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Miniaturized Capillary Electrophoresis for the Separation and Identification of Biomolecules

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1.1 Introduction

Microchip capillary electrophoresis (MCE) is one of the efficient bioanalytical tools for rapid separation and detection of bioactive molecules with high separation resolution [1–3]. It has proven to be a prominent tool for identification of nucleic acids and proteins in food and clinical microbiology [2]. Separation of biomolecules is a key platform for quantitative and qualitative analysis of target biomolecules in biological matrices. For the first time, Manz's group integrated a simple analytical procedure on a small glass chip for the separation and detection of target chemical species [4], collectively referred to as "lab-on-a-chip" or micro total analysis systems (μTAS). In this concept, MCE is included due to the separation mechanism in a microchip with very short channels. As a result, target molecules from the mixture are effectively separated by using high electric field strengths. MCE has been widely applied as a rapid separation tool in various fields of science, i.e. proteomics, genomics, biomarkers, and forensics [5–9]. These reviews reported that MCE has shown better performance for the separation of target analytes compared to traditional capillary electrophoresis. The MCE has successfully separated >30 000 proteins from a single cell [10].

In this chapter, we summarize the recent developments of MCE for separation and identification of nucleic acids and proteins from clinical and food samples. We briefly describe the history and role of MCE in clinical and food microbial research. A section is devoted to applications of MCE for separation and identification of nucleic acids, proteins, and biomarkers from clinical and food samples. The analytical features of MCE for rapid separation and detection of biomolecules are tabulated, which provides significant information to scientists to know potential advancements of MCE in molecular biology.

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1.2 Brief Summary of MCE

Generally, MCE consists of four core parts: microfluidic chip, electric field, separation, and detector. The electric field is applied for sample concentration and separation. Figure 1.1 displays a T-shaped microfluidic chip. The microfluidic chip contains few reservoirs such as sample and buffer reservoirs. These reservoirs should be filled with a background solution, and sieving gels and pipetting and syringe pumps are used for fluidic control. Once the microfluidic chip is set up with these parts, a high electric field is applied to the reservoirs (sample) to separate the target analytes. The detector is placed at the end of the separation channel, which results in registering the zones for separation and transmitting the data for signal processing unit, which generates an electropherogram. In this section, an overview of fabrication of microfluidic chips, sample preparation (on-microfluidic chip), separation, and analyte detection is given.

1.2.1 Fabrication of Microfluidic Chips

So far, several methods have been adopted for the fabrication of microfluidic chips, such as reactive ion etching, wet etching, photolithography, conventional

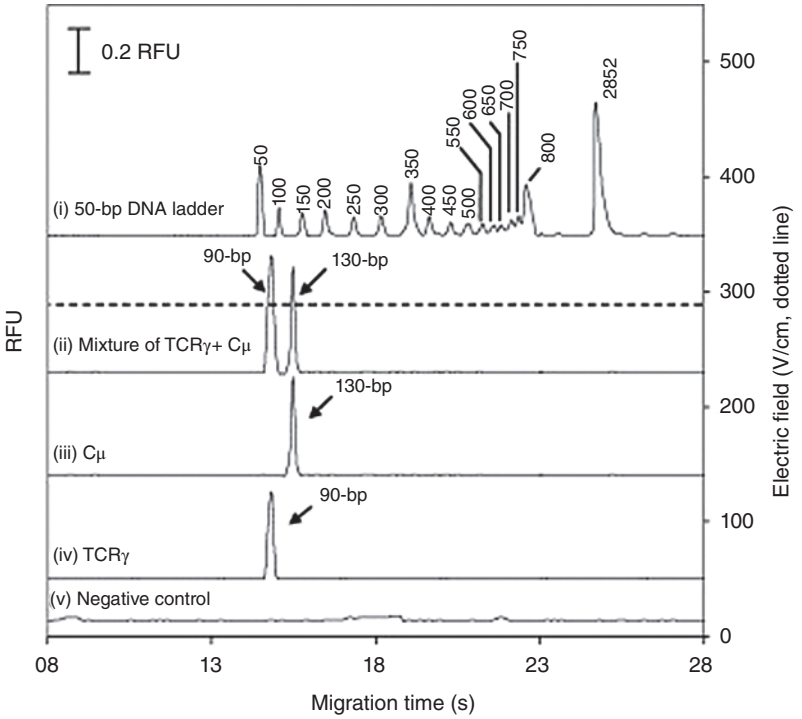

Figure 1.1 Separation and identification of amplified-PCR products of T-cell lymphoma. 50-bp DNA ladder (i); mixture of TCRγ and Cμ (ii); positive control Cμ(130-bp) (iii); TCRγ (90-bp) (iv); and negative control (v). The dotted linesrepresent the applied electric field. Figure reprinted from Ref. [25] with permission.

