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APPLICATIONS OF MICROFLUIDICS AND MICROCHIP ELECTROPHORESIS FOR POTENTIAL CLINICAL BIOMARKER ANALYSIS

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Abstract

This article reviews advances over the last 5 years in microfluidics and microchip electrophoresis techniques for detection of clinical biomarkers. The various advantages of miniaturization compared with conventional benchtop methods for detecting biomarkers have resulted in increased interest in developing cheap, fast, and sensitive platforms. We discuss the development of applications of microfluidics and microchip electrophoresis for analysis of various clinical samples for pathogen identification, personalized medicine, and biomarker detection. We highlight the advantages of microfluidics platforms over conventional methods that make them an attractive future diagnostic tool. We also discuss the versatility and adaptability of this technology for analysis of various biomarkers, including lipids, small molecules, carbohydrates, nucleic acids, proteins and cells. Finally, we conclude with a discussion of areas that need to be improved upon to move this technology towards routine clinical and point-of-care applications.

1. INTRODUCTION

The push for improved health care is increasing the need to develop diagnostic tools that are cost-effective, reliable, and fast. Lack of resources and expertise in many developing countries limit their ability to perform straightforward clinical diagnostic tests. On the other hand, developed countries often have a backlog of tests that results in longer waiting times for results to be dispensed to the physicians and ultimately to the patients. A causative factor in these problems with clinical diagnostics is that they are done using conventional benchtop analysis platforms that are effective yet slow, lab-bound, labor intensive, and consume large volumes of reagents and samples. Because of some of the disadvantages of conventional methods, researchers adapted photolithography and chemical etching techniques from the

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microelectronics industry to make microfluidic analysis systems starting in the early 1990's [1]. The goal of this review is to describe advances in microfluidics and microchip electrophoresis over the last 5 years in the analysis of clinically relevant biomarkers, including lipids, small molecules, carbohydrates, nucleic acids, proteins and cells. We further highlight the advantages of microfluidics and microchip electrophoresis over conventional benchtop methods in the analyses of clinical samples.

Commonly used disease diagnostic tools process complex bodily fluids [2,3]. Microfluidics and microchip electrophoresis offer advantages for clinical analysis like fast analysis, small sample volumes, low power, and integration of multiple sample manipulation processes into a compact format [4,5]. The manufacturing procedure for these devices is compatible with well-established semiconductor processing techniques. Moreover, microfluidic systems are compatible with point-of-care analysis that can be performed by semi-skilled workers in resource-limited locations [6-9].

Clinical diagnostics need to detect biological molecules that are disease indicators (biomarkers) in complex bodily fluidic samples. Thousands of biomarkers have been reported in literature, and nearly 100 of these are used in regular clinical practice [4,10]. Broadly, these biomarkers can be classified into five main categories: lipids, carbohydrates, nucleic acids, proteins, and cells. Clinical microfluidic and microchip electrophoresis work focuses on detecting one or more of these biomarkers and on developing ways to improve sensitivity, specificity, analysis time, and assay automation. This Critical Review highlights the contributions of microfluidic and microchip electrophoresis technology to the analysis of clinical biomarkers, and more generally to the field of healthcare diagnostics. Papers were selected on the basis of their promise to impact clinical diagnostics, and not necessarily with the intent to inform the reader of the best method to analyze for a specific biomarker. We first focus on microfluidic analysis of lipids, small molecules, nucleic acids, and cells in clinical samples. Information is provided about different approaches for device manufacturing, sensitivity and specificity enhancement, chip-scale integration of analysis steps and clinically accepted analyte detection. We next move on to discuss the contributions of microchip electrophoresis to clinical analyses of samples containing lipids, carbohydrates, nucleic acids, and proteins as disease biomarkers. We then conclude with a brief discussion of promising future directions for the field of point-of-care clinical analysis.

2. MICROFLUIDICS

2.1. LIPIDS

Lipids are biomolecules whose main functions are to store energy and provide structure in the cell membranes. Lipids can also be used as biomarkers for disease diagnosis. Some lipids, including cholesterol, acylglycerol, phospholipids, and prostaglandins have been approved by the world health organization as clinically relevant markers, primarily for cardiovascular disease [10]. Various microfluidic systems have been used for lipid analyses.

Wisitsoraat et al. [11] exploited the miniaturization potential of fluidic processes by including a cholesterol sensing platform inside an integrated bi-layer microfluidic device fabricated from poly(dimethylsiloxane) (PDMS) and glass substrates. In the electrochemical

detection setup functionalized carbon nanotubes grown over the gold surface were used for analyte sensing; cholesterol detection was moderated by cholesterol oxidase (ChOx) immobilized on the carbon nanotubes, and sample loading was accomplished through flow injection methods. In addition to a clinically relevant linear detection range (50 – 400 mg/dL) the analysis was fast (3 - 5 s) and required a low sample volume (15 μ L). Cholesterol detection was still specific in the presence of interferences of glucose, uric acid, ascorbic acid, and acetaminophen. Devices were stable (20% sensitivity variation over one month) and offered high throughput (60 sample/h).

