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RECENT DEVELOPMENTS IN MINIATURIZED PCR-MICROCHIPS, MICROARRAYS AND MICRODROPLETS

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ABSTRACT

Microminiaturization of assays and lab-on-a-chip devices hold considerable promise for the future of analysis, especially in point-of-care testing. This article focuses on developments that have occurred during the last five years in the specific area of microchip PCR and miniaturized PCR in arrays of reaction vessels and droplets. Although, this area continues to be an active focus of research and development and the variety and ingenuity of microchip PCR and integrated microchip PCR devices continue to increase, commercialization lags behind the progress being made in digital PCR and arrays for real-time PCR.

Key-words: PCR, RT-PCR, digital PCR, microchip, lab-on-a-chip, genetic testing, miniaturization

INTRODUCTION

PCR performed at a miniaturized scale in µL-nL volumes continue to attract the attention of the research community; a diverse range of "chip", "card'' and "array" devices have been created. This article focuses on developments that have occurred in this field during the last five years. A number of recent reviews have surveyed different aspects of miniaturized PCR and PCR microchips (1-4). More extensive coverage of the earlier literature on these types of analytical devices is presented elsewhere (5-7).

PCR MICROCHIPS

Chip fabrication materials and design - The first PCR microchips were fabricated from silicon and glass (8), but subsequently other types of material, especially polymers have been evaluated. This has been driven in part by issues concerning both ease of fabrication and production cost. In this context polyester has been utilized for PCR microchip fabrication by laser-printing of toner onto polyester films (9). This technique produces microfluidic devices with channel depths on the order of tens of micrometers. Deeper channels (e.g., 270 µm) can be produced using a CO2 laser to cut microchannels in a uniform layer of printed toner coated onto polyester sheets. The final chip is constructed by sandwiching the channel layer between uncoated cover sheets of polyester in which access holes have been precut. This type of chip has been used to perform solid phase extraction of DNA from blood (>65% recovery) and amplify a 520 bp fragment of lambda-phage DNA.

The low thermal conductivity of glass or polymers used to fabricate microchips limits both heat transfer rates and the speed of thermal cycling. Reducing the volume of a PCR reaction chamber can improve the speed of thermal cycling, but this strategy is not suited to analyzing samples with low analyte concentrations. Faster thermal transitionscan be achieved by providing a heat

sink. In the context of glass chips, a heat sink component has been shown to substantially reduce thermal resistance opposing heat dissipation into the ambient environment, and eliminates the parasitic thermal capacitance of other microchip regions that do not need to be heated (10).

On-chip components and integration - The scope of on-chip integration continues to expand and includes chips that combine solid phase extraction and PCR (~500 nL reaction chamber) (11); template purification, polymerase chain reaction (250 nL), post-PCR cleanup and inline injection, and capillary electrophoresis (CE) (12); cell lysis, DNA binding, washing, elution and PCR in the same reaction chamber (13); DNA extraction and PCR (14-16); and cell pre-concentration (via immunomagnetic beads), purification, PCR (100 nL), and capillary electrophoretic analysis (17,18).

Cell isolation is often required as part of an integrated PCR microchip assay and different strategies have been implemented (19) such as bead capture (20) and electrokinetic capture (21). Another option is to use on-chip electrokinetic capture (22). This has been accomplished on a chip by fabricating two interconnected chambers each containing electrodes. The first set of electrodes inside a larger chamber (0.6 µL) diverts bacterial cells from a flowing stream into a smaller chamber (0.4 nL) that contains interdigitated electrodes that actively trap and concentrate the bacterial cells using dielectrophoresis.

One strategy for microchip PCR has been to have separate zones for each of the steps in a PCR reaction (melting, annealing, extension) and move the reaction mixture between zones. However, this ultimately becomes a design challenge. A closed-loop ferrofluid-driven PCR chip provides an elegant solution to fluid movement in this type of multi-zone chip (23). In this prototype, the PCR reaction mixture was contained in a circular closed microchannel in a polymethyl methacrylate chip. Flow of the reaction mixture was driven by the magnetic force generated by an external magnet through a small oil-based ferrofluid plug and amplification of genetically modified soya or maize was achieved in <13 minutes.

Another aspect of integration is to store reagents on a chip. This has been achieved in a PCR chip by means of just-in-time releasable, paraffin-passivated, dry reagents (24). The paraffin protects the stored reagents during storage in the chip and during the sample preparation phase of the assay. In the final analytic phase, the PCR chamber is filled with target. Heating the PCR chamber to the denaturation temperature melts the paraffin and releases the reagents that then rehydrate in the target DNA solution and subsequently initiate PCR. A benefit of this strategy is that it reduces the number of analytical operations and simplifies the flow control on the chip.

Nanotechnology

Nanotechnology and the applications of nanofabricated objects have assumed considerable importance in many branches of science (25). Nanotechnology has also found application in PCR microchips (26-31).

Poly(quaternary ammonium)-modified gold nanoparticles have been used for efficient on-chip cell lysis prior to microchip PCR for the rapid detection of bacteria (3). The nanoparticles remain in the PCR solution thus facilitating integration of cell lysis and PCR as both processes are accomplished in the same reaction chamber; however, one problem was PCR inhibition caused by the gold nanoparticles which was eventually overcome by treating the PCR chamber with 1-10% BSA and increasing the annealing temperature. Gold nanorods can also be used to lyse cells for one step DNA extraction and real-time PCR of pathogens in a single chamber (32). The longitudinal resonance of gold nanorods transforms near infrared energy into thermal energy within a chip, and the heat generated causes cell lysis.

