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Suresh Kumar Kailasa[a]; Seong Ho Kang[a]
[a] Department of Chemistry and Basic Science Research Institute, Chonbuk National University, Jeonju, South Korea

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Microchip-Based Capillary Electrophoresis for DNA Analysis in Modern Biotechnology: A Review

Suresh Kumar Kailasa and Seong Ho Kang

Department of Chemistry and Basic Science Research Institute, Chonbuk National University, Jeonju, South Korea

Abstract: Microchip-based capillary electrophoresis (ME) raises fascinating possibilities for new analytical technologies particularly in the detection of DNA fragments in modern biology. The ME technique has gained considerable popularity with significant progress being made in the development of miniaturized microfluidic devices in micro-volume analysis methods. The ME offers the potential for highly efficient, simultaneous analysis of a large number of biologically important molecules in genomic, proteomic and genetically modified organisms (GMOs) in food stuffs. Recently, the ME technique has greatly impacted biotechnological research with potential applications in smart devices that can operate at the level of molecular manipulation. This review is focused on recent developments and applications of ME to the detection and separation of DNA fragments in various molecules i.e., biotechnological and clinical research.

Keywords: Microchip electrophoresis, DNA, programmed field strength gradients, genetically modified organisms

INTRODUCTION

Microchip-based capillary electrophoresis (ME) is a powerful analytical tool that is ideally suited for the ultra-fast separation and identification of

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Address correspondence to Seong Ho Kang, Department of Chemistry and Basic Science Research Institute, Chonbuk National University, Jeonju 561-756, South Korea. E-mail: shkang@chonbuk.ac.kr

Present address for Suresh Kumar Kailasa is Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan, Center for Nanoscience & Nanotechnology, National Sun Yat-Sen University, Kaohsiung, 80424, Taiwan.

biomolecules. It provides high efficient separation with a requirement of minimum sample volume. This demand becomes increasingly prominent in bioanalytical research, e.g., in biotechnology, clinical, diagnosis, genetic and forensic applications (1–3). The separation of DNA according to size is a fundamental analytical and preparative technique in biology, medicine and chemistry. Micro- and nano-fabricated structures offer many possibilities to improve the ability to manipulate and probe biological molecules. The instrumentation for microchip electrophoretic separations was responsible for a significant decrease in analysis time over previous methods. Such instrumentation is described.

High-speed electrophoretic separations have been performed in microchip-based capillaries for the fast detection of DNA in various biological molecules (3). Microchip electrophoresis offers several advantages such as greater speed, less cost, simplicity and reduced risk of cross-contamination. Moreover, ME allows for significant improvements in terms of separation efficiency and ease of operation. ME requires less sample volume than any other separation methods still giving high resolution and high sensitivity. ME is highly desirable for rapid analysis of various biomolecules in modern biotechnology.

THE ELECTROPHORETIC PROCESS

Separation of the molecules in electrophoresis is due to the differential migration of charged ions in an electric field. Electrophoresis was first introduced by Tiselius in 1937 (4). He separated a mixture of proteins (albumin, and α-, β-, and γ–globulins) by applying an electric field. The analytes migrated through a tube and the migration rate was depending on the protein charge. Electrophoresis has been traditionally performed in anticonvective support media (plates) by using various gel compositions such as semi-solid slab gel or in non-gel media such as paper or cellulose acetate. The gel composition provides the physical support and mechanical stability for the carrier electrolyte. In early days, gel electrophoresis to separate DNA fragments has been extensively used for the DNA manipulation. The DNA fragments migrate according to their size through the gel matrix.

However, gel electrophoresis is laborious, it requires large amount of sample and gives poor reproducibility. It is very difficult to separate proteins which are in low abundance level. Moreover, it is not possible to turn it into an automated tool for the high throughput analysis of biomolecules. Gel electrophoresis has been an invaluable analytical tool for modern biochemical research. However, to overcome the above problems, electrophoretic separations were soon performed in narrow-bore tubes or capillaries.

Open tubular capillary electrophoresis has been extensively discussed for several decades. Initial work on open tubular capillary electrophoresis has been reported by Hjerten in 1967 (4). He coated methylcellulose in capillary channels that were millimeter-bore quartz-glass tubing and the analytes were well separated and identified by UV detector. Using this approach, most of the problems of gel electrophoresis were eliminated. Later, Jorgenson and Lukacs used narrow fused silica capillaries with 75 μm i.d. and coupled them with fluorescence detection (5). After the initial and pioneer capillary design development of Hjerten, a number of theories and some column techniques that have been developed by various scientists that confirmed that open tubular electrochromatography is possible on chips. The first operation of electrochromatography on chips could be traced back to 1994 reported by Jacobson (6).

However, the preparation of a reproducible polymer bed and sample injection were two difficult points. To avoid such type of problems, Jacobson was first to operate electrochromatography on chips. After this pioneer development, several scientists developed a variety of capillary electrophoretic methods. It is important to give some brief definitions of various types of electrophoresis.

(i) Capillary electrophoresis (CE): CE is also known as capillary zone electrophoresis (CZE) or free-solution CE. The separation mechanism of the ionic species is based on the charge-to-mass ratio of the analytes. Ionic species are moved in a conductive liquid medium under the influence of an electric field. The separation of the analytes is mainly based on the pH controlled dissociation of acidic groups on the solute, or protonation of basic functions on the solute. CE requires low sample volume and shows good separation efficiency.

(ii) Capillary gel electrophoresis (CGE): It is a modification of traditional gel electrophoresis (on plates) into a capillary using polymer in solution to create a sieving gel. Analytes are separated to their charge-to-mass ratios. CGE is very useful for the analysis of proteins and identification of DNA sequencing and genotyping.

(iii) Capillary isoelectric focusing (CIEF): It separates amphoteric molecules such as proteins creating a pH gradient between the cathode and anode. The analytes migrate up to a pH point where their net charge becomes zero. Once all analytes have moved to their pI location, the capillary content is pushed so that its content passes in the detector. CIEF is commonly used in the characterization of protein molecules and identification of isoelectric points.

(iv) Isotachophoresis (ITP): A gradient of electrophoretic mobility is set in a capillary. ITP technique is based on the migration of the sample analytes between electrolytes of very different mobility. The analytes moves differently according to their electrophoretic

mobility and stack into sharp, focused zones of increasing mobilities.

(v) Electrokinetic chromatography (EKC): The analytes are also separated based on their electrokinetic properties but in a capillary coated by a stationary phase or containing an electrolyte with a pseudo-stationary phase. EKC combines electro-osmosis, electrophoresis and chromatography to separate molecules. It can be used for the separation of chiral molecules when cyclodextrins (a chiral selector) are added to the electrolyte. This technique has made significant impact on separation of drugs containing an asymmetric center (enantiomers).

(vi) Micellar electrokinetic capillary chromatography (MEKC): It is an EKC technique in which ionic surfactants are added to the buffer solution at concentrations that form charged micelles. The analytes are separated based on the differential partitioning between the micelles and the solvent and it can be applicable for charged and neutral molecules. This technique can be used for the separation of mixtures containing ionic as well as neutral species.

(vii) Microemulsion electrokinetic chromatography (MEEKC): It is a CE technique in which analytes partition with moving oil droplets in buffer. Heptane or octane can spontaneously form microdroplets in water when the surfactant sodium dodecyl sulfate (SDS) is associated at relatively high concentrations with a cosurfactant, mainly butanol, pentanol or hexanol. Microemulsions are able to solubilize hydrophilic (in the aqueous phase) and hydrophobic compounds (in the oily microdroplets). MEEKC is most useful in pharmaceutical industry.

(viii) Non-aqueous capillary electrophoresis (NACE): This CE method can separate analytes using an electrolyte medium made of organic solvents without water. The separation efficiency is very high (thin peaks) due to the low viscosity and dielectric constants of the organic solvents. NACE is most useful for the separation of hydrophobic compounds.

(ix) Capillary electrochromatography (CEC): Capillary electrophoresis is coupled with HPLC and applied an electric filed rather than hydraulic pressure to push the mobile phase through a packed bed. CEC is capable to separate the analytes by using small-diameter packing and it shows high separation efficiency. The analytes are detected on-line.

(x) Programmed field strength gradients (PFSG): It is a CE technique in which molecules are separated by applying different electric field strengths. In PFSG, a particular electric field profile is selected and applied for the ultra-fast separation of a specific DNA base pair. The charged DNA fragments migrate rapidly with the appropriate

resolution driven by the selected field gradient. PFSG-CE is most useful for the rapid separation and identification of biomolecules.

(xi) Microchip-based capillary electrophoresis (ME): In recent years remarkable efforts have been made on the area of automated DNA analysis and DNA sequencing. The generation of the genetic data of a species through DNA analysis has been a powerful incentive to develop modern biotechnology. DNA sequencing is dubbed to be the ''gold standard'' in detecting disease-causing genetic mutations. Such sequencing stays is very expensive and time- and energy consuming. Moreover, early methods for DNA analyses were slow, tedious, labor–intensive, and riddled with errors. To solve these problems, the concept of miniaturization began to have a significant impact on electrophoretic separation of biomolecules.

MICROCHIP ELECTROPHORESIS DEVELOPMENT

The following references give a brief history of the microchip and ME development. Manz and co-workers (7) first demonstrated and proposed a liquid-based miniaturized chemical analysis system based on CE. Several scientists have focused on CZE separation of calcein (a fluorescent cell marker) and fluorescein, as detected by laser-induced fluorescence (LIF), on a glass chip with channels 10-μm deep and 30-μm wide in less than 6 min.

The success was attributed to the effect of the electro-osmotic flow (EOF) able to pump reagents inside small capillaries without developing any high pressure, avoiding the use of a high performance liquid chromatography (HPLC) pump (8). The sample incorporation, pretreatment, separation and detection are based on silicon chip analyzers, which is the fundamental concept of a microchip. In 1992, the first silicon glass substrate was used in electrophoresis with planar chips for separation (9).

In recent years, various reviews have been published on different types of ME tools for DNA analysis. Reyes et al (8) reviewed the history of the total analysis on microchips, and discussed the theory of miniaturization, microfabrication, design, microfluidic interconnections, micro-valves and flow control and micro-pumps with mechanical and non-mechanical processes. One review focused on the latest research developments in integrating the electrochemical detection with ME. The types of detection channels were discussed along with their applications to the detection of various matrices (10).

Khandurina and Guttman (11) reviewed micro-fabricated devices for bioanalysis, and reported that ME is a highly efficient and suitable platform for the simultaneous analysis of a large number of biologically important molecules i.e., genomics and protein analysis. Li and Kricka

(3) reviewed the potential applications of ME for the rapid analysis of cancer susceptibility genes, and reported the use of ME as a diagnostic tool for cardiovascular, neurologic diseases, thyroid function, pathogens, immune disorders, diabetes and hereditary diseases.