machining, hot embossing, injection molding, soft lithography, in situ construction, laser ablation, and plasma etching [11, 12]. Silicon or glass is used as raw materials for the fabrication of microfluidic chips. Microfluidic electrophoresis chips consist of two reservoirs (sample and buffer) connected to the separation channel. A wide variety of materials including ceramics, glass, and polymers (poly(methyl methacrylate), cyclic olefin copolymers, polycarbonate, polystyrene, and fluorescent poly(*p*-xylylene) polymer (Parylene-C) have been used for preparation of microfluidic chips. Paper and fabric-based disposal chips have also received attention in MCE [7]. Electroosmotic flow (EOF) is generated in microfluidic chips as the reservoirs are filled with a background solution or electrolyte. Since EOF significantly obstructs separation, microfluidic chips are coated with various chemicals and hydrogels to suppress EOF.

1.2.2 Designing Microfluidic Channels

Crossed-channel and T-shaped microfluidic chips are widely used in MCE, and the microchip channel is connected perpendicularly to other channels (sample and buffer). Microfluidic chips are also prepared with different designs. For example, a microfluidic chip (Agilent Bioanalyzer™ chip) is prepared with 16 reservoirs, of which 12 are for sample reservoirs and four for references and reagents. Accordingly, MCE has been successfully applied for the analysis of various bioactive molecules with high precision and accuracy. Microfluidic chip channels with a width of 10–100 μm and a depth of 15–40 μm are considered the best design for separation of analytes. Also, separation channel area is designed to be 165 mm and 8×8 mm^2 and the number of channels of microchips is increased to 12–384 for separation of nucleic acids and multiple genotyping (384) molecules with reduced time and increased accuracy [3]. Furthermore, microchips are designed with 8, 12, 16, 48, and 384 parallel channels for rapid and efficient separation of a wide variety of analytes.

The electric field (voltage application) and hydrodynamic pressure are applied for sample injection in MCE. On-chip peristaltic pump is used for hydrodynamic injection [13]. Inkjet and array techniques are droplet injection systems, which provide high throughput and the sample injection volume ranges from nano- to picoliters [14]. As microfluidic chips are compact in size, electrokinetic injection is the preferred method of sample injection, where the injection volume of the sample is strongly dependent on the applied voltage and injection time [15]. Further, hydrodynamic injection system requires a pump or pipette, which limits its use in MCE. It is usually carried out by variations in pressure, vacuum, reservoir (sample waste), and fluid levels of sample.

Electrokinetic injection system contains several injection modes, i.e. floating, gated, dynamic, and pinched. In the pinched injection mode, a voltage is applied at various channels including sample, buffer waste, and buffer reservoirs, and, as a result, the sample is injected into the channel junctions, and further enters the separation channels. Although the pinched injection mode is well illustrated and low volume of analyte plugs, it decreases the sensitivity due to sample plug. In the floating injection mode, which is similar to the pinched design, the potential is not required in the buffer and buffer waste reservoirs, increasing the sample load due

to diffusion of the sample into the separation channel. Voltage can be applied at the sample and buffer reservoirs, and two waste reservoirs are grounded in this mode. The sample is injected into the separation channel by switching off the voltage at the reservoir (buffer), loading several amounts of the sample, which could help to improve the sensitivity of MCE. Then, the voltage is again switched on at the buffer reservoir. In dynamic injection mode, electroosmosis is required to inject the sample into separation channels, which can also improve the sample load.

1.2.3 Electrophoretic Separation

Target analytes are effectively separated in separation channels via electrophoretic separation by applying an electric field. Electrophoretic migration of analytes occurs due to the electric field and flow of liquid, collectively known as EOF. Various electrophoretic separations including electrokinetic chromatography, gel electrophoresis, and zone electrophoresis have been described in MCE [7]. Dielectrophoresis (DEP), gel electrophoresis, and zone electrophoresis modes are generally used in MCE. The DEP allows the separation of particles and cells by applying irregular electric fields (nonuniform) [7]. PC-3 human prostate cancer cells and polystyrene microbeads were separated by ionic liquid electrodes [16], and target analytes and particles were successfully separated via the on-chip procedure from human plasma [17]. The charged analytes migrate using the background solution (BGS) and are separated through electrophoretic mobility of target analytes, which leads to detection of analytes on the basis of descending mobility. As a result, small organic molecules including metabolites and drugs are effectively separated by the zone electrophoresis mode.

