Purification

Advances have also been made in the design of microdevices integrating PCR with other upstream and downstream sample handling and analysis steps (Table 2). The Wilding group has developed a series of microdevices incorporating micromachined 'weir-type' filtration structures

Table 2. Summary of microfluidic systems demonstrating integration of PCR with sample purification

Reference	Integrated Functions	Device Fabrication	Fluid Handling	Temperature Control	Templates	Targets
Wilding et al (1998) [40]	Cell filtration, PCR	Microfabricated chip (Si/glass)	External syringe	Thermoelectric elements affixed to chip	Human genomic	202 bp
Yuen et al (2001) [41]	Cell filtration, PCR	CNC machined Plexiglas chip	External syringe	Thermoelectric elements affixed to chip	Human genomic	226 bp
Panaro et al (2005) [42]	Cell filtration, PCR	Microfabricated chip (Si/glass)	External manifold syringe pump	Thermoelectric elements affixed to chip	Human genomic	379 bp
Lee et al (2005) [43]	Cell lysis, PCR	Microfabricated chip (PDMS/glass)	Electroosmotic pumping	Integrated micro-fabricated heaters and temperature sensors	Bacterial genomic (*Streptococcus pneumoniae*)	273 bp
Cady et al (2004) [44]	Purification, real-time PCR	Microfabricated chip (PDMS/Si)	Syringe pump	Thermoelectric elements affixed to chip	Bacterial genomic (*Listeria mono-cytogenes*)	544 bp

