

# Clinical Analysis by Microchip Capillary Electrophoresis

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**Clinical analysis often requires rapid, automated, and high-throughput analytical systems. Microchip capillary electrophoresis (CE) has the potential to achieve very rapid analysis (typically seconds), easy integration of multiple analytical steps, and parallel operation. Although it is currently still in an early stage of development, there are already many reports in the literature describing the applications of microchip CE in clinical analysis. At the same time, more fully automated and higher throughput commercial instruments for microchip CE are becoming available and are expected to further enhance the development of applications of microchip CE in routine clinical testing. To put into perspective its potential, we briefly compare microchip CE with conventional CE and review developments in this technique that may be useful in diagnosis of major diseases.**

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Since the development of capillary electrophoresis on a chip (microchip CE)<sup>3</sup> in the early 1990s (1, 2), this technology has been a focus of research in chemical and biochemical analysis and has been reviewed extensively (3–32). Potential advantages of microchip CE include miniaturization, integration, high speed, and reduced reagent consumption. We present an overview of the approaches and selected applications of microchip CE devices in the diagnosis of major diseases, including cancer (33–41); cardiovascular (42–45), renal (46, 47), neurologic (48–50), thyroid (51, 52), and infectious diseases (53–64); immune disorders (65–69); diabetes (70–72); and hereditary diseases (73–77). We also briefly

compare microchip CE with conventional CE and speculate on the future role of microchip CE in the clinical laboratory.

Commercial systems for microchip CE analysis have recently become available and are increasingly used for routine analysis. The introduction of more automated and higher throughput systems will likely make microchip CE technology more widely accepted in clinical analysis laboratories. Several companies are now supplying ready-made and/or custom-fabricated microchips, which will facilitate application of microchip CE devices. Some suppliers of commercial microchip CE systems and foundries for fabricating microchip CE devices are listed in Table 1. Approaches and selected applications of microchip CE for diagnosis of major diseases are described in the following sections.

## Cancer

Several approaches have been developed for the analysis of cancer susceptibility genes by microchip CE, including single-strand conformation polymorphism analysis (33) and a combination of allele-specific DNA amplification with heteroduplex analysis (34–37). Common mutations in the *BRCA1* and *BRCA2* genes show strong correlations with breast cancer, particularly in the Ashkenazi Jewish population (33). Using microchip CE, Landers' group decreased single-strand conformation polymorphism analysis time to ~130 s, at least a 100-fold improvement over conventional methods (33–37).

Another approach developed by the same group combines allele-specific DNA amplification with heteroduplex analysis for the detection of each mutation in the *BRCA1* and *BRCA2* genes (34–37). A schematic diagram of the system used is shown in Fig. 1, and results obtained for detection of mutations of *BRCA1* are shown in Fig. 2. The system was also used in assays for T- and B-cell lymphoproliferative disorders (38), and microchip CE

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<sup>3</sup> Nonstandard abbreviations: CE, capillary electrophoresis; CSF, cerebral spinal fluid; LIF, laser-induced fluorescence; and DMD, Duchenne muscular dystrophy.

**Table 1. Suppliers of microchip CE instrumentation and foundries for microchip CE devices.**

Suppliers/Foundries	Website	Microchip CE instrumentation
<b>Suppliers</b>		
Agilent (Palo Alto, CA)	www.agilent.com	Bioanalyzer 2100: microchip CE platform for analysis of DNA, RNA, proteins, and cells 5100 ALP: automated lab-on-a-chip platform handling up to 12-well plates
Caliper Life Science (Hopkinton, MA)	www.caliperls.com	Labchip automated electrophoresis systems
CE Resources (Singapore, Republic of Singapore)	www.ce-resources.com	Modular systems for microchip CE: multichannel HV power supply (HVPS) and different detectors
Hitachi (Tokyo, Japan)	www.hitachi.com.jp	SV110: microchip CE with LED confocal fluorescence detector
Shimadzu (Kyoto, Japan)	www.shimadzu.com.jp	MCE-2010: quartz microchip CE-based system with photodiode array detector
<b>Foundries</b>		
AMIC (Uppsala, Sweden)	www.amic.se	
Bartels Mikrotechnik (Dortmund, Germany)	www.bartels-mikrotechnik.de	
Epigem (Cleveland, UK)	http://www.epigem.co.uk	
Micalyne (Edmonton, Canada)	http://www.micalyne.com/index.html	
Microfluidic Chip Shop (Jena, Germany)	http://www.microfluidic-chipshop.com	
Scandinavian Micro Biodevices (Lyngby, Denmark)	http://www.smb.dk	