Another application of nanotechnology in the context of PCR microchips has been the development of a magnetic nanoparticle-based microheater embedded in polydimethylsiloxane (PDMS) microchips (29). Heat generated by the microheater could be controlled by the number of embedded particles and by the intensity of the applied AC magnetic field. This new type of chip with an embedded magnetic nanoparticle-based heater was shown to amplify a 732 bp target DNA with good efficiency (>90%) compared to a conventional PCR thermocycler.

Types of amplification reaction

Real time PCR - One target of microchip research has been the development of small portable or handheld instruments for point-of-care DNA analysis that could be used for medical diagnosis or environmental monitoring. To this end an electrochemical real- time PCR has been implemented on a silicon-glass microchip for simultaneous DNA amplification and detection (33). The onset thermal cycle at which the analytical signal became distinguishable from the background, was found to be much lower for the electrochemical real-time PCR compared to a fluorescence-based counterpart (template DNA 3 x 106 copies/µL).

A two-step ultra-rapid real-time (URRT) PCR has been described using a commercial silicon microchip system, the GenSpector TMC-1000 (34). Rapid detection of Enterohemorrhagic E. coli was accomplished in a chip with a 6 µL total reaction volume using 1 sec denaturation and 3 sec combined annealing/extension steps. The STX2 gene target of the Enterohemorrhagic E. coli was detected in ~7 minutes including melting point analysis (detection limit ~3 cfu/PCR) (35).

Reverse transcription PCR (RT-PCR) - RT-PCR in an integrated microdevice has also been developed for single-cell gene expression analysis. The microchip comprises integrated nanoliter metering pumps, a 200-nL RT-PCR reactor with a pad for single-cell capture, and an affinity capture matrix for the purification and concentration of products. The latter is coupled to a microfabricated capillary electrophoresis separation channel for analysis of the product. This microchip was successfully used to measure siRNA knockdown of the GAPDH gene in single Jurkat cells (26).

Point-of-care testing (POCT) detection of human immunodeficiency virus (HIV) has also motivated development of microchips for RT-PCR and an associated analyzer (36). This was achieved using a polymer lab-on-a-chip device coupled with a portable analyzer that provides non-contact infrared-based temperature control and chemiluminescence detection. Analysis of HIV (primer sets for p24 and gp120) with this system was completed in < 1 hour.
Similarly, development of a low cost (~$1000 in component costs), portable and integrated microfluidic instrument has been the motivation for other microchip-based RT- PCR devices (37). Reactions were performed in a tri-layered glass-PDMS microchip that consists of integrated pneumatically-actuated valves and pumps, a thin-film resistive combined heater and temperature sensor, and channels for capillary electrophoresis. The chip fits onto a platform that houses a laser diode and a charged coupled device (CCD) camera, circuitry for thermal control, and mini-pumps to operate the on-chip pumps and valves.

Miniaturized pcr on arrays and in droplets

One route to real-time PCR miniaturization has been to design cards that contain arrays of microwells linked to sample application reservoirs (e.g., TaqMan® Array Card,
384 wells, Applied Biosystems, Foster City, CA) (38). The Fluidigm Digital Array (Fluidigm Corp, South San Franscisco, CA) integrated fluidic circuit is another example of a device that partitions a PCR reaction mix into hundreds of individual PCR reactions in order to perform “digital PCR” (39-41). These devices have an on-chip network of
microfluidic channels, chambers, and valves that automatically assemble individual PCR
reactions ranging from 2304 to 39,960 reactions per device in volumes ranging from 0.85 - 10 nL. Partitioning of PCR reaction mixtures localizes individual nucleic acid molecules in separate regions so that each location will contain either zero molecules or one molecule, i.e., a negative or positive reaction. Quantification of target nucleic acid is then simply achieved by counting the positive regions (42).
Droplets of emulsified PCR reaction mixtures have become a popular choice for miniaturizing PCR to perform digital PCR. For example, the BioRad QX100 Droplet Digital PCR system (Bio-Rad Laboratories Inc, Hercules, CA) produces ~20,000 monodisperse droplets from a 20 µL sample of PCR reaction mixture (43). The RainDance system (RainDance Technologies, Inc., Lexington, MA) produces up to 80 million partitions per run with a volume of only 5 pL per partition (44). Another approach is the BEAMing (Beads, Emulsions, Amplification, and Magnetics) Technology (Inostics Inc., Baltimore, MD) (http://www.inostics.com) (45, 46). This performs single-molecule PCRs on magnetic beads in an emulsion droplet (average diameter of emulsion compartment 5 µm).

Conclusions

Miniaturization of PCR reactions, especially in emulsion droplets, has progressed rapidly in order to reap the benefits of digital PCR and several commercial systems are available. However, commercial development of PCR lab-on-a-chip devices has not kept pace with research and development activities in this area and the full potential of lab-on- a-chip devices for PCR-based analysis remains in the future. Thus, the question remains not if but when this future will materialize.

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