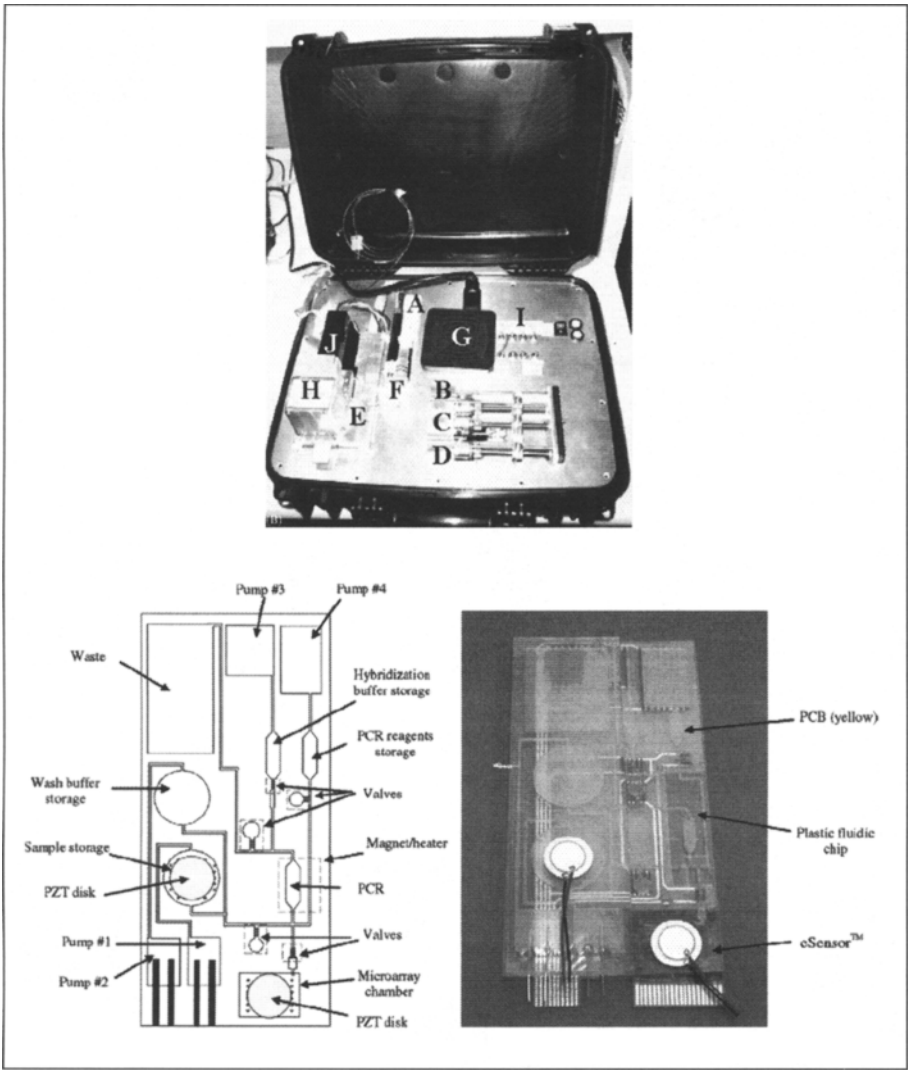

Figure 2. A) Microchip-based bacterial detection system integrating sample purification and real time PCR.[44] The system includes integrated syringe pumps (A-D), Moog micro valve (F), cooling fan (G), LED-based fluorescence excitation/detection system (H—dotted outline) with PMT detector (J), power toggle switches (I). The microfluidic purification/detection chip (E) is inserted into the unit directly above the thermoelectric heater cooler. The entire unit measures 36 cm x 28 cm x 15 cm (reproduced with permission, copyright 2004 Elsevier B.V). b) Self-contained bioanalysis device incorporating the ability to perform sequential sample preparation, PCR amplification and DNA microarray hybridization[46] (reproduced with permission, copyright 2004 American Chemical Society).

capable of isolating white blood cells from whole blood samples in the 1-10 μL range. Filtration occurred as samples were pumped through the chamber where the larger white blood cells were selectively retained by the filter, after which a PCR reagent mixture was injected and thermocycling was performed using a thermoelectric apparatus.[40,41] Both hybrid glass/silicon and Plexiglas

substrate materials have been used to construct the devices, and the potential for further integration has been demonstrated by performing product sizing analysis using separate electrophoresis microchips.[42] An integrated microdevice capable of performing cell lysis followed by PCR was reported by Lee et al.[43] The design consisted of a hybrid glass/PDMS configuration with PDMS thermal lysis and PCR chambers interconnected by a glass microchannel network in which the lysis products were mixed with PCR reagents and electroosmotically transported to the PCR reactor. The device was used to successfully amplify a 273 bp target from whole bacterial cells. Finally, a portable pathogen detection system integrating DNA purification and real-time PCR detection has been developed by Cady and coworkers (Fig. 2A).[44] Here, samples subjected to off-chip chemical lysis were pumped through a flow network containing arrays of etched silicon pillars that allowed purification to be performed via sequential binding and washing steps. The purified samples were then pumped into a PDMS PCR reactor for real-time amplification using a SYBR green fluorescence chemistry. The ability to detect between 10^4 and 10^7 bacterial cells in about 45 minutes was demonstrated in characterization studies involving amplification of a 544 bp target.

Integrated PCR and Hybridization

Several groups have also explored integration of microchip PCR with DNA hybridization analysis (Table 3). Anderson and coworkers demonstrated this concept using a device containing a credit card sized polycarbonate fluidic cartridge interfaced with an Affymetrix GeneChip microarray.[45] The integrated system was capable of performing sequential reverse transcription, PCR, enzymatic reactions and hybridization. Further progress in microarray integration was reported by Liu et al who presented a fully self-contained biochip device that incorporated the ability to perform sample preparation (cell capture, concentration, purification and lysis), PCR and hybridization using an integrated Motorola eSensor microarray (Fig. 2B).[46] This device was used to perform pathogenic bacteria detection from whole rabbit blood and single-nucleotide polymorphism analysis from diluted human blood samples. Another example of integration with hybridization involved employing an array of hybrid glass/silicon microreactors with integrated on-chip heaters and temperature sensors.[47] The bottom surface of each microreactor was patterned with hybridization oligonucleotides allowing reaction products to be detected with a confocal fluorescence scanner. Finally, Liu et al demonstrated a polycarbonate microdevice where PCR and hybridization are performed in separate fluidically interconnected reactors.[48] Here, reagents were sealed in the PCR chamber during thermocycling by employing phase change valves based on Pluronic F127, a block copolymer that liquefies at low temperature (~ 5°C) but becomes a solidified gel at higher temperatures. Operation of the device was demonstrated by using it to perform a bacterial detection assay.