was found to provide the same information as slab gel electrophoresis and conventional CE, but with much shorter analysis time (160 s vs 2.5 h on slab gel and 15 min on capillary CE).

Thomas et al. (39) performed microchip CE in uncoated polymer-based microchannels filled with various separation matrices for rapid analysis of ligase detection reaction products of low-abundance point mutations in genomic DNA. Diagnostic testing for point mutations in the human *k-ras* oncogene was performed on DNA obtained from colorectal tumors. The ligase detection reaction products were resolved from unligated primers in <120 s, ~17 times faster than capillary gel electrophoresis, with only a slight decrease in resolution.

Cantafora et al. (40) evaluated RNA messengers involved in lipid trafficking of human intestinal cells by reverse transcription-PCR with competitor technology and microchip CE. Their results showed that analysis of specific RNA messengers allows reliable evaluation of relative gene expression in CaCo-2 cells and confirmed

the role of cholesterol as a positive inducer of specific factors such as LXR- $\alpha$  and FXR.

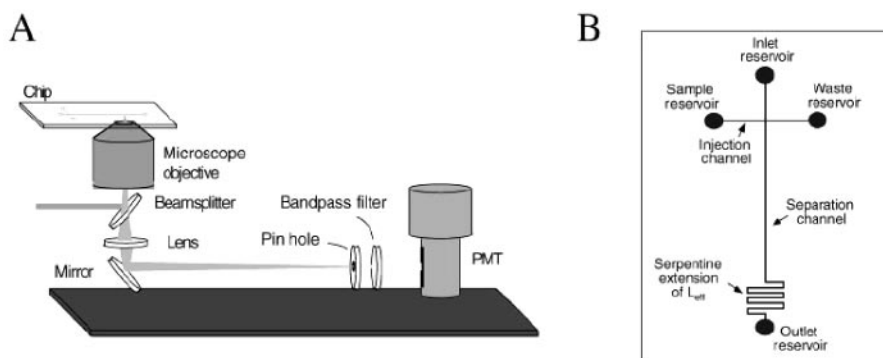
In a microchip-based enzyme assay for protein kinase A (41), the assay reagents were mixed in etched channels by electroosmotic flow. A fluorescein-labeled heptapeptide (LeuArgArgAlaSerLeuGly) was used as substrate for the enzymatic reaction. Substrate and the products of the reaction were separated electrophoretically, and the results demonstrated the usefulness of microchip CE for performing enzymatic assays for which a fluorogenic substrate is not readily available (41).

### Cardiovascular and Related Diseases

Microchip CE has been investigated as a diagnostic tool for assessment of arteriosclerosis via analysis of LDL (42, 43) and homocysteine (44). In addition, microchip CE has been used for the analysis of DNA fragmentation in cells to evaluate apoptosis in individual cardiomyocytes for the diagnosis of doxorubicin-induced cardiomyopathy, a life-threatening condition in patients undergoing

Fig. 1. LIF detection system for the electrophoretic microchip, based on an epifluorescent design (A), and single-channel chip, showing reservoirs, injection channel, and separation channel (B).

Reprinted with permission from Ferrance and Landers (36).