Electrophoretic mobility of analytes takes place when an electric field is applied along a sieving matrix. The analytes migrate through the matrix, and the degree of migration is dependent on the size/weight of the analytes. Porous gels are used for the preparation of the sieving matrix, and the size of the gel mesh affects separation efficiency of MCE. Generally, separation efficiency of MCE will be greater with the increasing concentration of cross-linked gels. Viscosity of gels also increases with increasing concentration of the sieving matrix, making it difficult to load the gels into microfluidic channels. Common gels are prepared by using starch, agarose, cellulose, and polyacrylamide. Large biomolecules such as nucleic acids (DNA and RNA), proteins, and biomarkers can be successfully separated by using gel electrophoresis based on size-dependent separation. Polymerase chain reaction (PCR) is often used for the amplification of RNA and DNA to concentrate nucleic acids prior to their separation by MCE. Importantly, MCE has ability to separate base pairs with reduced time and improved resolution, exhibiting better analytical features compared to slab gel electrophoresis, which requires <10 min for the analysis of base pairs.

1.2.4 Detectors

Target analytes can be detected by various detectors, once analytes are separated in separation channels of microfluidic chips. In MCE, various detectors including

absorbance, electrochemical, chemiluminescence, and laser-induced fluorescence (LIF) are widely used to detect a wide variety of analytes. Among them, LIF is a prominent detector for the detection of trace-level target analytes due to its simplicity and sensitivity. It has been widely applied for the analysis of nucleic acids from various sample matrices [7].

In the LIF detection, the analyte (fluorescent) is excited by a laser, and light is emitted when the excited molecule returns to the ground state, which can be useful for detection signal. To eliminate autofluorescence, fluorescent derivatization is done in LIF for effective detection of target analytes. Fluorescent derivatization is done in channel before sample injection [19], which can also extend for parallel detection of multiple analytes. For parallel excitation of molecules, two lasers at visible and near-infrared wavelengths are used for the excitation of three metabolites [20]. In LIF, laser and light emitting diodes have proven to be promising and inexpensive portable devices for the analysis of various target analytes. Although LIF is a sensitive detection device, microfluidic chips (narrow structures) and small volumes of injected samples decrease its analytical sensitivity. Furthermore, MCE was successfully used for the detection of interferon-gamma at the picomolar level by using the LIF detection mode [21].

In chemiluminescence, the target analyte is excited by absorbing energy generated from chemical reactions. In this detection mode, molecules are effectively detected, even target analytes in femto- and atto-molar concentrations. Oxidation of luminol is an example of chemiluminescence reaction where luminol oxidizes in a basic medium in the presence of hydrogen peroxide (oxidizer) and enzyme as a biocatalyst (horseradish peroxidase, HRP). To enhance the chemiluminescence signal, various chemicals are used as modifiers in the luminol reaction. Amperometric and capacitively are coupled with contactless conductivity for the detection of analytes by the electrochemical detection mode in MCE. Similarly, amperometric detection mechanism is based on measuring changes in electric current when the analyte reaches the detector. In recent years, electrochemical MCE approaches are considered promising biotools for point-of-care diagnostics in various clinical applications [22].

1.2.4.1 Capability of Microchip Electrophoresis for the Separation and Identification of Biomolecules

In this section, an overview of the potential applications of microchip electrophoresis for the separation of biomolecules is provided. One of the simplest ways of detecting DNA molecules is an assay based on MCE. The MCE is a promising miniaturized analytical device for the separation of target molecules in various fields of science including clinical, pharmaceuticals, forensic, biology, genomic, and biomedical sciences. The rapidity of ME was improved by applying an electric field with a programmed field strength gradient (PFSG), facilitating separation of DNA with high speed [23–26]. A novel rapid MCE approach was developed for the separation and detection of *Mycoplasma haemofelis* and *Candidatus M. Haemominutum* in Korean feral cats [24]. The developed method was able to separate and differentiate two polymerase chain reaction (PCR) products in <11 seconds. Another report illustrated the rapidity of MCE with PFSG for the detection of T-cell lymphoma in dogs [25]. In this

method, polyethylene oxide (PEO, Mr 8 000 000, 0.5%) was used as a sieving matrix for the separation of amplified-PCR products from canine T-cell lymphoma. This method successfully separated the PCR products of T-cell lymphoma within 7.0 s using PFSG (Figure 1.1).