Further Advancements in Integration

Increases in device complexity that accompanies simultaneous miniaturization and integration of multiple sample processing and analysis steps can pose daunting challenges, however progress is steadily being made toward addressing many of these issues resulting in the development of increasingly sophisticated designs (Table 4). An early compelling example illustrating the power of miniaturized genomic analysis systems was the hybrid glass-silicon design developed in the Burns group,[49] capable of performing a series of liquid metering, thermal reaction and analysis operations. This pioneering device was used to amplify a 106 bp DNA target from a bacterial genomic template via an isothermal strand displacement amplification (SDA) process followed by gel electrophoresis with integrated photodetection of the fluorescently labeled reaction products. A greatly improved version of this design was recently reported that is capable of performing a complete genotyping assay involving two sequential reactions followed by an electrophoretic separation in an ultra-compact 1.5 × 1.6 cm hybrid glass/silicon microfluidic chip (Fig. 3A).[50] Sample DNA and PCR reagents were loaded into the chip and pneumatically propelled into a reaction zone where they were sealed using paraffin phase change valves. Thermocycling was actuated using an array of integrated resistive heaters and temperature sensors, with the reaction zone thermally

Table 3. Summary of microfluidic systems demonstrating integration of PCR with DNA hybridization

Reference	Integrated Functions	Device Fabrication	Fluid Handling	Temperature Control	Templates	Targets
Anderson et al (2000) [45]	Purification, PCR, microarray hybridization	CNC machined polycarbonate cartridge	On-chip pneumatic silicone valves	Thermoelectric elements affixed to chip	Serum samples with HIV virus	1.6 kb
Liu et al (2004) [46]	Purification, cell lysis, PCR, microarray hybridization	CNC machined polycarbonate cartridge	On chip electro-chemical pumping, acoustic micro-mixing, paraffin phase change microvalves, and magnetic bead capture	Thermoelectric elements affixed to chip	Bacterial genomic (*E. coli*) in rabbit blood sample	221 bp
Trau et al (2002) [47]	PCR, hybridization	Microfabricated chip (Si/glass)	N/A	Integrated microfabricated heaters and temperature sensors	Plant genomic	Various
Liu et al (2002) [48]	PCR, hybridization	Laser machined polycarbonate	Syringe pump, Pluronic phase change microvalves	Thermoelectric elements affixed to chip	Bacterial genomic (*E. coli, E. faecalis*)	195, 221 bp

Table 4. Summary of highly integrated microfluidic DNA analysis systems

Reference	Integrated Functions	Device Fabrication	Fluid Handling	Temperature Control	Templates	Targets
Burns et al (1998) [49]	SDA, electrophoresis, integrated photodetection	Microfabricated chip (Si/glass)	Pneumatic pumping, drop placement at hydrophobically patterned zones	Integrated microfabricated heaters and temperature sensors	Bacterial genomic (Mycobacterium tuberculosis)	106 bp
Pal et al (2005) [50]	PCR, restriction enzyme digestion, electrophoresis	Microfabricated chip (Si/glass)	Pneumatic pumping, drop placement at hydrophobically patterned zones, paraffin phase change microvalves	Integrated microfabricated heaters and temperature sensors	Reverse transcribed viral genomic (Influenza)	690 bp
Blazej et al (2006) [51]	Sanger sequencing, product purification, electrophoresis	Multilayer microfabricated chip (PDMS/glass)	Electrokinetic with on-chip pneumatic PDMS valves	Integrated microfabricated heaters and temperature sensors	Plasmid DNA	N/A