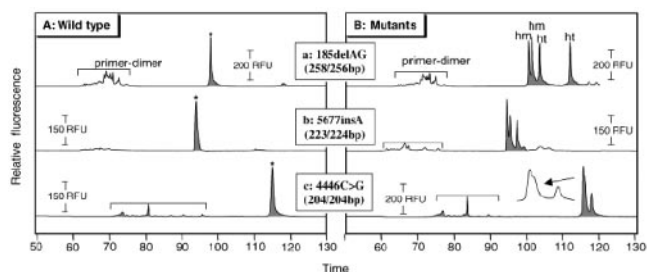


Fig. 2. Diagnostic screening of DNA mutations in the *BRCA1* and *BRCA2* breast cancer susceptibility genes, using heteroduplex analysis on a microchip.

The 3 types of DNA mutations—deletions, insertions, and substitutions—are all readily detected in separations taking <130 s. *RFU*, relative fluorescence units; *hm*, homozygote; *ht*, heterozygote. Reprinted with permission from Ferrance and Landers (36).

chemotherapy (45). For LDL analysis, Ceriotti et al. (42) used microchip CE with either glass microchannels dynamically coated with 40 mmol/L methylglucamine to prevent lipoprotein adsorption or uncoated glass microchannels with 0.3 mmol/L sodium dodecyl sulfate added to the sample buffer to sharpen the LDL peak.

A microchip CE-based method with electrochemical detection has been developed for the analysis of total and protein-bound homocysteine (44), which should be routinely tested in patients at risk for cardiovascular disease, according to the American Heart Association (44). Typical results obtained for the detection and separation of homocysteine and reduced glutathione by microchip CE are shown in Fig. 3.

Kleparnik et al. (45) used a compact disk-like microfluidic device to perform cell lysis, electrophoresis, and laser-induced fluorescence detection of doxorubicin-induced apoptosis of individual cardiomyocytes in the microchip device. Apoptosis was detected by analysis of

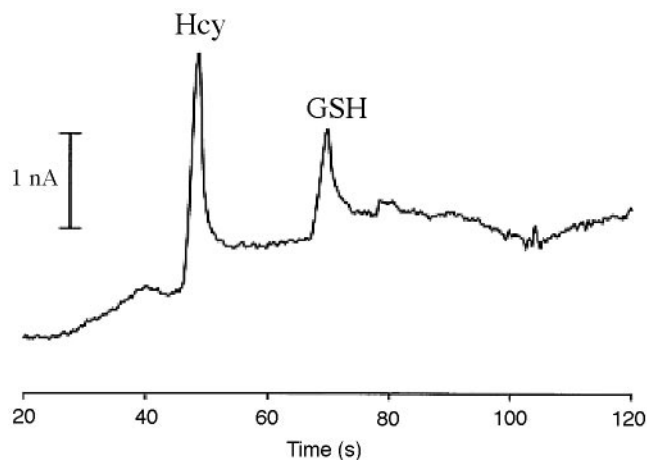


Fig. 3. Detection and separation of 200  $\mu\text{mol/L}$  homocysteine (*Hcy*) and reduced glutathione (*GSH*).

Elution buffer, 20 mmol/L TES (pH 8.0), 400  $\mu\text{mol/L}$  tris(2-carboxyethyl)phosphine (pH 7.5); applied potential, 1800 V (360 V/cm); injection, 4 s at 1200 V;  $E = +100$  mV with Au/Hg amalgamated electrode. Reprinted with permission from Pasas et al. (44).

DNA fragmentation in cells treated with doxorubicin for different durations. The results showed that prolonged exposure of cardiomyocytes to doxorubicin was associated with cellular necrosis (45).