Pumera (12) reviewed on microchip designs and described their various potential utility for genomic and proteomic analysis. Dolnik and Liu (1) reported that ME has expanded extremely over the last decade for the separation of biological samples. They cited a number of references for the analysis of DNA genotyping, DNA fragment sizing, enzymatic assays and immunoaffinity in various molecules. Feng and Babu (13) reviewed CE and ME, and examined the effect of various polymer solutions such as poly-*N*-*N*-dimethylacrylamide (PDMA), poly vinyl pyrrolidone (PVP), poly vinyl alcohol (PVA), polyethylene oxide (PEO), methylcellulose (MC), hydroxyl ethyl cellulose (HEC), hydroxyl propyl cellulose (HPC) and hydroxyl propyl methyl cellulose (HPMC) for the analysis of DNA electrophoresis models.

Some of the methods developed for DNA analysis with enhanced speed, sensitivity and high-throughput are vital to the efficient utilization of the sequence information generated from the human genome project as well as other genome sequencing efforts. At the same time, ME is a ubiquitous technique in molecular biological and medical analyses (14). A review on the published reviews on ME showed that novel advanced technology has been applied to micro fabrication and chip designs for the separation, detection schemes in various fields. This work examines the recent reports of novel microchip-based capillary methods used for the detection of DNA in various biological samples.

In this review, the attention was focused on the novel method developments for the rapid detection of DNA in various areas i.e., clinical, genetically modified organisms (GMO), point mutations and genomic analysis, respectively. The following sections give a brief summary of the microchip designs, advantages of detectors, PFSG for the ultra-fast detection of selected DNA fragments in modern biology. This review also describes the main principles of the various ME methods used and their applications to DNA fragments, point mutations and clinical analysis.

Designs of Microchip Devices

The development of microchip designs was boosted by the needs in DNA analysis using microchips for the amplification of PCR products at micro-level volumes in recent years (12, 15). The material composition of a microchip device can have a large influence on the efficiency of ME. Currently, researchers are still using chips composed of different

materials, which usually consist of channels micro-fabricated or etched on glass or silica wafers (16).

Recently, glass or silica substrates were used to prepare microchip on polymer substrates (17). These polymers are less costly and easier to manipulate than their glass or silica counterparts. Plexiglas and a wire-imprinting method have been used to fabricate chips. This method decreased the variability of the channel widths both in the same chip (6%) and between chips (10%). Wire-imprinted micro-fabricated chips have higher resolution than traditional microchips.

A review on nanoscale channels in electrophoresis was recently published (18). Liu et al. (19) employed a number of microchip separation channels for the separation of molecules using a high-throughput methodology. They described the possible variations in microchip design, and showed that the most effective designs incorporate the same features, including the effective use of chip space, simultaneous perpendicular scanning by the sample detector across all channels, and uniform injection capability.

The application and shapes of the channels are different, which might help obtain effective results from the capillary tubes and promote perfect separation for the detection of the DNA fragment sizes. They concluded that the chip layout for the DNA analysis and the channel lengths should not exceed half the diameter of the chip. For example, on a 10 cm-diameter chip; separation was achieved at 3.3 cm, even though short-length channels are ideal for restriction DNA fragments. Some researchers concluded that the resolution could be increased using long separation channels (20).

Straight channels have tremendous applications because curved channels reduce the resolution (19). The same authors described that the channels should be within the scanning range of the detector and be equal in length from the anode to cathode ends in order to produce electrical field strengths of equal magnitude in all channels. The widespread microchip fabrication as well as their novel applications and improvements for the DNA analysis can be expected in near future.

Format of the Microchip

Significant development has been directed on the design and format of the microchips by the several researchers (21). Moreover, several reviews appeared on the development of microchip format and their applications for the analysis of various molecules (12, 16, 18). However, a brief representation on the format of microchip is needed. Generally, micro channels lengths were effectively worked at width and depth 10–100 μm and 15–40 μm. In 1992, Harrsion and co-workers were first to fabricate a microchip on a planar glass material with dimensions of 14.8 × 3.9 ×

$1\,cm^3$ (22). After that a 165 mm long separation channel with a $8 \times 8\,mm^2$ area has been realized through serpentine column geometry (6). Later the chip channels were increased to 12 and even 384 for the high throughput analysis of DNA. 384 genotyping samples could be analyzed in 7 min with 98% accuracy (23).

Recently, several scientists introduced various microchip designs for the rapid identification of biomolecules with the corresponding detection methods (24, 25). Briefly, Lander's group designed an electrophoresis microchip with 8 independent microchannels for the separation of biomolecules and their detection by LIF (26). Liu and co-workers developed microchips with four-color sequencing in 16 parallel channels (19). Moreover, 48 parallel channels on a 165×100 mm piece of Borofloat glass has been designed by Simpson and co-workers (27).

Mathies's group was greatly involved in the development of 12-channel microchip electrophoresis (28), 48-channel (29), 96-channel (30), and up to 384-channel chips (23) allowing for the ultra-fast analysis of biomolecules. Recently, a method for the fabrication of poly(methyl methacrylate) chip has been developed for the separation of DNA and for the identification of multiplex PCR products of 18 and 36 cases with SARS and hepatitis B virus infection (31). All these applications indicate that the designs of microchips were greatly influenced by the need for fast separations and rapid identification of biomolecules.

BIOMOLECULE SEPARATIONS

Biomolecules are necessary for the existence of all known forms of life. Biomolecules chemical compounds formed by carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur. Proteins, polysaccharides, nucleic acids, amino acids, DNA and RNA are critical biomolecules for living organisms.

Ribonucleic Acid (RNA)

RNA is a type of molecule that consists of a long single-stranded chain of nucleotide units, alternating phosphate and ribose units with the bases adenine, guanine, cytosine, and uracil bonded to ribose. Most of RNA chains, including messenger RNA and transfer RNA, act as cellular intermediaries; they convert the genetic information stored in DNA into the proteins that provide cells with structure. RNA is very similar to DNA, but shows slight variations in terms of structural information: RNA is a single-stranded molecule, while DNA is double-stranded; RNA nucleotides contain ribose while DNA contains deoxyribose and RNA has the

base uracil rather than thymine that is exclusively present in DNA. Moreover, in some viruses RNA can act as the hereditary material (32, 33).

Deoxyribonucleic Acid (DNA)

DNA has been called the molecule of life. It is a long polymer molecule made from repeating units called nucleotides. Each nucleotide consists of a 5-carbon sugar called deoxyribose, a phosphate unit, and a nitrogenous base i.e., adenine, thymine, guanine or cytosine. The four nitrogenous bases in DNA actually form two pairs. A base linked to a sugar only is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. If multiple nucleotides are linked together, as in DNA, this polymer is called a polynucleotide (33).

DNA molecules are two long strands interlaced like vines, in the shape of a double helix. The nucleotide repeats contain both the segment of the backbone of the molecule, which holds the chain together, and a base, which interacts with the other DNA strand in the helix. It is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses (34). The main role of DNA molecules is the long-term storage of information. Generally, a DNA chain is 22 to 26 Å wide (2.2 to 2.6 nm), and one nucleotide unit is 3.3 Å (0.33 nm) long. The DNA segments have the genetic information, which is called genes.

Peptides and Proteins

Peptides play a significant role in fundamental physiological and biochemical functions of life. A peptide is a molecule formed by joining two or more amino acids (35). When the number of amino acids is less than about 50, these molecules are named peptides, while larger sequences are referred to as proteins. The type and the sequence of amino acids in a protein are specified by the DNA in the cell that produces them. The overall structure and function of a protein is mainly based on the sequence of amino acids. The protein may serve as a structural material (e.g. keratin), as enzymes, as transporters (e.g., hemoglobin), as antibodies, or as regulators of gene expression.

Oligonucleotide

An oligonucleotide is a short nucleic acid polymer, typically formed with 20 or fewer bases. Generally, the length of the oligonucleotide is usually

represented by "mer" (from Greek meros, "part"). For example, an oligonucleotide fragment of 25 bases would be called a 25-mer. Oligonucleotides are chemically synthesized using building blocks, protected phosphoramidites of natural or chemically modified nucleosides or, to a lesser extent, of non-nucleosidic compounds.

Oligonucleotides composed of DNA (deoxyoligonucleotides) are often used in the polymerase chain reaction, a procedure that can greatly amplify almost any small piece of DNA (36). Usually, oligonucleotides readily bind to their respective complementary nucleotide; they act as probes for the identification of DNA and RNA through DNA microarray, Southern blots, and fluorescent in situ hybridization, respectively.

Role of Gel Matrices

The composition of the used gel, nature of the polymer and ion concentrations all play a fundamental role for separation of biomolecules. The separation media must have high separation ability, low viscosity and self-coating ability to be usable in a microchip CE. Numerous gel matrices have been developed for the analysis of protein, RNA and DNA.

Feng and Baba prepared an excellent overview on the applications of polymer solutions for biomolecule analyses using capillary and microchip electrophoresis (13). The physical properties of polymer matrices are most important in terms of resolving single-stranded DNA. Quesada and Menchen proposed a theoretical discussion on the performance of polymer systems in capillary-based DNA separation (37).

Previously, capillary gel work involved the adaptation of cross-linked polyacrylamide gels (PAG), used for years in slab-based DNA sequencing separations, to the capillary format. In PAG cross-linked gels, a DNA mixture is sieved through pores formed by a network of polymer branches that are covalently fixed. Cross-linked gels were introduced into capillaries by initiating polymerization in an external vessel and then driving the flowable, prepolymerized solution into the capillary with a simple syringe. The cross-linked polymer matrices were extensively used in CE for DNA size-dependent separations (38). However, the cross-linked PAG matrices are limited to reuse, because the PAG matrices degrade rapidly and are able to produce bubbles under high electric field.

Degradation and bubbles greatly affect further DNA separation. These PAG matrix instability and unacceptably short average lifetime pushed for the search of different polymers. Various non-cross-linked polymer matrices have been evaluated for biomolecule separation (13). Several of these newly evaluated polymers have excellent self-coating

properties. They reduced surface interactions with biomolecules. Moreover, these polymer matrices have also low viscosity and rapid separation abilities for double-stranded DNA (39, 40).

The resolution of DNA or protein fragments depends on the polymer characteristics and ion concentration of the polymer solutions (11, 41), and the sequencing of amino acids varies according to the concentration of the borate buffer (42). At the same time, DNA samples with a higher ionic strengths produced faster results and a higher resolution. Moreover, ME was compared with CE for the detection of 6 heterozygous mutations, deletions and insertions, and ME has succeeded in detecting mutations with high ionic strength but at a lower resolution.

Heteroduplex analysis (HDA) located 2 homoduplexes, which indicates the capability of a microchip device for point mutations (43). The resolution of the fragments on ME indicates that ME is well suited to detect certain point mutations with a simple procedure. Microchip separation must be performed in solutions with a high salt concentration to achieve a acceptable resolution (43). In order to modernize the problem, HDA was carried out on ME with samples diluted with deionized water (20), and the ability of ME to detect DNA point mutations was reduced by diluting the sample. Various factors are affected in the separation of DNA fragments in ME i.e., the concentration of the sieving matrix, separation temperature, intercalated dye and electric field strength, which greatly influence the migration characteristics of DNA (44).