1.2.4.2 Detection of Cancer Biomarkers

Furthermore, MCE is applied to investigate cancer via the identification of various biomolecules (peptides, proteins, DNA, and RNA). Telomerase activity, carcinoembryonic antigen, and DNA mutations in p53 oncogenes are recognized as cancer biomarkers. A chip-based analytical approach was established for the analysis of genetic codes to assess the telomerase activity, separating the amplicons within 120 seconds [27]. An aptamer-based MCE with laser-induced fluorescence (LIF) was developed for the detection of thymidine kinase 1 [28] and carcinoembryonic antigen [29, 30]. To suppress the adsorption of biomolecules on the microchip channel, 30 mM sodium dodecyl sulfate (SDS) was added and target analytes were detected within 4.0 min, offering an impressive limit of detection (LOD) at 45.7 pg/ml. The p53 gene consists of more than 20 000 bp, 11 exons, and 10 introns, and p53 gene mutations are investigated by single-strand conformational polymorphism (SSCP) and heteroduplex analysis (HA) combined with MCE–LIF [31]. This method successfully analyzed 106 tissue samples, and p53 gene mutations were correctly identified with high accuracy. Similarly, MCE–LIF was applied to detect gene mutations in exons 19 and 21 of patients with non–small-cell lung cancer [32]. MCE with chemiluminescence resonance energy transfer mechanism was developed for the detection of neuron-specific enolase [33]. The developed method was able to detect neuron-specific enolase events at 4.5 pM, which confirms the potentiality of MCE for separation and detection of cancer biomarkers.

Low-abundance KRAS mutation detection, glycomic serum analysis, and microsatellite instability were used for the investigation of colorectal cancer. MCE method was developed for the detection of mutations in the KRAS gene in patients with colorectal cancer [34]. Figure 1.2 represents the use of MCE for the detection of the KRAS gene. This method involved the amplication of 107-bp fragment of KRAS from DNA templates extracted from cancer cells with mismatched primer PCR, generating two peaks for 77 and 30 bp fragments. Prostate cancer was examined by identifying the circulating cell-free DNA in patients with hormone-refractory prostate cancer [35]. The concentration of cell-free DNA was estimated during chemotherapy. The concentration of cell-free DNA increased from 13.3 to 46.8 ng/ml after treatment of the first cycle of chemotherapy. DNA methylation analysis and MCE–LIF were used for the identification of skin cancer in patients with oral squamous cell carcinoma [36]. Eight genes (transmembrane protein with epidermal growth factor-like and 2 follistatin-like domains 2 [TMEFF2], e-cadherin [ECAD], *O*-6 methyl-guanine DNA methyltransferase [MGMT], retinoic acid receptor beta [RARβ], fragile histidine triad gene, cyclin-dependent kinase inhibitor 2A, WNT inhibitory factor 1, and death-associated protein kinase 1) were used as signals for the estimation of elevated levels of DNA methylation. Among these, four hypermethylated genes (RARβ, TMEFF2, ECAD, and MGMT) were

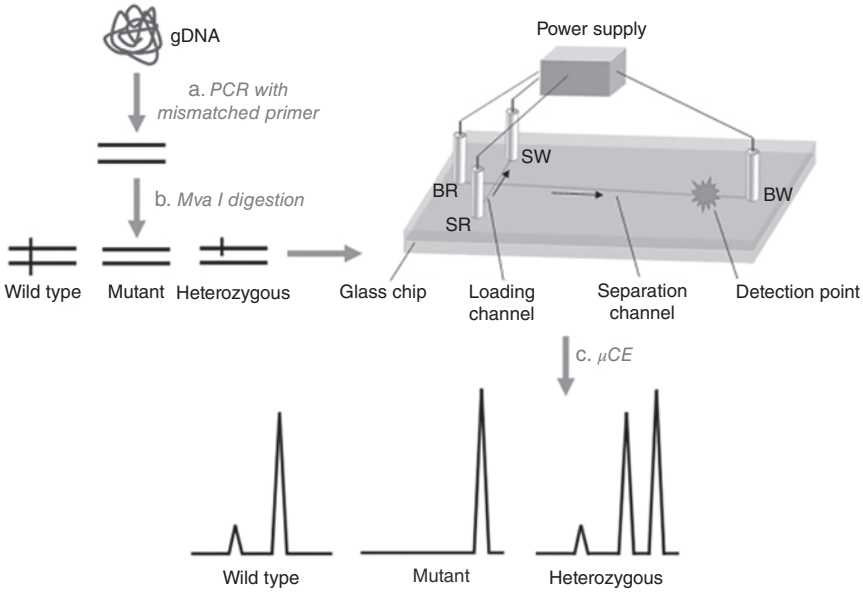

Figure 1.2 Schematic representation for the detection of low-abundance KRAS mutations in colorectalcancer. Figure reprinted from Ref. [2] with permission.