### Renal Markers

Analysis of renal markers such as creatinine, creatine, uric acid, urea, and *p*-aminohippuric acid in biological fluids allows evaluation of renal and muscular functions (46, 47). Microchip CE analysis of renal markers has been integrated with electrochemical detection (46, 47). One method was based on coupling of enzymatic bioassays using creatininase, creatinase, and sarcosine oxidase and electrophoretic separation and amperometric detection of reaction products (46). Gold-coated thick-film electrodes were used for the amperometric detection, and detection limits of  $2 \times 10^{-5}$  to  $4 \times 10^{-5}$  mol/L (signal-to-noise ratio = 3) were reported. An alternative method enabled direct measurement of the renal markers by pulsed amperometry for the detection of nitrogen-containing compounds and more easily oxidized uric acid (47). Four renal markers (creatine, creatinine, *p*-aminohippuric acid, and uric acid) were readily measured within 5 min. A creatinine:creatine ratio was determined in <2 min (compared with  $\sim 20$  min with conventional creatinine analysis methods). The microchip CE device allowed rapid, simple, and economical renal function testing. These devices have substantial potential advantages for decentralized clinical testing and point-of-care testing. Further developments to integrate additional functions (e.g., on-chip filtering of biological fluids) to analyze serum samples, and to perform parallel and multiple runs on a single-chip platform could enhance the acceptance of microchip CE for use in high-throughput clinical microanalyzers.

### Neurologic Diseases

Microchip CE has been used to analyze cerebral spinal fluid (CSF) samples for inflammatory cytokines and inhibitors of an enzyme for nerve function (48–50). Lapos et al. (48) demonstrated the applicability of a microchip CE device with dual laser-induced fluorescence (LIF) and electrochemical detectors for the analysis of CSF samples from patients with multiple sclerosis (48). Simultaneous LIF detection of 4-chloro-7-nitrobenzofurazan-derivatized amino acids (Arg, Phe, and Glu) and electrochemical detection of dopamine and catechol were demonstrated. Microfluidic assays for inhibitors of acetylcholinesterase, an enzyme essential for proper nerve function, have also been developed (49). Separation and detection of a mixture of 4 cationic inhibitors—tacrine, edrophonium, and tetramethyl- and tetraethyl-ammonium chloride—was accomplished within 70 s with a multiplexed screening device combining flow injection analysis and microchip CE separation.

Chip-based immunoaffinity isolation has been combined with CE for the rapid analysis of inflammatory cytokines in CSF (50). A panel of 6 immobilized antibody

ies was attached to the injection port of the chip to isolate the reactive cytokines from CSF samples obtained from patients with traumatic head injury.

### Thyroid Function

Schmalzing et al. (51) described a competitive immunoassay for the determination of serum thyroxine (3,5,3',5'-tetraiodo-L-thyronine) based on electrophoretic separation in fused-silica microchips and LIF detection. Analysis speed was substantially faster than conventional immunoassay and electrophoresis in capillaries, with separation of free from bound labeled thyroxine in ~15 s for serum samples. A microchip-based amperometric competitive immunoassay using a ferrocene redox label has been developed for the determination of 3,3',5'-triiodo-L-thyronine (52). The assay consisted of on-chip, precolumn reaction of the labeled antigen and the target antigen with the antibody, electrophoretic separation of the free and bound labeled antigen, and amperometric detection of the redox tag. A minimum detectable concentration of 1000 µg/L and an analysis time of 130 s were achieved.

Although the above protocols were developed for the testing of thyroid function, integration of immunologic reactions, electrophoretic separation, and a sensitive detection system on a microchip platform enables rapid immunoassay for a wide range of analytes.

### Infectious Diseases/Pathogens

High-speed, high-efficiency microchip CE devices are potentially powerful tools for the detection of pathogens and diagnosis of infectious diseases, an important function because of the acute nature of certain infectious diseases and the growing threat of bioterrorism. Analysis of specimens for the presence of viruses (53–58) and bacteria (59–64) by microchip CE has been demonstrated. To date, most of these studies have focused on the analysis of extracted and PCR-amplified nucleic acid fragments rather than on intact viral or bacterial particles.