Detection Methods

From all variants of electrophoresis, the ME technique has proven to be one of the most popular for analyzing DNA in various biological molecules. A survey of the literature revealed that various detection methods have been used for the detection of DNA in various samples. They are namely, chemiluminescence (45), electrochemiluminescence (ECL) (50), laser-induced fluorescence (LIF) (1, 3), electrochemical detection (1, 10, 46), Raman spectroscopy (75), thermal lens spectroscopy (76), surface plasmon resonance (77), refractive index detection (78) and mass spectrometry (MS) (80). The advantages and applications of these techniques for the sensitive detection of DNA in various samples are discussed thereafter.

Chemi- and bioluminescence measurements became extremely popular in recent years. They are often used to determine the amount of a specific unknown present in a sample. Over the last decade, they became extremely important in DNA detection, gene expression and gene regulation. Chemiluminescence is the light emitted from a chemical

reaction and bioluminescence is a type of chemiluminescence in which the chemical reaction is catalyzed by an enzyme.

The measurement of light from a chemical reaction is quite useful because the concentration of an unknown can be inferred from the rate at which the light is emitted. The rate of light output is directly related to the amount of light emitted, and accordingly, is proportional to the concentration of the luminescent material present. Therefore, the light measurement is a relative indicator of the amount of luminescent material present in the sample of interest (45, 47).

Chemiluminescence

Chemiluminescence is a sensitive and attractive analytical technique used to measure various chemi- and bioluminescent reactions, and has several advantages over other analytical techniques. The extraordinary sensitivity, wide dynamic range, inexpensive instrumentation and the emergence of novel luminescent assays make this technique quite popular. Aldo et al (48) reported a polymerase chain reaction (PCR) chemiluminescent enzyme immunoassay for the simultaneous detection and typing of seven types of high oncogenic risk human papillomavirus detecting human papillomavirus DNA in a single well. This method was specific and allowed the detection of 50 genome copies of the HPV 16, 18, 33 and 58, and 100 genome copies of HPV 31, 35, and 45. Wu and co-workers reported an assay that employs the differences in thermal stability between DNA hybrids in combination with a very sensitive chemiluminescence detection system (49).

The assay itself is versatile and can be applied broadly to the detection of other genetic changes. Huang and Ren reviewed CE and ME with chemiluminescence techniques for the detection of various molecules in many fields such as biological, biomedicine, environmental and food science (45). Electrochemiluminescence detection is similar to chemiluminescence; Zhao's group developed a electrochemiluminescence detection method with an integrated indium tin oxide electrode on electrophoretic microchip for direct bioanalysis of lincomycin in the urine (50).

Fluorescence

Since the use of fluorescence tags to label DNA oligonucleotides was first reported (51–54), fluorescence detection has been accepted as the main detection protocol in DNA sequencing. It has allowed DNA sequencing to be performed in an "automated" manner with base calling performed

during gel separation. More importantly, multiplexing capabilities, which allow multiple tracks of information to be processed in a single lane, has permitted high throughput applications. From all the variants of electrophoresis, microchip techniques have proven LIF detection to be one of the most popular techniques for the detection of DNA in various biological and clinical molecules.

The main advantage of fluorescence detection over absorption measurements is the greater sensitivity because the fluorescence signal has a very low background. For molecules that can be resonant excited, LIF provides selective excitation of the analyte without interference. LIF is useful in studies of DNA molecules and for making quantitative measurements of analyte concentrations (55–58). Gong et al. (59) described a ME method with LIF detection for a simple and accurate method of analyzing the protein-DNA interactions using frontal analysis.

The applicability of microfluidic devices for the separation of DNA with multilayer devices consist of 10 nm pore diameter membranes sandwiched between two layers of polydimethylsiloxane (PDMS) substrates with embedded microchannels. This method has been applied successfully to the rapid determination of reduced glutathione in human plasma and red blood cells without any off-chip deproteinization procedure. ME with a LIF detector has been progressively applied for rapid DNA sizing with high sensitivity (60).

Microchip offers a number of applications including rapid analysis, portability, ability to perform multiple analyses, and compatibility with integration allowing the development of sophisticated micro-analytical systems. In ME, LIF detection is becoming increasingly popular based on its close-to single molecule detection or high zeptomole (zM = 10^{-21} M) sensitivity (61). The high inherent sensitivity of fluorescence detection is further enhanced by confining the sample in the very small volume of a narrow capillary (56).

Electrochemical Detection

Electrochemical detection is another detection method with a potential associated with ME technology. Electrochemical detection provides a simple, inexpensive, accurate and sensitive platform for the detection of electroactive biomolecules. Direct electrochemical detection has been examined for three decades by making stable and miniature amperometric detection methods for biomedical applications. Electrochemical methods are well suited for DNA diagnostics in biomedical analysis. The electrochemical detection is ideally suited to the detection of substances using miniaturized analytical systems and is an attractive alternative mode for a ME mechanism (62–64).

The miniaturized total analysis system (μ-TAS) in ME device is a promising tool for bioanalytical purposes using electrochemical methods. μ-TAS-ME is made from various materials, e.g., poly(methylmethacrylate) (PMMA). Thermoplastic olefin polymer with an amorphous structure (TOPAS) and glass have been studied and evaluated with amperometric detection to describe its analytical applicability (65).

Amperometric detectors in ME are widely used for selective detection of DNA in various research areas. There are several advantages of amperometric detection over the other detection methods including selectivity and sensitivity. Several researchers selectively analyzed electroactive molecules with high sensitivity and quick response time (66–68). The electrochemical approach is well suited for the detection of catecholamine and neurotransmitter separations. DNA analysis was carried out using an integrated electrochemical detector on a microchip with a 10 μm wide electrode that was located 30 μm from the end of the ME separation channel (69).

The authors minimize the inference of a high separation voltage using an off-column detection method. Some restriction digest involves detection with an indirect detection mode at a detection limit of 28 zM (or 17,000 molecules per liter or 17 molecules in a single milliliter). The sensitivity of ME is enhanced considerably by carbon nanotube amperometry (70). The separation method along with sensitive amperometric detection was applied successfully for measuring the level of these compounds in the mouse brain and human urine sample (71). A specific application of the microchips is represented by isoelectric focusing measurements on a microchip, which is the subject of ongoing research in modern biology.

Recently, Shiddiky's group developed a ME process with electrochemical detection for simple and sensitive trace analysis of DNA. The authors used a HPC matrix modified with gold nanoparticles. The conducting polymer/gold nanoparticle layers act as an electro-catalyst for the detection of DNA by oxidation in a solution, in this method, they observed an improved sensitivity of ~25,000-fold compared to the conventional microchip gel electrophoresis with electrochemical detection (72) and a schematic diagram of the procedure is shown in Figure 1.

Kim and coworkers developed micro-fabricated CE with the electrochemical detection of DNA, this method successfully separated a 1 kb DNA ladder (8.4 ng/μL or about 23 nM) and the 500 bp to 10 kb DNA fragments migrated within 150 s using polydimethylsiloxane glass chip and an indium tin oxide electrode (73). Lin et al. developed a fabricated method on PMMA plates using PVP, PEO and gold nanoparticles for the detection of DNA. For this, 13-nm gold nanoparticles with 1.5% PEO are extremely important for obtaining a high resolution, sensitivity and reproducibility for the separation of DNA molecules (74).

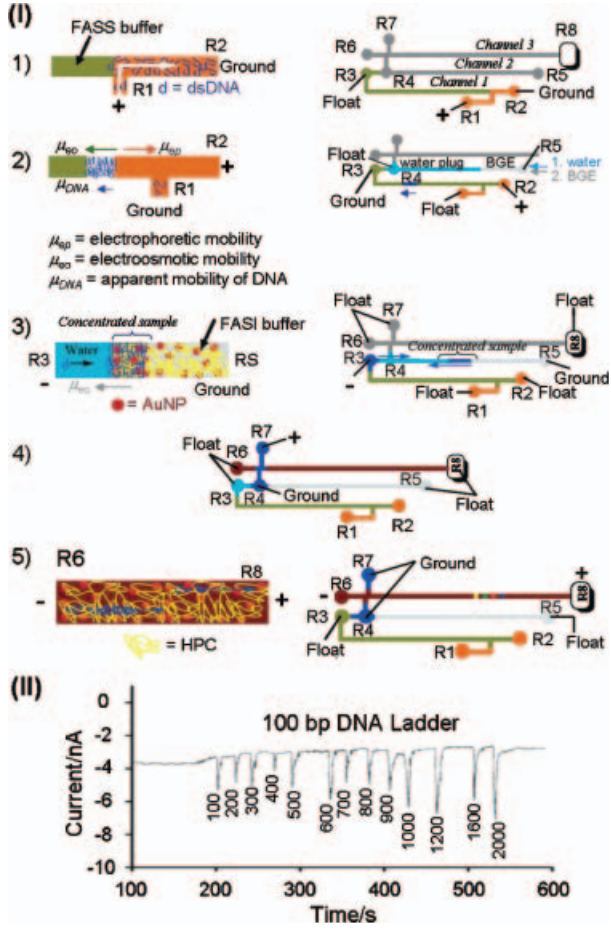

Figure 1. (I) Schematic diagram of the preconcentration, separation, and electrochemical detection of DNA: (1) Sample loading: +100 V/cm (R1) R2 is grounded and R3 is left floating. (2) FASS step: (i) +200 V/cm (R2) for 130 s, R3 is grounded and R1 and R4 were left floating. (ii) Water/stacking buffer injection: FASS step: a water plug was injected hydrodynamically from R5 to R4 at a flow rate of ~0.1 µL/min for 110 s. Next, the stacking buffer was injected for 60 s at ~0.1 µL/min. (3) FASI step: preconcentrated sample is injected into channel 2 at − 150 V/cm, R3 and R5 grounded, all other reservoirs floating. (4) Sample loading and injection: +100 V/cm to R7 for 40 s, R4 was grounded and R3, R5, R6, and R8 remain floating. Injection applying +200 V/cm for 5 s to R4. (5) Separation and detection: MGE-ED with −340 V to R6 and +1500 V to R8 with R3, R4, R5, and R7 floating. Amperometric detection potential: +0.8 V vs. Ag/AgCl. (II). Electropherograms showing the separation of 13 fragments present in a 100-bp DNA ladder with the pTTCA/AuNP-modified electrode. Concentrations: 100, 200, 300, 400, 600, 700, 800, 900, 1200, and 1600 bp at ~0.01 pg/µL; 500, 1000, and 2000 bp at ~0.03 pg/ µL. With permission from (72).

Despite the increase in alternative methods of optical detection such as Raman spectroscopy, thermal lens microscopy, surface plasmon resonance and refractive index detection systems were also playing an important role for the detection of biomolecules at very low levels in ME systems.

Raman Spectroscopy

Raman spectroscopy has a great ability to observe structural changes or the progress of a chemical reaction on microchip. Pan and Mathies developed Raman spectroscopy detection system for the identification of the chromophore structure of rhodopsin photointermediates as well as changes in protein–chromophore interactions in a glass microchip at room temperature (75). Thermal lens microscopy is more useful in immunoassay analysis. Tamaki's group was monitored a single cell analysis of cytochrome *c* distribution during apoptosis process by using scanning thermal lens microscope with a microchip (76).