selected for the diagnosis of oral squamous cell carcinoma with 87.5% specificity and 100% sensitivity. Further, α-fetoprotein acts as an important biomolecule for the detection of small tumors at the early stage of cancer growth, which could be used for the treatment of cancer. MCE with fluorescence detection and lectin-affinity chip electrophoresis were successfully applied to detect α-fetoprotein in various tumor cells [37, 38]. Lectin-affinity electrophoresis exhibited the limit of detection at 0.3 ng/ml in serum, whereas the transient isotachophoresis approach exhibited a detection limit of 5 pM. Both analytical techniques exhibited a much lower level of α-fetoprotein (280 pM). This immunoassay was also applied to detect lens culinaris agglutinin-reactive α-fetoprotein, des-γ-carboxy prothrombin, and total α-fetoprotein [39]. Three biomarkers fucosylated fraction of α-fetoprotein, α-fetoprotein, and prothrombin induced by vitamin K absence-II were used for the accurate diagnosis of liver cancer, allowing detection of small-sized tumor, early-stage hepatocellular carcinoma, and single tumor with an accuracy of 86.7%, 81.8%, and 91.7%, respectively [40].

The profiling of *N*-glycans in patient serum [41], human papillomavirus [42] and determination of β-subunits of human chorionic gonadotropin [43] can be used for the investigation of ovarian and cervical cancers. The glycans were extracted by solid-phase extraction using activated carbon columns followed by fluorescence labeling with APTS (8-aminopyrene-1,3,6-trisulfonic acid). The electropherogram provides the differentiation between the before and after drug treatment of patients. The assay of β-subunits of the hormone human chorionic gonadotropin was used as a tumor biomarker for choriocarcinoma. A noncompetitive immunoassay was developed for the detection of β-subunits [44]. The practical application of the

method was demonstrated for the detection of β-subunits in serum samples from healthy subjects and patients with ovarian cancer. The concentrations of β-subunits were found in the range of 9.5–15.7 and 160.9–210.4 mIU/ml in healthy and cancer samples, respectively. Several biomarkers such as lactate dehydrogenase B, galectin-1, and bladder cancer–specific genes (FGFR3, HRAS, TERT, RALL3, ALX4, MT1A, and RUNX3) were widely used for the detection of bladder cancer. In this connection, the cell lysate of bladder cancer cell lines RT4 and T24 were investigated for the protein expression of galectin-1 and lactate dehydrogenase B by microfluidic immunoassays [45, 46]. With this approach, the bladder cancer cells were rapidly extracted from human urine [46]. The amplicons were identified by MCE–LIF within 2 minutes.

1.2.4.3 Assays of Immune Disorders and Microbial Diseases by MCE

A reusable glass capillary array electrophoresis (CAE) chip was developed for the detection of standard K562 DNA [47]. In situ PCR was performed in the fabricated chip. The CAE chip consists of two DEA chips that are reversibly attached to polydimethylsiloxane (PDMS) blocks, thereby facilitating simple prewashing of