#### VIRAL INFECTIONS

Zhou et al. (53) developed a microfluidic system for the determination of severe acute respiratory syndrome coronavirus. For nasopharyngeal swabs from patients with a clinical diagnosis of severe acute respiratory syndrome, this system provided higher positivity rates more rapidly than conventional reverse transcription PCR (17 of 18 vs 12 of 18 positive identifications).

Chen's group analyzed hepatitis C virus qualitatively (54) and quantitatively (55). For qualitative analysis, they used a plastic (polymethylmethacrylate) microchip for the analysis of products from a 2-stage PCR with 2 pairs of primers designed to amplify the 5' noncoding region. The microchip CE device could resolve the 145-bp amplicon of hepatitis C virus in <1.5 min (54). For quantitative analysis, reverse transcription-competitive PCR was used (55). Co-reverse transcription and coamplification were performed for wild-type RNA extracted from serum with

a constant amount of recombinant internal standard RNA with the same primer binding region as the target template except for the removal of a centrally located 25-bp segment specific to the target RNA. Results were comparable to those obtained with a commercial hybridization-based detection method.

Diagnosis of herpes simplex encephalitis can be performed by analysis of PCR products of DNA extracted from CSF. Hofgartner et al. (56) used microchip CE to analyze archival DNA from 33 CSF specimens submitted for herpes simplex virus PCR testing. Microchip CE achieved 100% sensitivity and specificity with much shorter total analysis time than established methods (<100 s/sample vs 18 h for routine clinical liquid hybridization/gel retardation assay). Because the hybridization step is eliminated, the real-time, quantitative, fluorescence-based PCR assay also has the advantage of rapid analysis, but expensive instrumentation is required for this approach. Microchip CE-based technology has the advantage of versatility because it can detect a variety of fluorescently labeled clinical markers and integrate additional laboratory functions in addition to separation and detection steps.

Vegvari et al. (57) developed a hybrid microdevice based on a combination of a polyvinylchloride supporting plate and a fused-silica capillary fitted into a U-groove on the plate for the analysis of various samples, including peptides, proteins, DNA, viruses, and bacteria (e.g., Semliki Forest virus). Because of the high optical transparency of the fused-silica capillary, ultraviolet detection can be used, and this type of device is expected to be useful for a broad range of applications (57).

CE devices for DNA analysis have also been microfabricated by compression molding of polycarbonate (58). DNA separation in these devices provided good resolution and run-to-run reproducibility. In addition, on-chip PCR-CE of a 500-bp region of bacteriophage lambda DNA was demonstrated by thermally cycling the entire chip, with the sample reservoir of the CE device serving as the PCR chamber (58).

#### BACTERIAL INFECTIONS

Microchip devices integrating PCR with CE have been used for the analysis of bacteria such as *Escherichia coli* (59–63), *Staphylococcus* (59), *Salmonella* (62), and *Streptococcus* (64). Lagally et al. (59) developed an integrated portable genetic analysis system for detection of pathogens. The results obtained for the analysis of DNA from intact cells of different strains of *E. coli* are shown in Fig. 4. The system could be used to detect the serotype and pathogenic status of a given cell population simultaneously and to detect antibiotic resistance (59).

Other integrated microdevices have been developed for cell lysis, multiplex PCR amplification, and electrophoretic sizing of PCR products with a marker (60). For