Surface Plasmon Resonance (SPR)

SPR is a surface-sensitive detection method and chemical sensing was causes by refractive index changes on the surface of a metal film. Yager's group was developed a special microscope with SPR detection, and its application to characterization of protein absorption on the micro-channel surface of Mylar devices (77).

Refractive Index (RI)

RI detection is a sensitive to changes in temperature, pressure, and flow rate; it plays a role in other detection techniques. Costin and Synovec developed a microscale-molecular weight sensor method for the observation of probing molecular diffusions by refractive index gradient detection (78). Burggraf et al. introduced a holographic refractive index detector for the separation of biomolecules in microchip (79). Based on the above illustrations, optical detection techniques with microchip community was showed novel and unique applications for the separation of biomolecules at micro-and nanoscale level.

Microchip and Mass spectrometry

Mass spectrometry is an indispensable analytical tool in modern biology. Recently, several investigators have directed their efforts toward

improving coupling microchips with mass spectrometers for high-throughput detection and screening of various biomolecules. The first attempts have been made with chip-based electrospray ionization mass spectrometry (ESI-MS) by electro-spraying the sample solution directly from the channel terminus at the chip edge (80–81). Numerous biomolecules have been determined by microfabricated-based mass spectrometry (82–88).

In these reports, microfluidic platforms typically involved various procedures such as sample processing steps: infusion, cleanup, preconcentration, digestion, separation and detection. Wang's group developed efficient ESI-MS methods by using polymer microchannels with integrated hydrophobic membranes (89). A simple chip-based nano-ESI-MS was developed for the on-line monitoring of supramolecular interactions (90).

Separation and identification of peptides have been performed on microfabricated device coupled with nanoelectrospray mass spectrometry by Li and co-workers (91). The same group developed a microfabricated devices coupled with CE-ESI-MS. They used very low sample volume to the rapid identification of proteolytic digest (92). A facile peptide and protein analysis was performed on a microdevice with integrated liquid junction by CE-ESI-MS (93).

DeVoe and Lee described microfluidic technologies in matrix-assisted laser desorption/ionization (MALDI)-MS for proteomics (88). The Gustafsson's group introduced an integrated sample preparation procedure and the experiments were performed with MALDI-MS on a microfluidic compact disk (94). Electrowetting-based microfluidic device with MALDI-MS has been developed for the analysis of peptides and proteins (95). An integrated digital microfluidic chip-based MALDI-MS was used for the multiplexed proteomic sample analysis and their identification (96–97).

Marko-Varga's group was able to develop a microchip device for the identification of proteins via MALDI-TOF-MS (98). The same group described a disposable polymeric high-density nano-vial array devices coupled with MALDI-TOF-MS for biomolecule analyses (99). Scholarly, microfluidic device-based mass spectrometry is a multidisciplinary modern bioanalytical tool, which is involving fundamental biotechnologies targeted at improving performance in practical applications through size reduction and as well as identification of peptide, protein sequences and DNA mutations.

APPLICATIONS OF ME IN MODERN BIOLOGY

One of the simplest ways of detecting DNA molecules is an assay based on ME. Recently, it was reported that ME provides highly sensitive and

ultra-fast separation of DNA at femtomolar (10^{-15} M) concentrations (about 400 femtograms per mL) (72). The performance of microchip separation devices in electrophoresis has become a trend in modern separation science, particularly in clinical, forensic, DNA, genomic, biology and biomedical analysis in recent years.

The separation of DNA on a microchip has evident benefits such as speed, simplicity, low reagent consumption, high throughput analysis and sensitivity. The development of selective and sensitive detection, often involving expensive reagents, or the rapid development of a separation method can be listed as examples of such applications. Specific measurements for particular purposes represent further useful applications of microchips. The applications of microchips in DNA, point mutations, nucleic acids and clinical analysis are discussed in the following sections.

DNA Analysis

In recent years, a number of papers on the applications of ME to the DNA analysis in a variety of biomolecules have been published. Modern molecular biology research had evolved through the development of the technologies used especially for DNA analysis. DNA microchip gel electrophoresis is one such technology, which enables researchers to examine and address the issues that were once believed to be non traceable. One can analyze the expression of many genes in a single run quickly and in an efficient manner. DNA microchip gel electrophoresis on technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of a method as well as to examine the genetic diagnosis and genotyping in various species.

The migration time of the DNA or target genes are much shorter than with the traditional techniques. An analysis of the single strand conformation polymorphism (SSCP) of wild-type and normal 185delAG, 5382insC, and 6174delT alleles was performed on a microchip within 120 s, which is 4 times faster than the conventional method and 100 times faster than traditional methods (43). Another report on ME was compared with conventional slab gel separation for the detection of point mutations in clinical samples (100). The authors showed that results could be obtained 50 times faster than with slab gel electrophoresis.

Biomolecules are separated by a hydrophilic polymer on chitosan modified poly(dimethylsiloxane) ME using end-channel amperometric detection method (101). Dang et al. reported that the ME and CE behavior of linear, super-coiled and nicked circular conformers of pGL3 plasmid DNA in the presence of various intercalating dyes (102). This is a

promising alternative method for evaluating the transfection efficiency of DNA delivery systems in modern biology.

Recently, ME with the polymer matrixes of polyacrylamide (PAM), copolymers of acrylamide (AM) and *N,N*-dimethylacrylamide (DMA) was used for the separation of DNA, and has been used to the separate pBR322/*Hae*III digest with good resolution and fast speed (103) and electropherogram was shown in Figure 2. This method demonstrates that ME can separate 10 bp DNA ladder with good separation resolution and speed.

Novel miniaturized isotachophoresis was developed to examine the behavior of DNA under the conditions of miniaturized isotachophoresis. An electrolyte system was devised, which enabled DNA to be isolated and migrated isotachophoretically on a PMMA chip device. The developed system was tested with samples of salmon sperm DNA and human genomic DNA extracted from whole blood (104).

Recently, a multi-channel microfluidic network with 16 separation channels on polycarbonate (PC) was developed using a high precision micro-milled metal master (105). Each channel was 40 μm deep and 60 μm wide with an effective separation length of 40 mm, and used a gold sensor array lithographically patterned onto a PC cover plate. This device analyzed amino acids, proteins and oligonucleotide ladder with good resolution and speed. Another multi-channel ME using a multiplex PCR method has been used for the rapid diagnosis of genetic disorders in human genes (106).

In this paper, multiplex PCR of six DNA markers located on the Y-chromosome of human and the subsequent analysis of multi-channel ME technology was used to evaluate a molecular disorder in human genes. In addition, 72 DNA markers were screened in a single-run in about 3 minutes. PCR microchips have been improved combining the two steps: amplification and subsequent analysis of PCR products by ME with micro-volumes of sample. The PCR products could be analyzed in less than 2 minutes with good sensitivity. Observed electropherograms are presented by Figures 3-I and II (15). A novel multi-channel ME tool was developed with 12 lane channels for the screening of multiplex PCR products of the DNA sequence-tagged sites (STS) located on the human Y-chromosome (107). This system allows for 36 DNA markers to be detected within 3 minutes. A typical generated electropherogram is shown by Figure 3-III.

A review of the literature pulled various ME methods for the rapid DNA size detection in various biomolecules. Our group described the rapid detection and separation of target DNA fragments by ME with PFSG (41, 57–58). The PCR amplified-DNA products of the GMO of soybean were separated and detected (100 and 250 bp) within 11 s by applying programmed field strength gradients in a ME. A typical PFSG

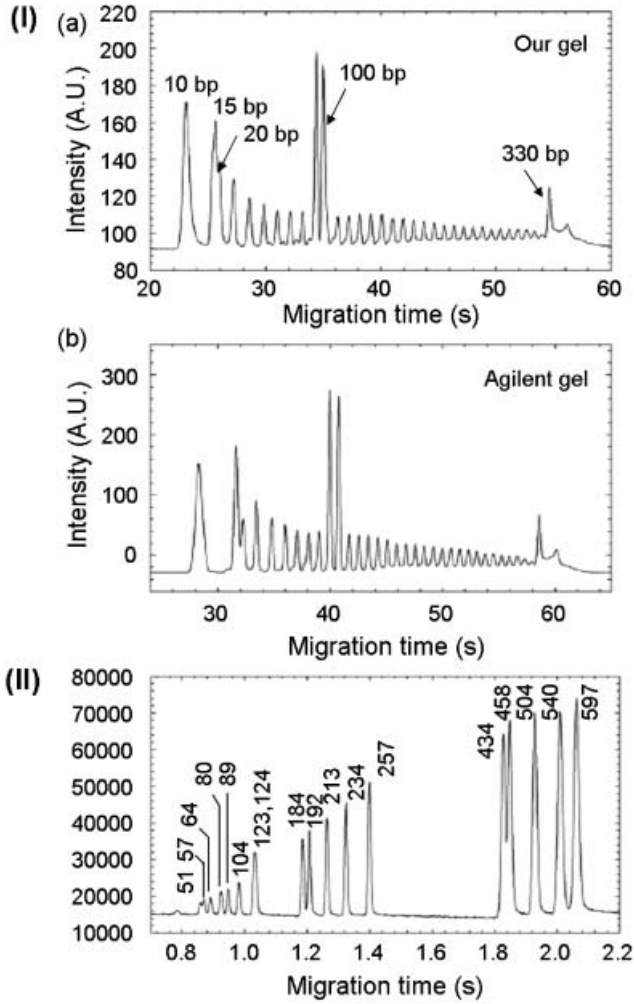

Figure 2. (I) Electropherogram of 10 bp DNA ladder using (a) 10% (g/mL) PAMco- PDMA random copolymer in 200 mM Taps (pH ~8). Copolymer 500 kDa; molar ratio AM/DMA 7/3. (b) Agilent gel for DNA 500 reagent kit; 300 V/cm. (II). Electropherograms of pBR322/HaeIII digest in 3% PAMco-PDMA in 1 × TBE buffer. Copolymer 2.2 MDa; molar ratio AM/DMA 7/3; 100 V/cm. With permission from (103).

electropherogram is shown in Figure 4. The authors state that a higher electric field (294.1 V/cm) is unsuitable for the rapid separation and detection of the PCR products.