In another application of nanomaterials to enhance the sensitivity of microfluidic sensors, Ali et al. [12] deposited nanocrystalline anatase TiO₂ on indium tin oxide (ITO) electrodes. Then ChOx was immobilized on the TiO₂ layer to react with cholesterol. Sensitivity of the modified ITO electrode was increased due to greater overall surface area and favorable electron transfer kinetics at the ChOx-TiO₂-ITO electrode. This microfluidic device used a small sample volume (1.6 μ L) to give fast (~5 s) and stable results (3% electrochemical current drop over 35 days). The analysis was still specific with normal concentrations of interferents such as glucose, ascorbic acid, uric acid, and lactic acid in serum, and had a linear detection range from 25 – 400 mg/dL.

Recently, graphene has become a popular material in multiple fields including healthcare diagnostics. Labroo et al. [13] used graphene ink electrical biosensing arrays in a paper microfluidic device for multiplex metabolite detection, including cholesterol. This device had four graphene electrodes (Figure 1A), each with an analyte-specific oxidase immobilized (Figure 1B), and the electrodes were connected to a common inlet through microfluidic channels. Fast (~2 min) and sensitive (0.3 μ M detection limit, with 0.3-15 μ M dynamic range) results were obtained using a 2 μ L sample containing model analytes. The same device used with real blood gave detection limits of 1 μ M, 1 mM, 4 mM, and 5 mM for xanthine, lactate, glucose, and cholesterol respectively, which was comparable with results from commercially used screen printed electrodes.

Bai et al. [14] developed an integrated microfluidic chip/nano liquid chromatography platform for the analysis of 8-isoprostaglandin F₂, an oxidative stress biomarker in human urine. The polyimide chip-nano liquid chromatography unit integrates a microfluidic switch, on-chip sample enrichment column, analytical separation column, and a nanospray emitter for mass spectrometer based detection. A linear detection range of 0.01–2 ng/mL was obtained with <6% precision, >99% accuracy, and a detection limit of 0.005 ng/mL.

Takai et al. [15] developed a miniaturized device for home-based medical diagnosis that colorimetrically detected triglycerides, total cholesterol, and high-density lipoprotein in a blood sample. This integrated device contained a stainless steel needle for blood collection and a three layered microfluidic chip for sample processing and detection. The centrifugal pumping microfluidic chip part had three layers of poly(methyl methacrylate): one layer separated plasma from blood, the second transported reagents across the chip, and the third helped in mixing and optical measurements. A 6 μ L blood sample processed for ~12 min showed normal analyte levels for a healthy subject. Integrated chip results for serum

correlated very well with commercial tests, with P values of 0.002, 0.006, and <0.001, respectively, for triglycerides, total cholesterol, and high density lipoproteins.

2.2 SMALL MOLECULES

Small molecules are another category of biomarkers for clinical diagnosis. One of the most commonly measured analytes is glucose because levels are monitored routinely in diabetes [4,12,16]. Accurate blood glucose analysis is critical for diabetic patients and provides vital information for insulin delivery needs to control glucose levels. Microfluidic devices for glucose analysis offer promise to improve diabetes management.

Cai et al. [17] developed a simple paper-based microfluidic device for the colorimetric detection of glucose in human serum. As shown in Figure 2, the flower-like design had fluidic channels, detection points, and a central unit (Figure 2G), all of which were fabricated by the transfer of a paper mask pattern (Figure 2B) to unpatterned paper (Figure 2A) using silanization and heating (Figure 2C-F). A colorimetric detection signal was generated by the reaction between potassium iodide and electrogenerated hydrogen peroxide in the presence of horseradish peroxidase catalyst. A 0.6 μ L sample volume was analyzed in ~20 min, and glucose concentrations of 7 – 15 mM were determined, comparable to standard glucose analysis techniques.

Yu et al. [18] used a simple, inexpensive paper-based microfluidic device to simultaneously detect glucose and uric acid in artificial urine samples. The detection principle was based upon the generation of an enzyme oxidase reaction byproduct and its subsequent chemiluminescence reaction with a rhodamine derivative. The device was a three layer structure with patterned chromatography paper sandwiched between two water impermeable adhesive tape layers. The paper layer contained a sample injection area, two bioactive channels for glucose oxidase and uric oxidase enzyme immobilization, and chemiluminescent detection areas. The linear range was 0.4 to 50 mM for glucose and 1.4 – 47 mM for uric acid. Detection limits were 0.14 mM for glucose and 0.52 mM for uric acid. This sensor was reproducible (8.9% RSD of signal for 11 replicate measurements), stable (retained 90% of signal intensity over 10 weeks), and insensitive to numerous interferents, including lactic acid, citric acid, sodium bicarbonate, calcium chloride, sodium chloride, magnesium sulfate, sodium sulfate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and ammonium chloride.