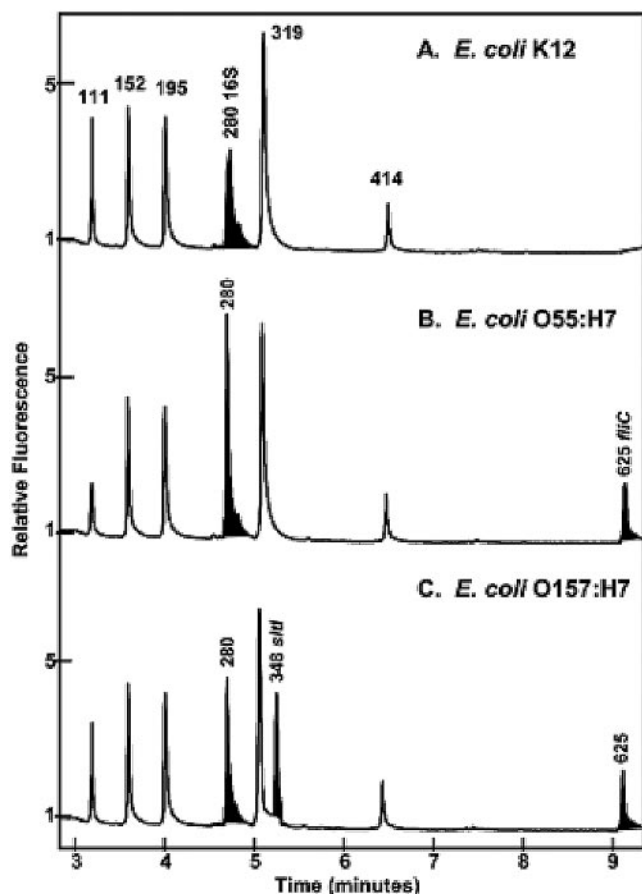


Fig. 4. Pathogenic organism analysis conducted directly from intact *E. coli* cells on the portable PCR-CE microsystem.

(A), analysis of *E. coli* K12 cells, showing only the presence of the coinjected DNA ladder and the 280-bp 16S species-specific amplicon. (B), analysis of *E. coli* O55:H7 cells, showing the ladder, the 280-bp 16S species-specific amplicon, and the 625-bp *flIC* amplicon characteristic of cells presenting the H7 surface antigen. (C), analysis of *E. coli* O157:H7 cells, showing the DNA ladder, the 16S species-specific amplicon, the 625-bp *flIC* amplicon, and the 348-bp *sII* amplicon, characteristic of *E. coli* both possessing an H7 antigen and expressing shigatoxin. Each analysis was conducted with a starting concentration of 40 cells in the reactor in a time of 30 min. Reprinted with permission from Lagally et al. (59).

example, plastic microfluidic devices have been fabricated that integrate PCR, microfluidic valving, and electrophoresis for bacterial detection and identification (62). Amplicons generated in the plastic device from PCR reactions with genomic DNA from *E. coli* (232 bp) and *Salmonella* (429 and 539 bp) were successfully analyzed. Woolley et al. (63) used an integrated PCR-CE device for rapid assay of genomic *Salmonella* DNA. The entire assay was accomplished in <45 min, including both the PCR and the CE separation steps.

Microchip CE detection of cariogenic bacterial genes has also been achieved (64). Allele-specific PCR primers were designed based on the dextranase gene to identify *Streptococcus mutans* and *Streptococcus sorbrinus* in dental plaque. A polymer mixture consisting of hydroxypropyl methylcellulose and polyethylene oxide served as the separation medium for microchip CE. Rapid (85 s), pre-

cise (CV = 0.3%; n = 7), high-resolution (resolution = 2.67 for 226 bp/202 bp), and sensitive (10- to 100-fold better than agarose gel electrophoresis) analysis was achieved.

### Immune Disorders

Several microchip CE methods have been developed to detect increased concentrations of IgG, which may be associated with chronic infection (polyclonal increases) or cancer (monoclonal increase) (65–68). Linder et al. (65) developed a heterogeneous competitive immunoassay of human IgG that used Cy5-human IgG as tracer and Cy3-mouse IgG as internal standard. Quantification of human IgG in serum was difficult because of the high relative SD at a low human IgG concentration and the weak concentration dependence at high IgG concentrations. Nevertheless, it was possible to unambiguously distinguish patient serum samples with increased IgG (35.5 g/L in one patient with chronic infection and 64.7 g/L in another patient with myeloma) from those with IgG concentrations within the reference interval (8–16 g/L).