At the same time, the same target DNA base pairs were migrated rapidly and separated within 11 s by applying the following non-uniform electric fields in the microchip (470.6 V/cm for 9 s, 294.1 V/cm for 1 s,

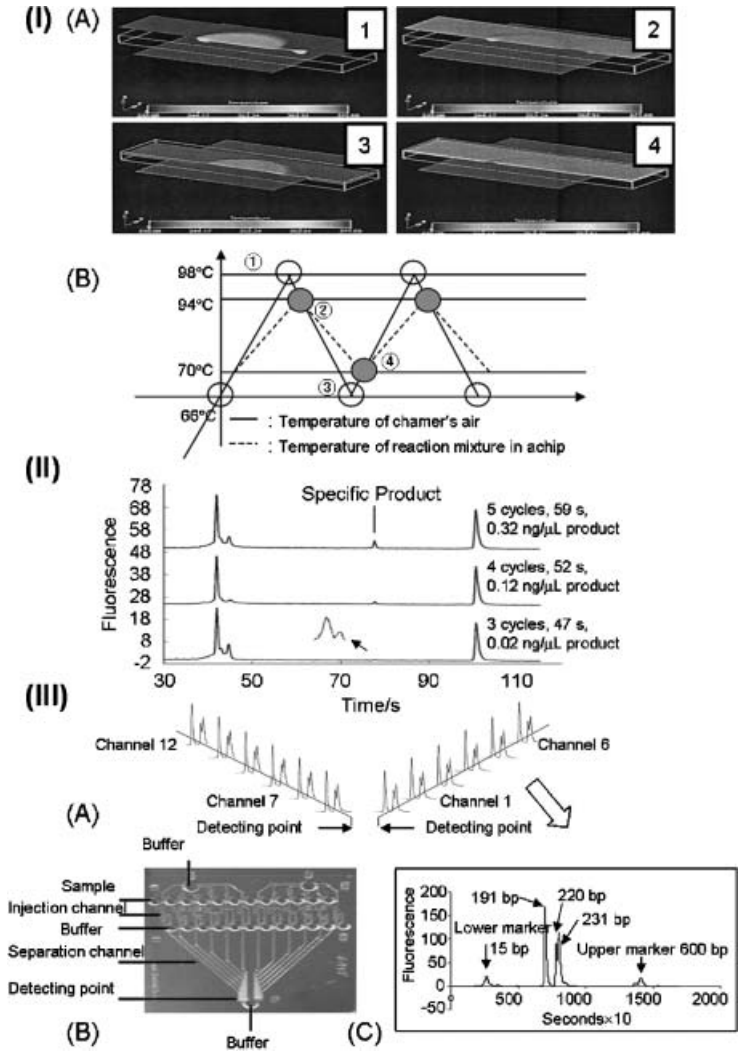

Figure 3. (I) Heat transfer of the PCR microchips with a circular reaction chamber using CoventorWare (MEMS). (A) Temperatures inside and outside of the circle reaction chamber; (B) calibrated temperature curves. (II) Fast amplification of a 300-bp fragment of λ-DNA with ME. PCR performed on LightCycler: initial denaturation, 94°C; annealing and extension, 70°C; template concentration, 10 ng/μL; polymerase concentration, 5 units/μL. All other conditions see experimental part. With permission from (15). (III) Separation of three Y Chromosome DNA STS (DYS211, DYS252, and DYS241) with the 12-Lane Hitachi Microchip, (B) the 12-Lane Multichannel Chip and (C) Chromatogram of an anormal YYY Chromosome DNA, STS (DYS211, DYS252, and DYS241) amplified with Multiplex PCR and separated with the 12-Lane Hitachi Microchip. With permission from (107).

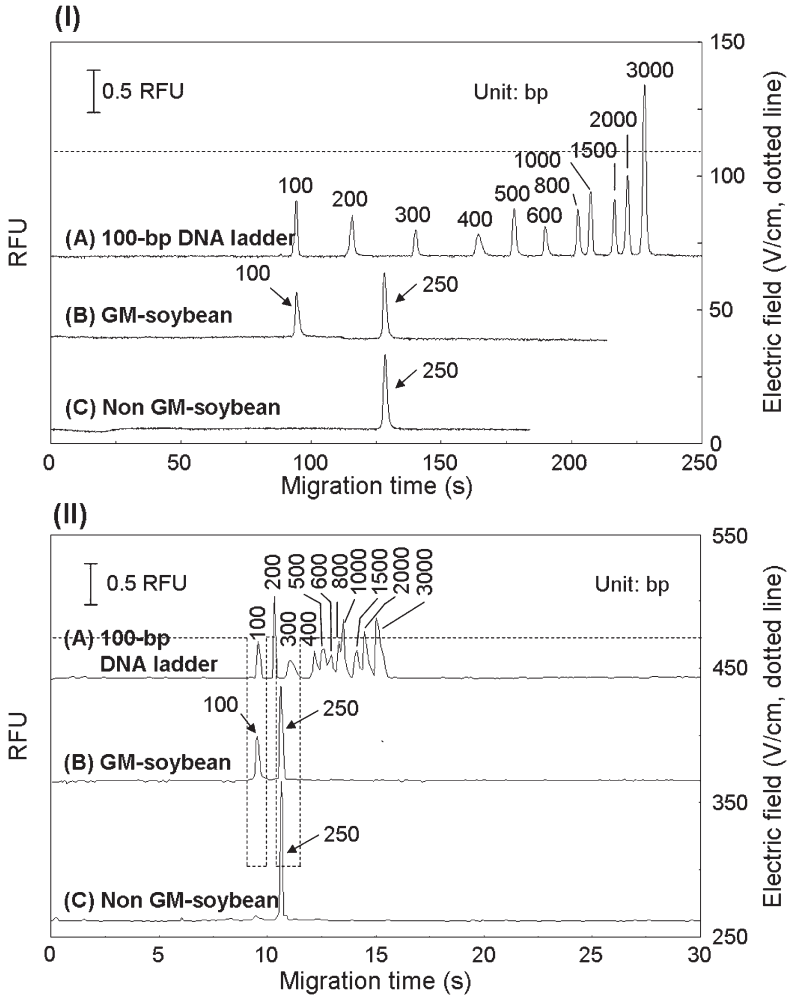

Figure 4. (I) MCGE separation of the PCR products, 100- and 250-bp DNA fragments; constant field 117.6 V/cm; electrokinetic injection, 0.48 kV for 60 s; run buffer, 1 × TBE buffer (pH 8.3) with 0.5 ppm EtBr; coating matrix, 0.5% PVP (M_r 1,000,000); sieving matrix, 0.3% PEO (M_r 8,000,000); sample, PCR amplified 100-bp DNA ladder, GM-soybean and non GM soybean; *RFU: relative fluorescence unit. **(II)** MCGE separation of PCR products, 100- and 250-bp DNA fragments by PFSG. 100-bp DNA ladder; running buffer, 1 × TBE (pH 8.3) with 0.5 μg/ml EtBr; coating matrix, 0.5% PVP (M_r 1,000,000); sieving matrix, 0.3% PEO (M_r 8,000,000); injection 60 s at 0.48 kV; separation voltage, 470.6 V/cm for 9 s, 294.1 V/cm for 1 s, 470.6 kV for 0.5 s, 294.1 V/cm for 1.5 s and 470.6 V/cm for 20 s. With permission from (58).

470.6 kV for 0.5 s, 294.1 V/cm for 1.5 s and 470.6 V/cm for 20 s, respectively). The rapid separation and diagnosis of PCR products of 591 and 1191 bp DNA fragments from the 18S rRNA of *Babesia gibsoni* and *Babesia caballi* by ME using programmed field strength gradients has been reported (Figure 5). In this paper, ME with PFSG applied to the ultra-rapid separation and enhanced resolving power of target PCR fragments. They report that there is a relationship between the applied electric field and the efficiency of the some DNA fragments.

More recently, ME using the PFSG technique was introduced to reduce the migration time of the selected DNA base pairs keeping an appropriate resolution (41, 108–109). The PCR fragments of five GMO maizes (Event176, 100-bp; MON810, 113-bp; Bt11, 127-bp; GA21, 133-bp; and T25, 149-bp), GM maize (101-bp for35S promoter and 151-bp for tNOS) and non-GM maize (151-bp) were examined within 30 s using a non-uniform electric field strength in ME. The effect of the sieving matrix of PEO with a M_r of 8,000,000 and effect of the electric field strength on the separation of DNA fragments have been reported.

Very recently, Lee et al. described applications of ME with PFSG for simultaneous detection of obesity-related coenzymes [(hepatic acyl-coenzyme A synthetase (ACS), carnitine palmitoyltransferase-I (CPT-I) and acetyl coenzymeAcarboxylase (ACC)] in mice (110). DNA fragments ranging from 50 to 2652 bp were well separated with a running buffer (1 × TBE), 0.5% polyvinylpyrrolidone (M_r 1,000,000) as the coating gel and 0.7% PEO (M_r 8,000,000) as the sieving gel at pH 8.30. The separation of the three RT-PCR products was achieved by ME in a single run within 17 s (470 Vcm^{-1} for 9 s, 205.8 Vcm^{-1} for 2 s, 411.6 Vcm^{-1} for 4 s, 117.6 Vcm^{-1} for 2 s and 470.4 Vcm^{-1} for 8 s). The efficiency of the PFSG for rapid separation and detection of biomolecules is much better with programmed field than with constant electric field (Figure 6).

Kailasa et al. developed a novel approach for the ultra-fast detection and differentiation of *Mycoplasma haemofelis* and *Candidatus M. Haemominutum* in Korean feral cats by ME with gel and PFSG (108). The detection and differentiation of two PCR products were obtained within 11 s. The same technique was applied to the 33 positive samples. The same group developed another ME-PFSG technique for the rapid detection of T-cell lymphoma in dogs (109). In this approach the authors used 0.5% PEO of M_r 8 000 000 as sieving matrix for the ultra-fast separation and detection of the amplified-PCR products (90 and 130-bp DNA fragments) from canine T-cell lymphoma. Results of the PCR products (90 and 130-bp DNA) of the T-cell lymphoma were generated within 7.0 s using PFSG, 41.7 s for low constant field strength and 15.5 s for high-constant field strength, respectively (Figure 7). The developed method gave results that correlated at 100% with those obtained results using conventional slab gel.