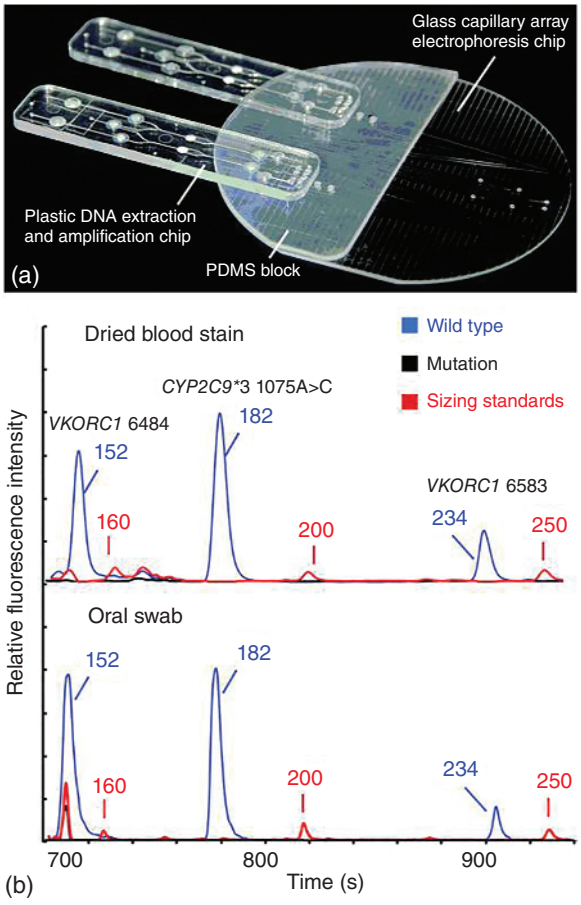

Figure 1.3 (a) Photographic image of microfluidic chip for the rapid pharmacogenetic typing of multiple warfarin-related single-nucleotide polymorphisms. (b) Electropherogram of pharmacogenetic analyses carried out using a dried blood stain and buccal swab. Reprinted with permission from Ref. [2].

PDMS surface (Figure 1.3a). As a result, effective electrophoretic separation was achieved (Figure 1.3b), suggesting that this method was successfully applied to detect target analytes directly from blood, buccal swabs, and dried blood spots. A new portable microfluidic chip was developed for the detection of uric acid in urine of gout patients [48]. MCE was successfully developed for the identification of proteins for different patients [49]. This method successfully differentiated several proteins including albumins and α-, β-, and γ-globulins from cerebrospinal fluid and serum samples of patients and healthy persons. A novel and rapid immunoassay was developed for the detection of phenobarbital in blood samples of epileptic patients [50, 51]. In this approach, liquid–liquid extraction was performed for the extraction of phenobarbital, and effectively analyzed target analytes within the therapeutic range from 43 to 172 μM.

Recently, MCE has also been widely applied to detect various pathogenic microorganisms such as viruses (influenza and hepatitis B) and bacterial species. MCE was used to detect influenza A (H1N1) virus [52]. A high-throughput separation was achieved for hemagglutinin (HA) and nucleocapsid protein (NP) gene PCR products (116 and 195 bp, respectively) by multichannel MCE. A spatially multiplexed microdevice was applied to detect influenza A and influenza B in nasopharyngeal and throat swabs of humans [53]. This method enables to complete the analysis within 40 min, offering an LOD of 10 RNA copies per reaction. Similarly, MCE–LIF method evaluated alanine aminotransferase (ALT) enzyme kinetics [54]. This method exhibited LOD of 4.0×10^{-7} and 2.0×10^{-7} M for l-Glu and l-Ala, respectively. This method was effectively applied to evaluate ALT enzyme kinetics in serum samples.

A novel paper-based CE microdevice was fabricated for point-of-care (POC) pathogen diagnostics [55]. This newly designed paper-based CE was successfully applied to differentiate single-stranded DNA (ssDNA) with 4 bp resolution. PCR amplicons of two target genes of *Escherichia coli O157:H7* (rrsH gene, 121 bp) and *Staphylococcus aureus* (glnA gene, 225 bp) were successfully separated and identified within 3 minutes. Figure 1.4 shows the design of a paper-based CE microdevice. Figure 1.5 shows the electropherogram of bracket ladders of genomic DNA (from 0.5 ng to 0.5 pg). The developed paper-based CE achieved impressive LOD of 9.3×10^{1} and 1.6×10^{2} copies for *E. coli O157:H7* and *S. aureus*, respectively. A dual-channel PDMS-based microchip was fabricated for the detection of *o*-aminophenol, *p*-aminophenol, and *m*-aminophenol using a modified indium tin oxide (ITO) microelectrode detector [56]. This method exhibited LOD of 0.41, 0.24, and 0.42 μM for *o*-, *p*-, and *m*-aminophenols, respectively. A portable MCE combined with on-chip contactless conductivity detector was developed for the analysis of vancomycin in human plasma [57]. This method was applied to detect vancomycin within 5.0 min, offering an LOD of 1.2 μg/ml. This method exhibited good recovery ranges of 99.00–99.20% in plasma samples.