Conventional CE with a short capillary and a glass microchip CE device has been used to analyze fluorescein isothiocyanate-labeled anti-human IgG (66). The microchip device had several advantages, including high efficiency, fast analysis time, and low requirements for samples and solvents. Wang et al. (67) described an electrochemical enzyme immunoassay on microchip platforms. Precolumn reactions of alkaline phosphatase-labeled antibody with antigen, electrophoretic separation of the free antibody and antibody-antigen complex, postcolumn reaction of the enzyme tracer with the 4-aminophenyl phosphate substrate, and amperometric detection of the liberated 4-aminophenol product were performed on the same device. A very low detection limit [ $1.7 \times 10^{-18}$  mol/L ( $2.5 \times 10^{-16}$  g/mL)] was reported for the model analyte (mouse IgG). In another study, direct measurement of antibodies and monitoring of immunologic interactions was achieved with a microchip CE system with contactless conductivity detection (68). With a glass microchip CE device, separation and detection of IgG (anti-IgM), IgM, and the complex were performed simultaneously.

### Diabetes

Insulin and glucose analyses are important in the diagnosis of several conditions, including diabetes, pancreatic islet cell malfunction, hypoglycemia, and insulinoma. Several studies have been devoted to the use of microchip CE devices for the measurement of insulin (69, 70) and glucose (69, 71). Dual immunologic and enzymatic microchip-based assays for simultaneous measurements of insulin and glucose have been described by Wang et al. (69). Insulin immunodetection was performed with alkaline phosphatase-labeled antibody with postcolumn addition of *p*-nitrophenylphosphate substrate, and glucose analysis with glucose dehydrogenase and NAD<sup>+</sup>.

A microfluidic chip has been developed for continuous electrophoresis-based immunoassay monitoring of hormone secretion from live cells (70). Insulin secreted from islets of Langerhans was detected in a CE competitive immunoassay. Insulin secretion profiles could be obtained, and characteristics of first- and second-phase insulin secretion could be observed. Microchip CE systems are highly suited for monitoring the chemical environment of live cells with high temporal resolution, and such devices may be used for cell-based sensing and diagnostic systems in routine clinical laboratories.

Du et al. (71) described a microchip CE device for electrophoretic detection of glucose in human plasma. Separation and injection channels were fabricated on a poly(dimethylsiloxane) layer. Copper microelectrodes were fabricated on the electrode plate by selective electrodeless deposition. In pilot studies, glucose in human plasma from 3 healthy individuals and 2 individuals with diabetes was successfully determined.

### Hereditary Diseases

Microchip CE has been used for the analysis of genetic diseases such as Duchenne muscular dystrophy (DMD) (72–74) and hemochromatosis (75), and for genomic DNA analysis (76). DMD is caused by mutations in the dystrophin gene on the X chromosome. Carriers of the disease are identified by the detection of duplicated or deleted exons in the gene (72–74). The deletions/duplications associated with DMD tend to be located at certain regions of the gene, making the diagnosis of DMD (and the related, less severe Becker muscular dystrophy) a straightforward process involving analysis of a limited number of PCR-amplified DNA fragments (72–74). An integrated microdevice for infrared-mediated PCR amplifications directly coupled to microchip CE DNA separations was developed for this purpose. In this approach, infrared radiation directly heats the PCR mixture rather than heating the microchip (72), allowing fast temperature cycling and shorter analysis time. Unfortunately, separation efficiency was hampered by heating of the microchip

sieving matrix buffer solution during PCR cycling. Because heating is integral to PCR, this problem must be solved before this approach can become useful. Nevertheless, PCR-CE provided considerable savings in time, labor, and materials compared with traditional methods (e.g., Southern blot). Solution of the heating problem could make feasible a fully integrated diagnostic device for genetic diseases based on microchip CE.