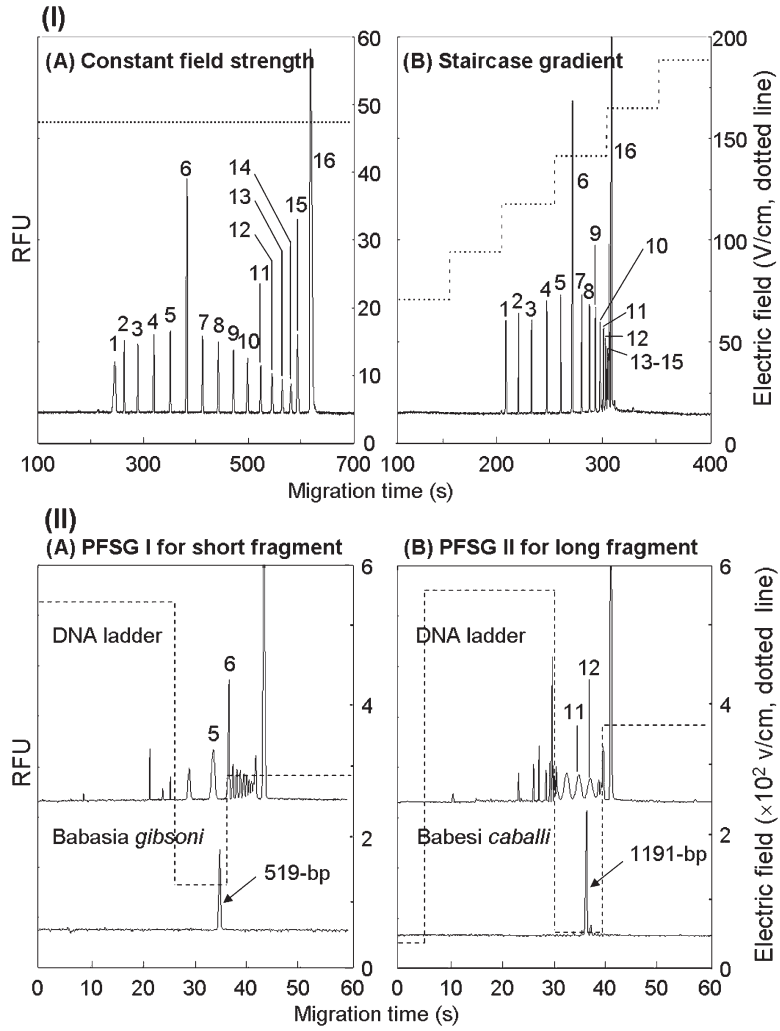

Figure 5. (I) ME separation of 100 bp DNA ladder fragments under (A) a constant field strength and (B) the staircase FSG method. ME conditions: applied separation (A) constant electric field, 47.1 V/cm and (B) FSG at regular field strength, 23.5 V/cm from 0 to 50 s, 47.1 V/cm from 50 to 100 s, 70.6 V/cm from 100 to 150 s, 94.1 V/cm from 150 to 200 s, 117.6 V/cm from 200 to 250 s, 141.2 V/cm from 250 to 400 s (dotted line). Peak numbering corresponds to the hundredths base pairs: 1 = 100, 2 = 200… except for 16 = 2070 bp. Other ME conditions as in Figure 2. (II) PFSG separation of PCR products, 519 and 1191 bp DNA fragments from the 18S rRNA of *B. gibsoni* and (B) *B. caballi* for the fast diagnosis of Babasia in ME. *The arrows indicate the amplified DNA peaks. With permission from (57).

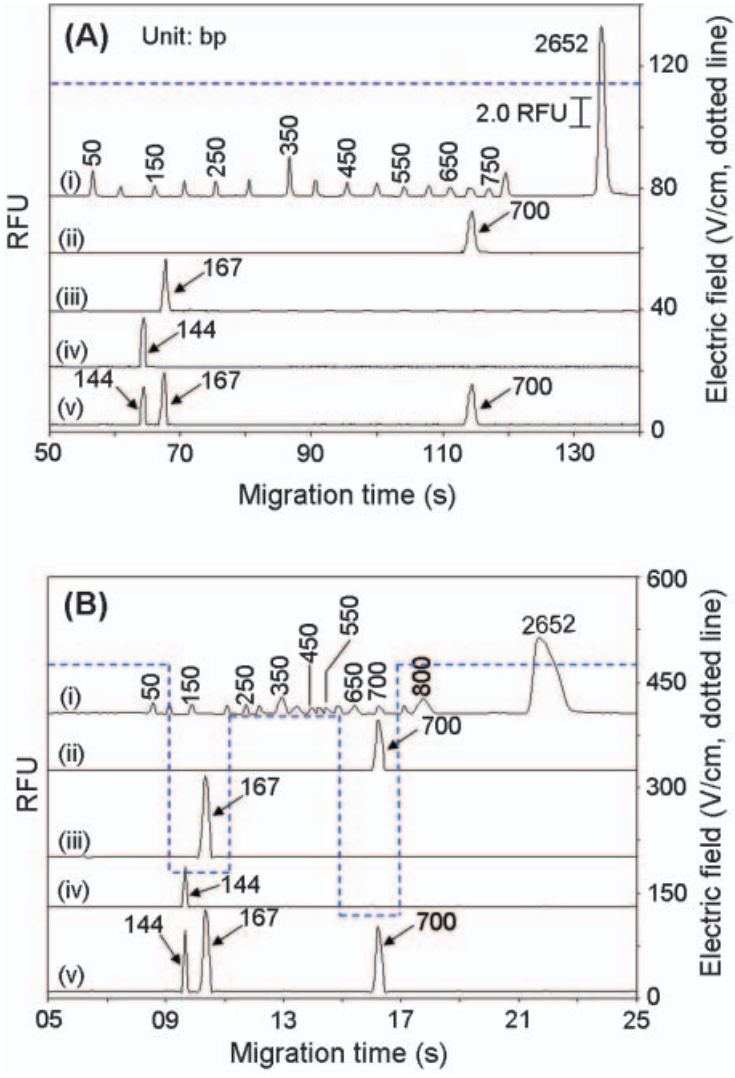

Figure 6. Electropherograms of the RT-PCR products of mRNA along with the 50-bp DNA ladder. (A) RT-PCR products of (i) 50-bp DNA ladder; (ii) hepatic acyl-coenzyme A synthetase (ACS, 700 bp); (iii) acetyl coenzyme A carboxylase (ACC, 167 bp); (iv) carnitine palmitoyltransferase-I (CPT-I, 144 bp); (v) mixture of three RT-PCR products. (B) RT-PCR products of (i) 50 bp DNA ladder; (ii) ACS (700 bp); (iii) ACC (167 bp); (iv) CPT-I (144-bp); (v) mixture of the three RT-PCR products. Separation conditions; electrokinetic injection 58.8 Vcm^{-1} for 60 s; applied electric field; constant electric field for 117.6 Vcm^{-1}; PFSG; 470.6 Vcm^{-1} for 9 s, 205.8 Vcm^{-1} for 2 s, 411.6 Vcm^{-1} for 4 s, 117.6 Vcm^{-1} for 2 s and 470.6 Vcm^{-1} for 8 s; ME conditions are the same as those shown in Fig. 4. With permission from (110).

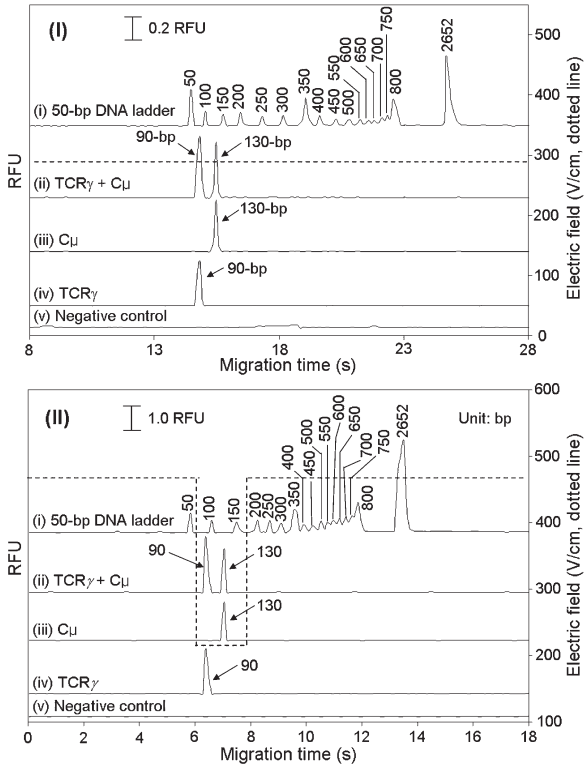

Figure 7. (I). MGE separation of PCR products of T-cell lymphoma. (i) 50-bp DNA ladder; (ii) mixture of TCRγ and Cμ; (iii) positive control Cμ (130-bp); (iv) TCRγ (90-bp); and (v) negative control. ME condition: running buffer 1 × TBE buffer (pH 8.31) with 0.5 ppm EtBr; coating matrix, 0.5% PVP (M_r 1 000 000); sieving matrix, 0.5% PEO (M_r 8 000 000); electrokinetic injection 56.50 V/cm for 60 s, applied separation voltage, 294.0 V/cm; effective length, 20 mm; RFU, relative fluorescence unit. The dotted lines represent the applied electric field. **(II)** Electropherograms of the amplified-PCR products of a T-cell lymphoma in dogs along with the 50-bp DNA ladder by MGE-PFSG. (i) 50-bp DNA ladder; (ii) mixture of TCRγ and Cμ; (iii) positive control Cμ (130-bp); (iv) TCRγ (90-bp); and (v) negative control. MGE-PFSG condition: running buffer 1 × TBE buffer (pH 8.31) with 0.5 ppm EtBr; coating matrix, 0.5% PVP (M_r 1 000 000); sieving matrix, 0.5% PEO (M_r 8 000 000); electrokinetic injection 56.50 V/cm for 60 s, applied PFSG, 470.4 V/cm for 6 s, 205.8 V/cm for 2 s and 470.4 for 20 s; effective length, 20 mm; RFU, relative fluorescence unit. The dotted lines represent the applied electric field. With permission from (109).

Several parameters, such as pH, buffer, separation medium, concentration, composition and electric field strength, have a significant influence on the separation of DNA in ME (111). Some researchers have focused on

a microchip to bring about the modern application of PCR reaction conditions for the amplification of DNA fragments (15, 112). Tabuchi and Baba reported an alternative novel triple injection method for the DNA peaks examined by ME with higher intensity (113). The peak intensity was controlled by applying a separation voltage in the sample loading process.

A 40% higher peak intensity was obtained using this method than with the conventional methods. ME has been evaluated for the identification of mitochondrial DNA in forensic and ancient DNA samples (114). HVR1 and HVR2 haplotypes regions were amplified for PCR reactions in the mitochondrial DNA analysis in blood and hair samples. ME was reported to be rapid and sensitive identification method for the accurate detection of 16 186T→C transitions in HVR1 and 309.1 and 309.2 C-insertions in HVR2.

The detection techniques for circle-to-circle amplification (C2CA) are quite limited for double-stranded DNA. However, a novel ME technique was introduced to overcome the low analytical speed, high labor intensity, and difficulty in automation with other systems. This method has been applied successfully for the fast and precise analysis real sample of bacterial pathogen (*V. Cholerae*) at single nucleotide level by ME (115). Stable C2CA products were produced by applying a new enzymatic step to C2CA, and detection was achieved within 55 s with a RSD of the migration time of 3.6% ($n = 6$). ME has been applied to the analysis of DNA by examining various parameters such as the sieving matrix (116) and self-associating block copolymers networks to enhance DNA separation using ''Inchworm'' chain dynamics (117).

Mohamadi and co-workers reported a novel attractive DNA microchip electrophoresis technique for the sources of errors in quantifying purified DNA fragments and unpurified PCR products (118). A novel and integrated DNA extraction microchip was developed for the extraction of a specific range of DNA molecules. A DNA extraction microchip was designed and fabricated, which was composed of a micro-pillar array, 6 micro-channels, 6 electrodes with probe windows, 1 loading window and 5 unloading windows with entropic barriers, respectively. Xie and co-workers presented an integrated microdevice combining temperature-controlled enzymatic reactor, analyte delivery, CE separation, and fluorescence detection. They successfully used it to analyze on-line digested DNA fragments of *Bam*HI and *Fok*I. They used different polymer matrix compositions, namely, 0.5% hydroxypropylmethyl cellulose, 0.1% PVP and 6% mannitol for the generation of good electropherograms. The analysis was completed within 160 sec (119).