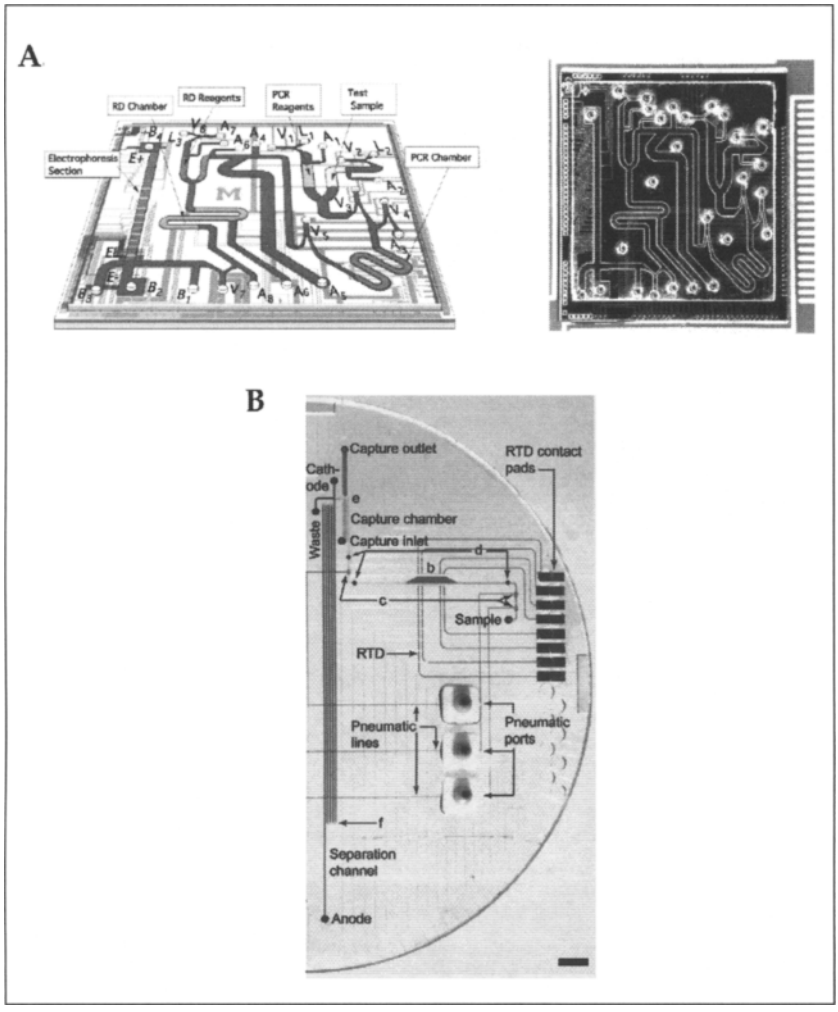

Figure 3. A) Schematic (left) and photograph (right) of a microfluidic device capable of performing a sequential PCR, restriction enzyme digestion and gel electrophoresis.[50] An etched glass microchannel network is bonded to a silicon chip containing integrated electrodes, heaters and temperature sensors. Fluid handling is accomplished using an array of addressable on-chip phase change valves. The chip measures 1.5 x 1.6 cm (reproduced with permission, copyright 2006 Royal Society of Chemistry). B) Layout of a microfluidic bioprocessor for performing Sanger cycle sequencing of DNA (scale bar = 5 mm).[51] Thermocycling reactors are interconnected with post reaction purification and gel electrophoresis components. A multilayer glass/PDMS design is used to incorporate an array of pneumatically actuated microvalves (reproduced with permission, copyright 2006 National Academy of Sciences, USA).

isolated from the rest of the chip. Upon completion of the PCR reaction, the sealing valves were opened and the sample was propelled forward, mixed with additional reagents and incubated to perform a restriction enzyme digestion reaction. Upon completion of the second reaction, the products were directed into a gel electrophoresis column for analysis. This system was used to perform a PCR-RFLP assay for influenza A virus detection, illustrating tremendous potential as

a generic platform suitable for use in a wide range of genotyping applications. Another notable recent advancement has been reported by the Mathies group, where a multilayer glass/PDMS device was used to perform integrated Sanger cycle sequencing of DNA—a reaction requiring similar thermal cycling parameters as PCR (Fig. 3B).[51] Sequencing reactions were performed in 250 nL reactors, after which the products were electrokinetically transported into a novel capture gel for purification. Here, oligonucleotides complementary to the region immediately adjacent to one of the sequencing primers were immobilized in a sparsely crosslinked polyacrylamide gel plug, such that the sequencing products could be retained inside the gel while unincorporated primers and other reagents passed through. Upon heating the gel to a temperature above the primer melting point, the hybridized products were released and electrokinetically injected into the separation channel. Read lengths of 556 bases were achieved with a 99% base call accuracy, demonstrating tremendous potential for significant reductions in cost and time scales associated with genome sequencing.