Ertl et al. (75) developed a sheath-flow supported electrochemical detection system for microchip CE analysis of an allele-specific, PCR-based single-nucleotide polymorphism typing assay and demonstrated the application of the system for diagnosis of hereditary hemochromatosis by detection of the C282Y polymorphism. The sheath-flow design minimized interferences of the detection system from electrophoresis potentials. Unlike optical detection systems, the electrochemical detector is easier to miniaturize because bulky optical components (i.e., light source, lens, and filters) are not required. The use of multiple electrodes may make this system a portable micro total-analysis system for analyzing complex analyte mixtures. A semiautomated sample preparation, amplification, and electrophoretic separation platform has also been developed for analysis of human genomic DNA to detect hereditary and infectious diseases based on microchannel CE with 2-color optical detection (excitation wavelengths at 488 and 532 nm). Two-base pair resolution of single-stranded DNA was achieved in the analysis of PCR products from leukocyte lysates (76).

### Comparison of Microchip CE and Conventional CE

Conventional CE revolutionized DNA analysis and was vital to the success of the Human Genome Project (77, 78). Introduction of microchip CE technology represents another major step in the development of miniaturized, rapid, automated, and integrated analytical systems with potential to meet the requirements of lower cost, faster, more sensitive, and more selective analytical systems to solve complex clinical analysis problems. The most commonly available features of conventional and microchip

**Table 2. Comparison of conventional and microchip CE.**

Feature	Conventional CE	Microchip CE
Injection	Hydrodynamic; electrokinetic	Mainly electrokinetic
Detection	Mainly ultraviolet and LIF	Mainly LIF
Separation channels	Mainly silica; single capillary or capillary array	Glass or polymer
Separation media	Buffers, gels, sieving polymers, microparticles	Buffers, sieving polymers, microparticles
Analysis speed	Fast (typically minutes)	Very fast (typically seconds)
Peak capacity	More peaks because of longer capillaries	Fewer peaks because of short channels
Integration	Hard to connect capillaries without dead volume	Easy to integrate multiple functions, e.g., PCR-CE
Automation	Highly automated	Highly automated in some commercial systems
Throughput	Very high for multicapillary systems	Very high for multichannel systems
Sample amount	Very small (nanoliters to microliters)	Very small (nanoliters to microliters)
Reagent usage	Very small (typically microliters to milliliters/day)	Very small (typically microliters to milliliters/day)
Potential for growth	Relatively mature	Emerging technology with potential for novel microchip designs and new applications

CE are compared in Table 2. Microchip CE has some disadvantages compared with conventional CE, such as lower peak capacity because of the shorter separation channels and a lack of compatibility with versatile ultraviolet detectors because microchip fabrication materials are typically not transparent to ultraviolet light. Important advantages of microchip CE, however, include rapid analysis (typically ~10 times faster) and easy integration of sample preparation and derivatization steps, which allow further reduction of analysis time and labor costs. Because cost and speed are crucial concerns for modern clinical analysis, these advantages are expected to become more important when new generations of commercial microchip CE systems become available, and eventually microchip CE may overtake conventional CE as the technology continues to mature.

### Future Prospects

Although microchip CE is still in the early stages of development, the technique has already shown applicability in many areas of clinical analysis. Compared with conventional techniques, microchip CE-based molecular diagnostic methods have demonstrated advantages in terms of analysis speed, cost savings, and detection sensitivity. As this review has shown, there is already a rapidly growing collection of new applications based on microchip CE. At the same time, many on-column pre-concentration methods have been developed to improve detection sensitivity in CE separations that are readily transferable to the microchip CE format (79, 80). With the introduction of highly automated, high-throughput commercial instrumentation, microchip CE is likely to replace many of the complex and slower analytical systems used in routine analyses. Further improvements in automation and an increase in sample throughput along with development of new testing protocols and enhancement of detection sensitivity for certain analytes could make microchip CE systems key instruments for clinical analyses.

S.F.Y. Li, is a founder and shareholder of CE Resources Pte Ltd. L.J. Kricka is a scientific cofounder of ChemCore, a company acquired by Caliper Technologies Corporation, but he has no financial interest in any of the companies mentioned in this review.

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