Recently, Wang's group developed a sensitive, simple and robust on-chip transient ITP (TITP) method for the analysis of PCR DNA samples. They performed TITP analysis on a microchip device with PMMA as a

polymer matrix. The developed system has a 20-fold signal enhancement compared to classical ME. The limit of detection was as low as 0.24 pg/mL (600 attoM or 6×10^{-16} M at S/N = 3) (120).

ME in Clinical and Modern Biotechnology

ME is a high-throughput analytical system for clinical analysis and modern biotechnology on account of its rapid analysis (typically seconds), easy integration of analytical steps and routine analysis (3). Currently, studies on ME are focused on cheaper and faster separation assays for DNA analysis than traditional slab gel electrophoresis techniques such as heteroduplex analysis (HDA) and single-strand conformation polymorphism (SSCP), which are typically applied slab gels (19). Slab gel techniques have notable faults, including a slow rate of analysis, a high cost, and the difficulty to identify heterozygous DNA in the samples (20). Recent strategies of separation technology such as ME have focused on reducing the analysis time with micro-volumes of the DNA samples by miniaturizing the procedure, and as well as increasing the precision with micro-volumes of samples (19).

Our group described the potential of ME for the SSCP in the rapid detection of point mutations in the human obesity gene (56). First, the obesity genes (Leu72Met) were detected by CE and the method was then translated to ME. In this technique, the SSCP peak was achieved within 85 s under the following conditions, 1.75% of PVP (M_r 1,300,000), 1% PEO (M_r 600,000) and 5% (w/w) glycerol, respectively. For this, the effect of PVP, PEO, glycerol and electric field were examined (Figure 8-I).

Tian and co-workers reported a novel method for the rapid detection of mutations through heteroduplex analysis with CE and ME (121). In this paper, the authors were able to analyze heteroduplex DNA, BRCA11 and BRCA2, as well as homoduplex DNA (wild type and mutant) within two minutes. They proved to be 4 to 6 times faster than CE (Figure 8-II). A rapid and accurate ME analytical tool was developed for the fast diagnosis of the Herpes simplex virus (HSV) by PCR amplification. In this method, ME provided the results within 110 s with 100% sensitivity and specificity (122). Chen et al. reported the clinical application of ME for an analysis of the Hepatitis C Virus using PCR (123). Here, they improved the sensitivity using ME. CE resolved the 145-bp of PCR product of HCV in 15 min when ME could resolve the same PCR products in less than two minutes. This indicates the great potential of microchip for clinical analysis.

Recently, researchers focused on plastic ME and its capability for the PCR products analysis in clinical samples (124). The authors described the high throughput plastic ME method for an analysis of the PCR

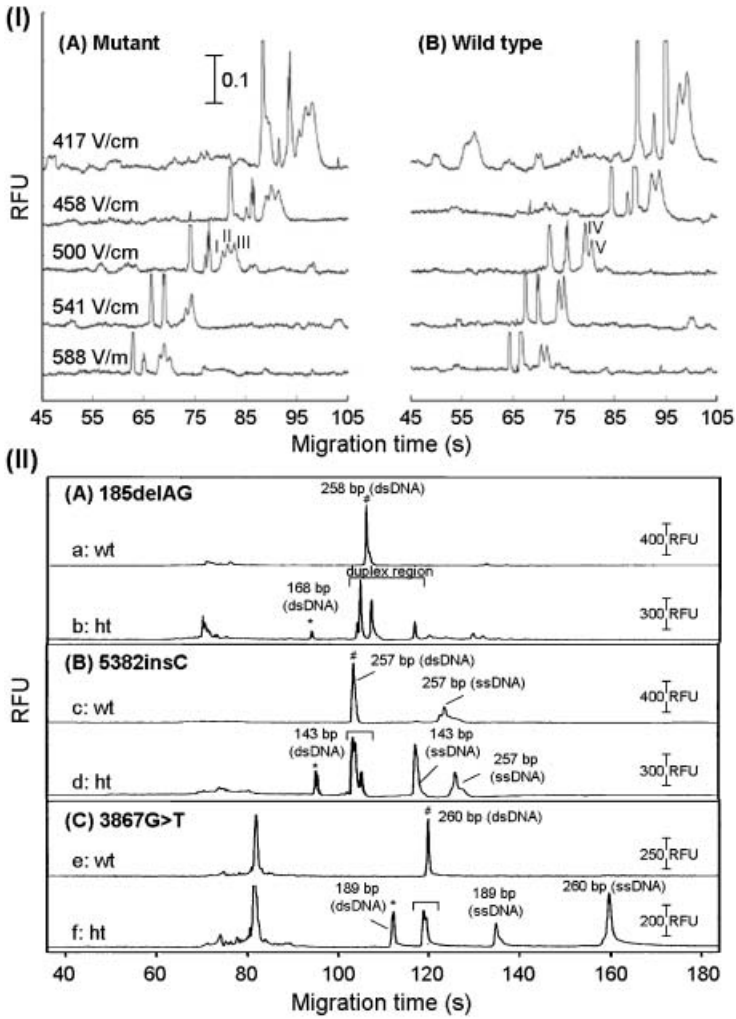

Figure 8. (I) Representative SSCP-ME electropherograms showing the effect of the electric field. (A) Mutant. (B) Wild type. 417–588 V/cm. Other SSCP-ME conditions, see Figure 3 caption. With permission from (56). (II) Fast mutation detection through allele-specific amplification-HD analysis with ME. Panels A, B, and C: AS-PCR-HD analysis for the wild-type (wt) and the heterozygous mutants (ht) specified. Separation buffer: (A and B) 25 g/L HEC with 100 g/L glycerol in 13 TBE (pH 8.6); (C) 45 g/L HEC with 100 g/L glycerol and 150 g/L urea in 13 TBE (pH 8.6). Microchannel coated with PVP, LIF detection (emission/excitation 520/488 nm). PCR products injected for 100 s at 333 V/cm, separation voltage: 573 V/cm, effective microchannel length, 55 mm. With permission from (121).

products of the fragile X (CGG)*n* alleles to facilitate a rapid exclusion test of fragile X syndrome (FXS), and showed a 100% correlation with already existing methods. ME successfully analyzed the amplified PCR fragments from the variable region of the T-cell receptor-γ gene (150–250 bp range) and immunoglobulin heavy chain gene (80–140 bp range). These PCR products were resolved effectively and with a decrease in analysis time from 2.5 h to less than 3 minutes.

More automated and high throughput systems have been developed for the analysis of cancer susceptibility genes using ME methods such as SSCP (43) and allele-specific DNA amplification (20, 61, 121, 125). Reverse transcription (RT)-PCR products have been developed for an evaluation of the RNA messengers in lipid trafficking of human intestinal cell by ME. These results showed that an analysis of specific RNA messengers allows a reliable evaluation of relative gene expression in caCo-2 cells (126).

Point mutations in the human *k-ras* oncogene were analyzed on ME by the DNA obtained from colorectal tumors. The ligase detection reaction products migrated from the unligated primers within < 120 s, which is $\sim$17 times faster than CE (127). ME has been used to assess arteriosclerosis though an analysis of LDL and homocysteine (128–130). More recently, several groups have focused on ME with PCR for an analysis of bacteria i.e., *Streptococcus* (131), *Staphylococcus* (132), *Salmonella* (133), *Escherichia coli* (132, 133–136) and bacterial pathogen DNA (137). A modern and an attractive ME has been used for the analysis of genetic disorders i.e., hemochromatosis (138), genomic DNA (139) and Duchenne muscular dystrophy (140–142).

More recently, a novel microchip device coupled with multiplex PCR was designed for typing multiple single nucleotides polymorphisms (SNP) (143). This device allows for the simultaneous analysis of five SNP of 100C $>$ T, 1661G $>$ C, 1758G $>$ T, 2470T $>$ C and 2850 C $>$ T in cytochrome P 4502D6 (CYP2D6) with reduced sample amounts. ME is a modern technique for the rapid sizing of DNA fragments, and this experiment was carried out with a 50 pL sample volume. This technique is promising for the analysis of biomolecules (31).

A PMMA chip was used for the analysis of the multiplex PCR products of 18 and 36 cases with the SARS and hepatitis B virus. Microfluidic technology is an important component in DNA analysis and a maternal circulatory extra-cellular DNA of pregnant woman in blood plasma was analyzed using microchip electrophoresis (144). A PCR product of polymorphisms on the human Y-chromosome was detected in single step by ME using linear imaging UV detection and fluorescence detection (145).

Munro et al. described the separation capability of the B-and T-cell genes of Lymphoma by PCR using slab gel, CE and ME. Among them ME

could separate the PCR products of B-and T-cells within 160 s (146). Recently, a novel ME with a single-photon avalanche diode detector was used for the molecular characterization of mutations in disease genes. The ME allowed to assess the optimal analytical conditions for ultra-fast detection of DNA variations with diagnostic samples (147). Chen and co-workers monitored monoclonal antibody protein quality using a ME offering unique applications with regard to resolution and sensitivity. This ME was 70 times faster than their conventional CE instrument (148).

Very recently, a rapid chip-based capillary electrophoretic mobility shift assay with negative pressure injection method has been developed for the binding study of transcription factor Abf1 in *Saccharomyces cerevisiae*. The authors analyzed the captured DNA samples within 100 s and the method LOD was found to be 156 nM (149).

The Kartalov group developed a disposable elastomeric microfluidic device with fluorescence sandwich immunoassays detection method for the quantification of proteins in human serum. This device is a very important bioanalytical tool for the consequent immunoassay applications in biomedical diagnostics (150). A PDMS ME with LIF detection has been used for the identification of single cell in biomolecules. Both precision and accuracy of the method were confirmed by analysis of glutathione and rhodamine 123 in single K562 cells (151).

Very recently, an effective ME chip has been developed for the sequential analysis of synthesized RNA and the results were obtained with in 160 s (152). The Li group developed a Special ME for the ultra sensitive simultaneous analysis of multiplex PCR products of food-borne pathogenic bacteria. In this method four pairs of oligonucleotide primers were determined for the amplification of target genes of *Vibrio parahemolyticus, Salmonella, Escherichia coli (E. coli) O157:H7, Shigella*. The PCR products were simultaneously detected within 8 min (153).

Shim and co-workers observed the dispersion of protein bands through horseshoe microchannels (154). An accurate ME was introduced for the quantitative estimation of allele frequencies of 5 single nucleotide polymorphism in a DNA pool composed of 141 genotype health controls and a DNA pool composition of 96 genotype gastric cancer patients with a frequency representation of 10%–90% for the variant allele (155).

Osiri and co-workers reported a high peak capacity 2-D protein separation system combining SDS micro-CGE with microchip MEKC using a PMMA microfluidic chip for the analysis of fetal calf serum proteins (156). The specific sequence of Phage Lambda DNA amplified PCR products was analyzed with electrochemical detector (working electrodes; hanging mercury drop, carbon paste and carbon screen-printed electrodes) within 100 s (157). Table 1 summarizes the significant information for the rapid analysis of biomolecules using CE, ME and ME

Table 1. Rapid identification of various biomolecules in capillary and microchip electrophoresis.