Novel Micro-PCR Methods

Buoyancy driven natural convection phenomena have also been investigated as a means of accelerating the thermocycling process. By designing reaction chambers that harness an imposed static temperature gradient to generate a circulatory convective flow field, PCR reagents can be automatically transported through temperature zones associated with denaturing, annealing and extension conditions. Convective flow thermocyclers may be broadly classified as *cavity-based* or *loop-based* systems, depending on the nature of the flow field generated. Cavity-based designs typically consist of reactor geometries in which the PCR reagents are enclosed between upper and lower surfaces maintained at annealing and denaturing temperatures, respectively.[52-54] When the aqueous PCR reagent mixture is heated from below, an unstable "top heavy" arrangement is created which can provide sufficient driving force to establish a continuous circulatory flow in much the same fashion as in an ordinary lava lamp. Here, thermocycling parameters (e.g., cycling time, residence time within each temperature zone) are controlled by an interplay between reactor geometry (height to diameter aspect ratio) and the magnitude of the imposed temperature gradient. Krishnan et al successfully demonstrated this concept by amplifying a 295 bp β-actin target from a human genomic DNA template in a 35 μL cylindrical reactor (Fig. 4A).[52] In subsequent work, this simple design has been adapted to perform amplification of a 191 bp target associated with membrane channel proteins M1 and M2 of the influenza-A virus with cycling times ranging from 15 to 40 minutes in a multiwell cartridge format that offers potential for use in high throughput settings.[53,55] Convectively driven PCR of a 96 bp target from a bacterial genomic template has also been demonstrated by Braun et al in low aspect ratio cylindrical cavities using either focused infrared heating or a micro immersion heater inserted at the center of a low aspect ratio cavity to drive the flow (Fig. 4B).[56-58]

Successful PCR amplification has also been demonstrated in closed-loop convective flow systems. These designs are attractive because of their ability to generate unidirectional flows along a closed path enabling cycling parameters to be precisely controlled. Wheeler and coworkers designed a novel disposable polypropylene reactor in which opposite sides of a racetrack-shaped flow loop were maintained at 94 and 55°C respectively to amplify targets ranging from 58 to 160 bp from a bacterial genomic template (Fig. 4C),[59] while Chen et al employed a triangular arrangement with three independently controlled temperature zones maintained at 94, 55 and 72°C to amplify 305 and 700 bp targets from a bacterial genomic template (Fig. 4D).[60] Krishnan et al employed both triangular and racetrack shaped designs to amplify 191 and 297 bp targets from control and human genomic DNA targets respectively.[55] Most recently, Agrawal et al have demonstrated a triangular design in which flow loops are constructed using disposable plastic tubing (Fig. 4E).[61] Here, two opposing sides of the loop are maintained at denaturing and extension temperatures using independently controlled thermoelectric heaters while the third side passively attains annealing conditions. This system is capable of performing single and multiplex PCR for targets ranging from 191 bp to 1.3 kb within 10 to 50 minutes using 10 to 25 μL reaction volumes, highlighting

