Microchip Electrophoresis and the Analysis of Polymerase Chain Reaction Products

Performing capillary electrophoresis (CE) on microchips has made great progress toward practical applications in recent years. This article describes using microchip-based CE, with and without the integrated function for polymerase chain reaction (PCR), for the analysis of PCR products. The author compares these chip-based electrophoretic methods with several existing methods and other potential competitors. She also discusses the methods’ advantages and limitations and summarizes the future of microchip platforms with fully integrated functions for genetic analysis.

The performance and costs of genetic assays can be improved in the microscale because of the reduced analysis time and reagent consumption and the automation and control provided by miniature electronic devices (7). However, DNA samples in biological fluids often are present at concentration levels that are too low for any direct test. Therefore, analysts normally use enzyme-induced amplification such as polymerase chain reaction (PCR) to increase the sample concentration before analysis (8,9). In this article, I will describe the use of microchip-based CE, with and without the integrated function for PCR, for the analysis of PCR products and discuss its advantages and limitations.

Polymerase Chain Reaction
PCR is an enzyme-induced DNA amplification process designed to increase the concentration of a specific base sequence in various DNA samples by replication (8,9). A typical amplification uses the high-temperature-resistant Taq polymerase enzyme mixed with the unknown DNA sample (or template), an adequate supply of nucleotides (deoxyribonucleic acid [DNA], proteins, and many organic compounds. Among these applications, DNA assays have an enormous scope within the biotechnology and medical fields, ranging from agriculture and farming applications to the detection of pathogens in foods to drug discovery and genetic diagnostics of human subjects (7).

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Simultaneous amplification of more than one target sequence can be achieved by adding different primer pairs for flanking different target sequences. This method is called multiplex PCR. Restriction enzyme digestion sometimes is performed after PCR for analyzing chromosome structure and sequencing very long DNA molecules. Restriction enzymes are used to cleave DNA molecules into specific fragments, and the size distribution of these fragments can serve as a fingerprint for a DNA molecule. The analysis of replicated DNA often involves separations based upon sizes, and their length is determined by comparisons with a calibration ladder.

Conventionally, analysts have used slab-gel electrophoresis or blotting methods in the detection of PCR products, but these techniques are time-consuming and labor-intensive. The required time for these post-PCR analyses could exceed the time for PCR. Another method is to monitor the amplification of specific sequences using the TaqMan technique (10–12). This method uses a fluorogenic oligonucleotide probe that contains reporter dye and quencher located on proximal nucleotides, so the fluorescence of the reporter dye is quenched. The probe is designed to hybridize to the PCR target sequence where it is degraded by the 5′-exonuclease activity of the Taq polymerase during amplification. This process allows the chromophores to be separated spatially and results in an increase in fluorescent signal from the reporter dye. This method is unique in its real-time monitoring, which is useful for quantitative analysis before the reaction is completed (12). However, because no separation is involved, the detection of the products from multiplex PCR by this method is limited by the number of reporter dyes that can be distinguished spectrally. Moreover, the instrument is expensive because of the optical component.

Another relatively new alternative is an array hybridization technique with fluorescence or chemiluminescence detection (13). This method also is a nonseparation protocol that uses DNA probes attached to a rigid surface by a linker molecule (14). The recognition is based upon the matching of complementary strands between the probe and the analyte. Hybridization arrays also can be miniaturized using the microelectro-mechanical systems technique (DNA chips) (15). Scientists have reported many new approaches for performance improvements such as electric field-controlled hybridization (16) and gold particle-bonded sites for detection (17).

Alternatively, electrophoretic analysis resolves the amplified products in a sieving medium, and detection normally is performed using a single dye. As a result, the detection instrumentation is simpler and the number of amplified products that can be detected is limited only by the resolution of the separation. Because of the speed in an automated format and superior separation efficiency, CE becomes an alternative for slab-gel electrophoresis, and it rapidly is becoming an important tool for the analysis of PCR products (18–20). As the electrophoresis technique advances to microchip devices, the use of microchip electrophoresis for DNA analysis is emerging as a promising method (2–6). As Figure 1 shows, the analysis time for DNA fragments is reduced greatly from the slab-gel to the capillary to the microchip format without substantial loss of efficiency.