No.	Name of the biomolecule	Analysis time	Reference
1.	Detection of the amplified 300-bp fragment of λ-DNA	~120 sec[b]	(15)
2.	Separation of a DNA size marker, HaeIII digest of φX174	<3.0 min[b]	(17)
3.	DNA sequencing on multiple channel microchips	15–18 min[b]	(19)
4.	Identification of 6 heterozygous mutations,*185delAG, E1250X (3867GT), R1443G(4446CG), 5382insC, 5677insA in BRCA1, and 6174delT in BRCA2*	8–14 min[a] 130 sec[b]	(20)
5.	Rapid detection of DNA genotyping	<160 sec[b]	(28)
6.	High throughput genetic analysis	<8.0 min[b]	(29)
7.	Analysis of DNA sequencing	24 min[b]	(30)
8.	A multiplex PCR for the detection of SARS and hepatitis B virus	<600 sec[b]	(31)
9.	Genetically modified PCR products of maize (Event176, MON810, Bt11, GA21, and T25) and non-genetically modified maize	122.76 sec[b] 30.45 sec[c]	(41)
10.	Single-strand conformation polymorphism profile for common mutations in BRCA1 and BRCA2	10 min[a] 120 sec[b]	(43)
11.	Detection of 50 genome copies of HPV 16, 18, 33, and 58, and 100 genome copies of HPV 31, 35, and 45	5–10 min[b]	(48)
12.	Diagnosis of bovine theileriosis using whole blood without DNA purification by direct PCR	71 sec[b]	(55)
13.	Single strand conformation polymorphism (SSCP) analysis to rapidly detect the point mutation, Leu72Met, in a human obesity gene	8.5 min[a] 79.6 sec[b]	(56)
14.	Rapid separation of PCR products, 519 and 1191 bp DNA fragments from the 18S rRNA of *B. gibsoni* and *B. caballi.*	36 sec[c]	(57)
15.	Rapid detection of genetically modified organisms (GMOs) in soybeans	<150 sec[b] 11 sec[c]	(58)
16.	DNA mutation detection analysis was carried out using heteroduplex analysis (HDA) for the detection of the BRCA1 gene (a breast cancersusceptibility gene)	250 sec[b]	(61)
17.	Separation of 100-bp DNA ladder	<500 sec[b]	(72)
18.	Separation of double-stranded DNA	7.0 min[b]	(74)
19.	Single nucleotide polymorphism in the p53 suppressor gene	68.4–100.2 sec[b]	(100)

Table 1. Continued.

No.	Name of the biomolecule	Analysis time	Reference
20.	Separation of amino acids	80 sec[b]	(101)
21.	Separation of pGL3 plasmid DNA and *HindIII*-digested pGL3 plasmid DNA	140 sec[b]	(102)
22.	Fast separation of *pBR322*/*HaeIII* digest	1.7 min[b]	(103)
23.	Rapid separation of amino acids (alanine; valine; glutamine and tryptophan)	140 sec[b]	(105)
	Peptides analysis (leucine enkephalin; methionine enkephalin and oxytocin)	75 sec[b]	
	Separation of proteins (chymotrypsinogen A; cytochrome C and bovine serum albumin)	45 sec[b]	
	Identification of 1 kbp oligonucleotide ladder comprised of 517, 1018, 1636, 2036, 3054, 4072, 5090, 6108, 7126, 8144, 10 000, 15 000, 20 000, and 40 000 bp fragments.	200 sec[b]	
24.	The multiplex PCR for 6 DNA markers located on human Y-chromosome and their analysis with 12 channel microchip electrophoresis system	180 sec[b]	(106)
25.	A multiplex PCR for amplifying 3 DNA sequence-tagged sites (STS) located on the human Y chromosome and their detection with 12 channel microchip electrophoresis system	180 sec[b]	(107)
26.	Ultra-fast detection of *Candidatus* Mycoplasma haemominutum and Mycoplasma haemofelis in Korean feral cats	75 sec[b] 11 sec[c]	(108)
27.	A polymerase chain reaction (PCR) for the ultra-fast diagnosis of canine T-cell lymphoma	41.7 sec[b] 7.0 sec[c]	(109)
28.	Detection of hepatic acyl-coenzyme A synthetase (ACS), carnitine palmitoyltransferase-I (CPT-I) and acetyl coenzymeAcarboxylase (ACC) are coenzymes associated with the genetic type of obesity in animal models	114.5 sec[b] 16.2 sec[c]	(110)
29.	Study of the DNA separation at various buffers and pHs	240–100 sec[b]	(111)
30.	20-cycle PCR amplification of a 176-base pair fragment from the DNA gyrase gene of *Neisseria gonorrhoeae*	1.5–18.7 min[b]	(112)
31.	Identification of mitochondrial DNA targets including the haplotype analysis of HVR1 and HVR2 and the study of interspecies diversity of cytochrome *b* and 16S ribosomal RNA (16S rRNA) mitochondrial Genes	~120 sec[b]	(114)

Table 1. Continued.

No.	Name of the biomolecule	Analysis time	Reference
32.	Separation and detection of circle-to-circle amplification products	55 sec[b]	(115)
33.	Separation of small DNA molecules in high polymer concentrations	260 sec[b]	(117)
34.	Detection of PCR products of DNA from pUC118 cloning vector	110 sec[b]	(118)
35.	Analysis of on-line digested fragment of restriction endonucleases *BamHI* and *FokI*	~150 sec[b]	(119)
36.	Detection of PCR products by on-chip transient ITP (TITP) method	159 sec[b]	(120)
37.	Fast mutations detection via allele-specific amplification-HD analysis; (185delAG, 5382insC, 3867G3T, and 6174delT) in BRCA1 and BRCA2	<24 min[a] <170 sec[b]	(121)
38.	Identification of Herpes simplex virus PCR products	8.3 min[a] <115 sec[b]	(122)
39.	Post-PCR analysis of hepatitis C virus	~14.0 min[a] <140 sec[b]	(123)
40.	PCR products of fragile X (CGG)n alleles to facilitate a fast exclusion test of FXS	< 3 min[b]	(124)
41.	Separation of standard DNA digest Separation of 50-bp DNA ladder Separation of viral agents (Cytomegalovirus; Herpes simplex virus; Varicella-Zoster virus; Epstein–Barr virus) Detection of B cell lymphoma Screening of DNA mutations in the BRCA1 and BRCA2 breast cancer susceptibility genes	< 32 min[a] < 155 sec[b] <197.5 sec[b] <160 sec[b] <130 sec[b]	(125)
42.	Analysis of β-actin, apolipoproteins and Caco-2 cell	110 sec[b]	(126)
43.	Separation of single-stranded DNA (ssDNA) products generated from an allele-specific ligation to screen for a singlebase mutation at codon 12 in the K-*ras* oncogene	23–40 min[a] <145 sec[b]	(127)
44.	Separation of low density lipoprotein	17.1 sec[b]	(128)
45.	Separation of low density lipoproteins Separation of high density lipoproteins	~11 min[a] ~25 sec[b] 12.5 min[a]	(130)
46.	Rapid identification of *Streptococcus mutans* and *Streptococcus sobrinus*	72.1–84.0 sec[b]	(131)
47.	Detection of pathogen and genotyping directly from whole *Escherichia coli* and *Staphylococcus aureus* cells	<9 min[b]	(132)

Table 1. Continued.

No.	Name of the biomolecule	Analysis time	Reference
48.	Detection of bacteria species i.e., *Escherichia coli O157* and *Salmonella typhimurium*	<2.5 min[b]	(133)
49.	Ultra-fast analysis of PCR amplification of β-globin target cloned in M13	20 min[a] 120 sec[b]	(134)
	Rapid detection of genomic *Salmonella* DNA	45 min[a] 51 sec[b]	
50.	Detection of PCR products of multiplexed amplification of *E. coli* genomic and plasmid DNAs	<3 min[b]	(135)
51.	Rapid detection of bacteriophage λ DNA, 346- and 410- bp regions of *E. coli* genomic and plasmid DNAs	<200 sec[b]	(136)
52.	Detection of bacterial pathogen DNA *Escherichia coli*	<40 min[b]	(137)
53.	Analysis of human genomic DNA	960 sec[b]	(139)
54.	The diagnosis of Duchenne muscular dystrophy genetic disease	<230 sec[b]	(140)
55.	Detection of mutations in the X chromosome, caused Duchenne muscular dystrophy genetic disease	<100 sec[b]	(142)
56.	Separation of 100-bp DNA ladder	<200 sec[b]	(145)
57.	Detection of T- and B-cell lymphoproliferative disorders	12 min[a] 100–200 sec[b]	(146)
58.	Molecular characterization of mutations in disease genes	<600 sec[b]	(147)
59.	Screening of monoclonal antibody product	30 min[a] <33 sec[b]	(148)
60.	Binding study of transcription factor Abf1 in *Saccharomyces cerevisiae*	100 sec[b]	(149)
61.	Identification of proteins in human serum	20 sec[b]	(150)
62.	Identification of K562 cells	650 sec[b]	(151)
63.	Identification of synthesized RNA	<160 sec[b]	(152)
64.	Detection of foodborne pathogenic bacteria	~8.0 min[b]	(153)
65.	Genetic association analysis through single nucleotide polymorphism (SNP)	<115 sec[b]	(155)
66.	Separation of fetal calf serum proteins	<160 sec[b]	(156)
67.	Analysis of specific sequence of Phage Lambda DNA	<100 sec[b]	(157)

[a]Capillary electrophoresis; [b]Microchip electrophoresis; [c]Microchip electrophoresis with programmed field strength gradients.

with PFSG. All the above developed microchip-based electrophoresis methods provide high sensitivity, selectivity, good reproducibility and less time (sec or min) for the rapid separation and identification of biomolecules.

CONCLUSIONS

ME has attracted considerable attention as the next advancement in DNA analysis in modern biotechnology. DNA detection on the microchip-based electrophoretic analysis of biomolecules with a variety of biomedical applications has been reported, and all variants, ME have overcome many of the challenges for the DNA analysis. The various applications of ME for DNA analysis have shown reduced analysis time, automation, lower cost and better sensitivity over other methods.

Recent reports on ME have generated great insights into the variables affecting the sample resolution and usefulness. These results suggest that the ME can be a single consistent and reproducible method for DNA analysis. This method holds great potential over proven traditional slab gel electrophoresis techniques with regard to its fast and inexpensive analyses, the requirement for only minute amounts of sample, and possible automation (20, 43, 61, 127, 128). Further developments of the microchip device, including automated matrix replacement and sample loading, are needed.

The large number of publications on ME in DNA analysis suggests its widespread analytical applications for the separation of PCR products, point mutations and nucleotides and as well as simultaneous analysis in a single run for routine analysis (41, 57, 58, 61). In the future, more improvements of ME analytical technology with tremendous applications for high-throughput methods in DNA analysis, PCR products and DNA screening will certainly provide important and novel tools for rapid DNA analysis and might be an important analytical technique for the diagnosis of many diseases. At present, most scientists have been directed to further improvements in automation, an increase in sample throughput and enhancement of detection sensitivity for biomolecule analysis at trace level. It can be expected that, in the near future, ME will be widely accepted and present in most of the academic and commercial laboratories.

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