1.2.4.4 Assays of Biomarkers by MCE

Assay of biomarkers plays a key role in diagnosing various disorders by MCE. Multichannel chips were fabricated using poly(methyl methacrylate) (PMMA)

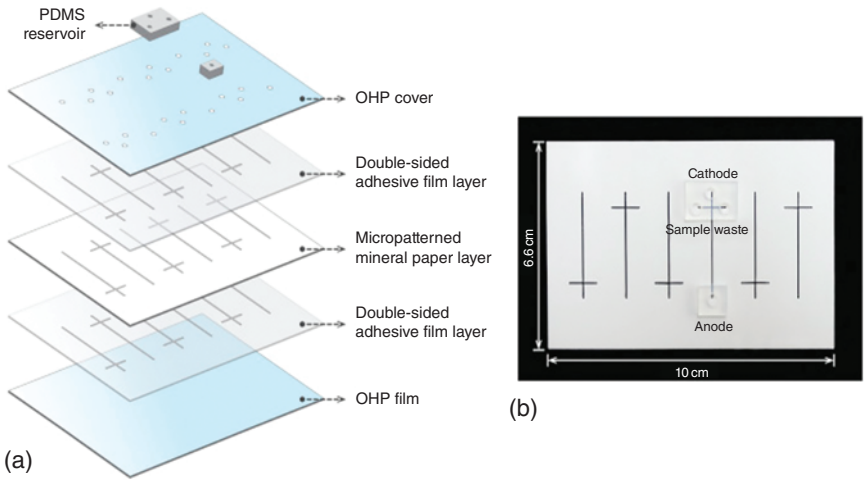

Figure 1.4 (a) An view of paper-based capillary electrophoresis that consists six layers. (b) Photographic image of paper-based CE. Reprinted with permission from Ref. [55].

and applied for the separation and identification of free bilirubin in biofluids [58]. The developed method exhibited a wider linear range from 10 to 200 µM with an LOD of 9.0 µM. This method was successfully applied to detect total bilirubin (118.3–119.4 µM) from the samples of jaundiced infants. An MCE with fluorescence detector was established for the detection of creatinine in urine [59]. The creatinine was quantified by using exciting and emission wavelengths at 490 and 523 nm, respectively. Authors obtained an impressive LOD of 2.87 µM and good recovery in the range of 96.0–107.0%. MCE has been used as a promising analytical tool for the rapid and selective detection of urine proteins in clinical samples [60]. This method exhibited a wider linear range in the concentration of 1.0–15.0 and 1.0–10.0 g/l for human albumin and human transferrin with an LOD of 0.4 g/l. This method has the ability to detect urine proteins in various clinical samples, i.e. 105 nonselective proteinuria, 60 selective proteinuria, and 6 overflow proteinuria. Thyroxine (T4) was successfully quantified by MCE method [61]. The detection principle was based on the immunoreaction between T4 and HRP-labeled T4 (HRP-T4) with anti-T4 mouse monoclonal antibody (Ab), which favored the formation of HRP–T4 and the HRP-T4–Ab complex, which can be easily separated and identified by MCE with the chemiluminescence technique. The MCE separated the target analytes within 60 seconds, exhibiting an LOD of 2.2 nM. Furthermore, Au/chitosan/Fe_3O_4 nanocomposites were synthesized and inserted into the microfluidic device [62]. The Au/chitosan/Fe_3O_4 nanocomposites inserted microfluidic device was connected to Au microelectrode for immunoassay of hemoglobin A1c (HbA1c). In this work, Au/chitosan/Fe_3O_4 nanocomposites were functionalized with anti-human hemoglobin-A1c antibody (HbA1c mAb), enabling rapid immunoreactions between the sequence of HbA1c, Hb mAb, and secondary alkaline phosphatase-conjugated antibodies. This method exhibited an LOD of 0.025 µg/ml and successfully evaluated assay7 of HbA1c in blood.