**Design and Instrumentation of Microchip Electrophoresis**

CE chips are fabricated mainly using various glass substrates, from inexpensive soda-lime glass to high-quality quartz (21,22). Glass substrates are the most common substrates because of their good optical properties, well-understood surface characteristics, and well-developed microfabrication methods adapted from the microelectronics industry. However, glass substrates are relatively more expensive compared with polymer materials. Recently, analysts have used various polymer materials to fabricate microchips for CE separations. Polymer microchips are of increasing interest because their potentially low manufacturing costs might allow them to be disposable. Moreover, no column coating is needed for DNA analysis, which otherwise would require tedious derivatization procedures (23,24). Fabrication procedures for glass and polymer materials are quite different. Structures on glass substrates usually are generated using standard photolithographic technologies (2). Methods for the fabrication of plastic microchips include laser ablation (25), injection molding (26), silicon rubber casting (27), and hot embossing (28).
The design of microchips for CE has undergone significant development from simple, single-channel structures to increasingly complex ones with multiple channels (29). The most common miniaturized chips contain two crossed channels and four reservoirs for the injection cathodes (sample), injection anodes (waste), buffer cathodes, and buffer anodes (Figure 2). This cross-configuration enables a sample-injection system to be integrated with the separation channel on a planar chip device (30). Two major drawbacks with conventional CE are that the sample-introduction method of exchanging sample and buffer reservoirs is time-consuming and lacks precision and ions with greater mobilities are disproportionately introduced in larger quantities with an electrokinetic injection scheme. This integrated device on microchip electrophoresis has solved these two major drawbacks. Consecutive injections can be performed simply by voltage switching among the four reservoirs (31). Moreover, the volume of sample plug can be controlled accurately with pinched-mode loading, and it is time independent and enables the injection of a constant volume without any electrophoretic mobility-based bias (32).

Although the separation performance of microchips measured per unit length is similar to or exceeds that of conventional CE, the absolute resolution and peak capacity are lower, primarily because of shorter separation lengths (33).

To maintain the compact footprint of the microchip, however, the lengthened separation channel will need to incorporate serpentine turns that will add a geometrical contribution to analyte dispersion (34). Researchers recently reported several novel designs of channel geometry such as a spiral-shaped channel (35) and tapered turns (36) that increase the separation length in a small footprint area. Unlike DNA sequencing, however, a high peak capacity and resolution are not critical for the sizing of amplicons because usually a limited number of PCR products is available (24,37–39). Hence, the cross-configuration has been used most often thus far, including multiplex PCR (24,37–39).

Sensitive detection schemes are essential in microfabricated devices for CE because of the extremely small size of the detection cell. The most common detection system for microchip electrophoresis is laser-induced fluorescence, which provides sensitivity, particularly for DNA analysis (27) (Figure 3). Both the covalent dye and intercalating dye have been used for the analysis of PCR products. Covalent dyes such as Cy5 can be incorporated into the PCR process by using fluorophore-labeled primers or fluorophore-labeled dNTP reagents. These fluorescence-emitting labels are extremely sensitive and permit the detection of individual molecules in femtoliter-volume samples.

Alternatively, the intercalating dyes such as YO-PRO or TO-PRO can be added into the separation buffer before analysis. Intercalating dyes fluoresce when excited by ultraviolet (UV) light only when bound between two nucleotides in double-stranded DNA. Because a fragment can accommodate one intercalating label per base pair, a single DNA molecule can contain hundreds of fluorophores and emit a strong signal. The fluorescence signal can be observed using epi-fluorescence microscopes (Figure 3) with a photomultiplier tube or a charge-coupled device. The output from a photomultiplier tube or a charge-coupled device can be preamplified, digitized, and acquired by a computer for processing.

Laser-induced fluorescence detection of multichannel chips presents additional requirements compared with a single-channel chip (27). Analysts can scan a laser or microchip microchannel, which reduces the duty cycle, or use continuous illumination, which reduces laser power density at each microchannel. However, either method leads to a reduced signal-to-noise ratio. Recently, commercial instruments designed mainly for DNA analysis have come on the market; they include power supplies, fluorescence readers, disposable single-channel glass chips, reagents and standards, and a computer for data acquisition and system control (40). Mueller and co-workers (40) reported validated results obtained using the commercial system, and...
Dolník and colleagues (29) published a review article about microchip electrophoresis.

**Microchip Electrophoresis for Post-PCR Analysis**

Although an increased number of research groups are reporting their production and use, applications of electrophoretic microchips in real-world analyses are very few. The low usage primarily is because of the technique's early stage and the fact that commercial instrumentation has become available only recently. Only four reports have been published since 1998, and most applications are for clinical diagnostics. In these approaches, the researchers used microchip electrophoresis to size and detect the PCR products that were made independently using a conventional PCR instrument or another microchip.