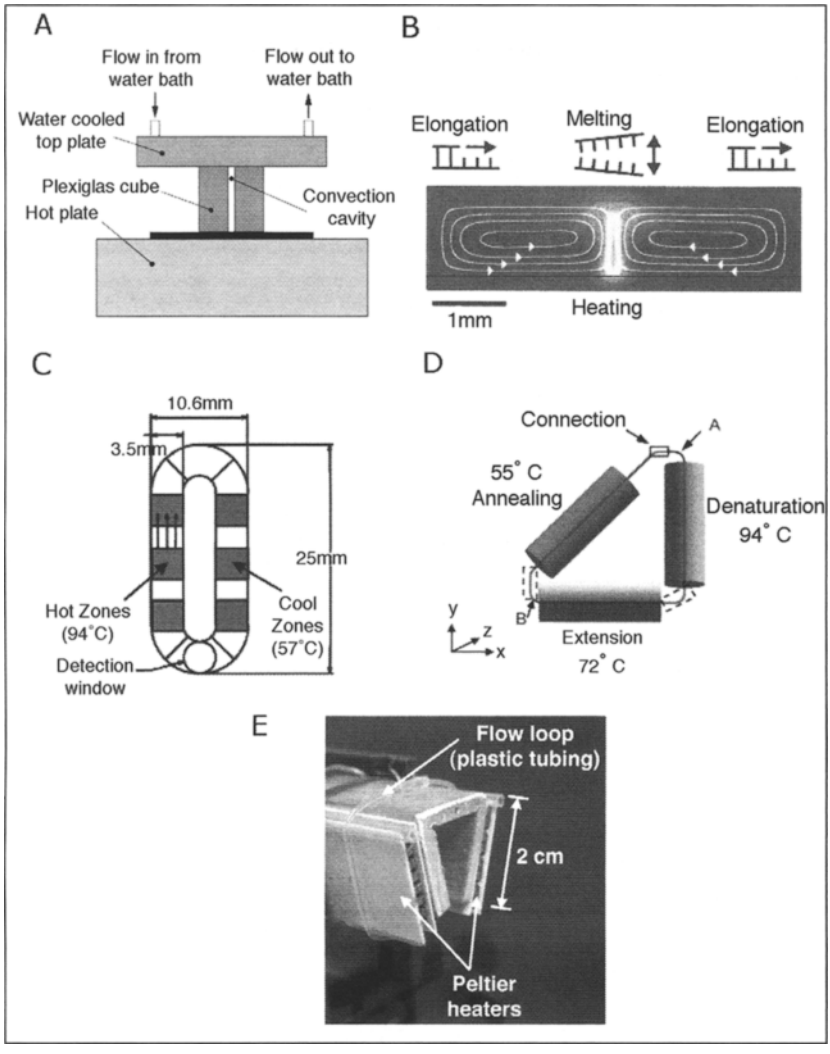

Figure 4. Examples of convective flow-based PCR thermocycler designs. Cavity-based systems may consist of (A) high aspect ratio reactor geometries subjected to a vertical temperature gradient,[52] or (B) low aspect ratio reactor geometries heated at the center[57] (reproduced with permission, copyright 2003 The American Physical Society). Examples of loop-based systems include (C) racetrack shaped designs[59] (reproduced with permission, copyright 2004 American Chemical Society) and triangular loop designs heated along (D) three[60] (reproduced with permission, copyright 2004 American Chemical Society) or (E) two sides of the flow path.[61]

the attractive combination of simplified hardware design and rapid cycling performance possible in convective flow-based systems.

Conclusions and Future Outlook

Impressive progress continues to be made in the development of integrated microfluidic systems capable of performing PCR-based bioanalysis assays. These advances are largely driven by the compelling benefits offered by miniaturization, including lower hardware costs arising from

mass production using microfabrication technology, extremely rapid analysis times and greatly reduced reagent requirements. A number of challenges remain, however, including development of improved miniaturized fluid handling technology capable of precisely metering, transporting and sealing nanoliter liquid volumes to prevent evaporation without relying on bulky off-chip mechanical hardware. Potential inhibitory effects that arise under ultra-high surface to volume geometric conditions are also likely to remain a serious consideration as reactor volumes continue to shrink. Finally, improvements are still needed in the area of product detection, where the necessity to employ benchtop-scale fluorescence imaging systems often limits portability. Developments in these areas are ongoing and ultimately have the potential to greatly expand the use of genomic analysis technology by making the capability to perform an increasingly sophisticated array of assays accessible for use in a wide range of settings by those who need it most.

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