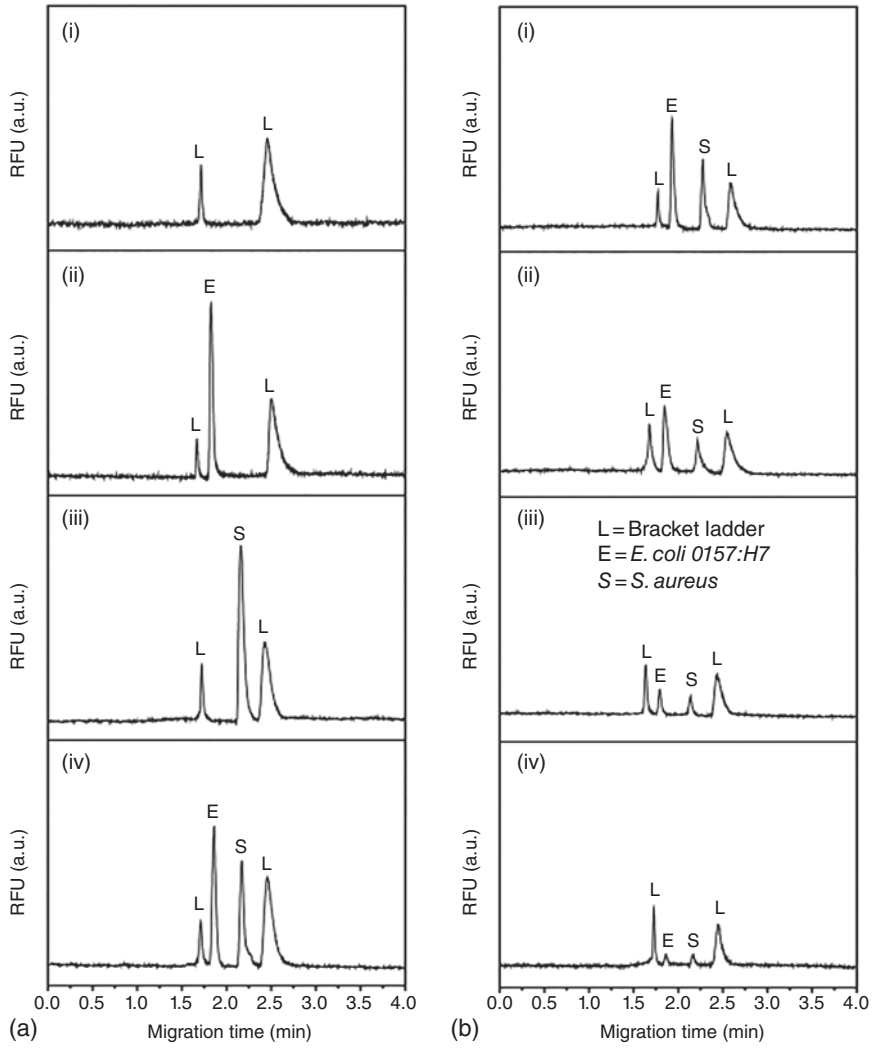

Figure 1.5 Separation and identification of pathogens (i) bracket ladders, (ii) *E. coli O157:H7* with bracket ladders, (iii) *S. aureus* with bracket ladders, and (iv) *E. coli O157:H7* and *S. aureus* with bracket ladders by paper-based CE. (b) Electropherogram of *E. coli O157:H7* and *S. aureus* at different DNA concentrations (i) 0.5 ng, (ii) 0.05 ng, (iii) 5 pg, and (iv) 0.5 pg. Reprinted with permission from Ref. [55].

Four urinary proteins, namely, immunoglobulin G (IgG), transferrin, human serum albumin (HSA), and β2-microglobulin were successfully separated and identified by isotachophoresis/capillary zone electrophoresis [63]. Using this approach, sample desalting/preconcentration was carried out on-chip, achieving low LOD with high selectivity. Testosterone was sensitively detected using MCE with chemiluminescence detectors [64]. The detection mechanism was based on the immunoreaction between testosterone and *N*-(4-aminobutyl)-*N*-ethylisoluminol-labeled T

(ABEI-T) with antibody (Ab). The formed products ABEI-T–Ab complex and free ABEI-T were efficiently separated and detected. This method displayed an LOD of 1.0 nM in human serum. An electrokinetic-based microfluidic device was developed for the detection of preterm birth biomarkers [65]. In this approach, solid-phase extraction was used for the extraction of preterm birth biomarkers. The developed method required ~10 μl sample volume and exhibited 30-fold faster time for the extraction and preconcentration of preterm birth biomarkers (lactoferrin and ferritin) in the clinical samples. A microfluidic device was developed for the extraction and detection of preterm birth biomarkers using pH-mediated solid-phase extraction [66]. In this method, the preterm birth biomarker peptide was effectively separated using nM of biomarkers, demonstrating the detection of preterm birth biomarkers even at 400 pg (0.2 pM). These methods have illustrated that MCE can be successfully applied to separate and detect trace-level target analytes from complex samples.

1.3 Summary

In summary, MCE has significantly proven to be a miniaturized analytical device for separation and identification of trace-level organic molecules and biomolecules from various sample matrices. Microfluidic devices enable on-line sample preparation procedures where trace-level analytes are isolated and enriched, thereby achieving low LOD in microvolume samples of various biomarkers. To enhance sensitivity and selectivity of MCE, nanocomposites have been inserted into microfluidic devices, facilitating immunoreactions in microfluidic channels, which enables detection of target biomarkers with high selectivity and rapidity. Overall, microfluidic capillary electrophoresis has proven to be a miniaturized analytical tool for the rapid analysis of various target molecules with small sample volumes.

Acknowledgments

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