In Landers and colleagues' work (37), capillary-based separations were replicated on microchip electrophoresis to replace slab-gel electrophoresis in separating B- and T-cell gene rearrangement PCR products. When they used multiplexed PCR and subsequent fragment separation, a normal cell population displayed a large variety of DNA fragment sizes, whereas a malignant population displayed a predominance of one DNA fragment size. Their results indicate that the separation of B- and T-cell gene rearrangement PCR products on microchips provides diagnostic information in dramatically reduced time — 160 s versus 2.5 h — with no loss of diagnostic capacity when compared with current methodologies. In this study, they found that poly(vinylpyrrolidone) might provide an adequate sieving matrix without a microchannel coating on a silica-based substrate.

Kant and co-workers (38) compared the microchip electrophoresis method with the liquid hybridization-gel retardation method as an established clinical PCR product detection for the diagnosis of herpes simplex encephalitis. This study examined archival DNA from 33 selected cerebrospinal fluid specimens, and the herpes simplex virus PCR products were transferred to microchips without any desalting step. Results indicate that microchip electrophoresis provided identical results with less than 110 s/sample and achieved 100% sensitivity and specificity, compared with 33 min/sample using the established method.

Chen and colleagues (24) investigated fused-silica CE and plastic microchip electrophoresis on a poly(methyl methacrylate) substrate for clinical post-PCR analysis of hepatitis C virus. They found that the hepatitis C virus assay established on the wall-coated, fused-silica tubing could be transferred directly to an uncoated poly(methyl methacrylate) microchip with comparable separation power and separation time reduced by one order of magnitude. In conclusion, the polymer microchip provides advantages such as no column coating, fast processing time, simple operation, and disposable use, and it holds great potential for clinical analysis of PCR products.

Kricka and co-workers (39) performed random nonspecific amplifications of the human genome on one microchip and then loaded aliquots into another microchip for a locus-specific, multiplex PCR as part of a Duchenne-Becher muscular dystrophy study. They analyzed the amplicons by traditional CE and microchip electrophoresis on a silicon-glass substrate and obtained similar results.

![Figure 4](https://chromatographyonline.com)

**Figure 4:** Real-time analysis of a β-globin PCR amplification using an integrated PCR-CE microdevice. Chip CE separations of the same sample were performed sequentially in the integrated PCR-CE microdevice after (a) 15, (b) 20, (c) 25, and (d) 30 cycles at 96 °C for 30 s and 60 °C for 30 s. (Reprinted with permission from reference 41.)
Escherichia coli genomic DNA, and plasmid DNA can be amplified from E. coli cells on a microchip and then electrophoretically separated in less than 3 min.

The device developed by Burns and colleagues (43) incorporated simple injectors, a mixer, a heating chamber, and a separation channel with detectors. They constructed diode detectors in a silicon substrate with a thick optical filter deposited on the surface that blocked UV excitation. In this device, the sample and reagent could be driven through the system pneumatically or by heated gas trapped in pockets behind the sample, and the sample spread was controlled by a series of hydrophobic stops. This device demonstrated the motion, amplification, separation, and detection of DNA samples integrated in a single part.

The latest device from Lagally, Paegel, and Mathies (36) allows submicroliter-scale PCR reactors to be coupled directly with separation microchannels that enables a 30-cycle amplification reaction to be completed in only 15 min. In this device, novel tapered-turn geometries for the microfabrication of folded long channels provide increased device performance and feature density. Although the PCR–CE chips have been microfabricated and investigated for model reactions, these devices have not been applied to real-world analysis (36, 41–43).

**Conclusion and Outlook**

Despite the fact that few reports have demonstrated the application of electrophoretic microchips to real-world analysis, it is convincing that this technology is well-suited to the rapid analysis of PCR-amplified DNA with minimum consumption. I foresee a substantial increase in published reports in the coming years as the instrument becomes popular. However, a complete DNA protocol in the microscale requires several steps, including extraction or purification of nucleic acid from a cell followed by amplification, labeling, and electrophoretic separation. These functions must be either implemented in the microchip or acquired through interfacing with the outside world by hardware connections. Presently, none of the established techniques can automate the whole process, even in the macroscale.

Perhaps the greatest advantage of the electrophoretic chip platform over the slab-gel and even the capillary formats is its potential for directly integrating sample-processing steps. Although many novel approaches have been demonstrated or proposed, their practical implementation presents more problems. If these and other challenges can be overcome, CE performed on microchips will become a major technology for general life science applications.

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**References**