In a different approach Lin et al. [19] combined a solid-state sensor and microfluidic chip with enzyme carrying alginate microbeads to detect glucose, urea, and creatinine in human blood serum. The device had four different layers containing sensor, fluidic, control, and reference electrode parts. A step-like obstacle in the fluidic microchannel and an external magnetic field immobilized a magnetic powder containing analyte-specific enzyme-carrying alginate microbeads on the surface of an electrolyte-insulator-semiconductor sensor. Injected samples reacted with the enzyme on the beads to release hydrogen ions resulting in a change in voltage signal of the sensor that could be related to the sample concentration. Results showed a detection range of 2–8 mM, 1–16 mM and 0.01 – 10 mM, respectively, for glucose, urea and creatinine, with coefficients of variation of 7%, 11%, and 12%,

respectively. The measured values of the three analytes deviated from those obtained using a commercial kit by 5-14% which was acceptable for these preliminary results.

2.3 NUCLEIC ACIDS

Nucleic acids are an important category of biomarkers used to assess cancer, cardiovascular, and viral diseases [4]. Sample preparation is critical for the analysis in complex biological fluids such as blood, and this process is primarily performed using conventional benchtop techniques.

Chen et al. [20] detected nucleic acid from pathogens in saliva on a polycarbonate-based microfluidic cassette where fluid flow was facilitated by on-chip valves and pouches. This cassette integrated sample introduction and lysis; solid-phase nucleic acid extraction, concentration, and elution of purified sample; PCR or reverse transcription-PCR nucleic acid amplification; and lateral flow assay detection modules, all on the same platform. The device was used to detect bacteria in a 100 μ L saliva sample spiked with *B. cereus*, and a detection limit of 10^3 cells/mL was obtained. Another analysis with human immunodeficiency virus spiked saliva gave a detection limit of 10^5 virions/mL.

Ferguson et al. [21] developed an integrated microfluidic device for the analysis of H1N1 influenza virus (Figure 3A) from throat swab samples (Figure 3B). This disposable device integrated modules for sample preparation (Figure 3C-G) (immunomagnetic target capture, reverse transcriptase (RT)-PCR and single-stranded DNA generation) and nucleic acid specific electrochemical detection in an automated fashion (Figure 3H), completing a whole analysis in ~3.5 h. Levels as low as 10 TCID₅₀ (tissue culture infectious dose) were detected for the H1N1 virus sequence, which was well below the infectious dose.

Wang et al. [22] detected influenza and performed virus sub-typing in a pneumatically controlled microfluidic device having three different modules. The first comprised analyte specific, probe conjugated magnetic beads for RNA extraction. The next module performed RT-PCR on the extracted RNA. Finally in a third module, fluorescence-based detection of the amplified nucleic acid was done. Complete analysis of a 10 μ L sample was accomplished in 110 min with the ability to detect as little as 100 viral copies.

2.4 CELLS

Cells can also serve as indicators for many diseases [23]. Thierry et al. [24] developed a pneumatically controlled PDMS based microfluidic device to capture cancer specific cells over-expressing human epidermal growth factor receptor 2 (HER 2), a clinical biomarker for breast cancer. Cell capture involved immobilization of a HER 2 specific monoclonal antibody (Herceptin) on a modified PDMS surface. The device was capable of capturing ~80% of the HER 2 positive cells from a 5 mL control blood sample that was spiked with breast cancer cells.

Issadore et al. [25] used a high throughput (10^7 cells/min) miniaturized Hall effect sensor chip to detect magnetically labeled cells in blood. This microdevice contained a micro Hall sensor layer and a microfluidic control layer constructed on the PDMS substrate. Figure 4A-B demonstrates the working mechanism and Hall voltage measurement scheme,

respectively, while Figure 4C shows the device design including sensing and hydrodynamic focusing modules for the analytes. The device was successfully used to detect circulating tumor cells in whole blood of ovarian cancer patients as well as for the simultaneous detection of epithelial growth factor receptor, HER 2/neural, epithelial cell adhesion molecule, and mucin 1 on single cells [25].

2.5 CONCLUSIONS

With ongoing advances in materials science and miniaturization techniques, the potential applications of microfluidic technology for lipid analysis will continue to expand. The demonstrated advantages offered by this technology such as small sample volumes, multi-process integration, and portability will further help to provide quick and inexpensive diagnostic information for future clinical analysis. These systems also have potential to assist physicians in remote locations in making decisions about therapeutic interventions. The rapid detection of small molecules is an area in which microfluidic systems, including paper and flow-based devices, have shown great promise for clinical applications. For example, the demonstrated sensitivity and selectivity of microfluidic devices for glucose measurement may help to improve diabetes management. The ability to integrate sample preparation with detection on a single chip platform makes microfluidic systems promising for clinical analysis in a more compact package than those typically used in a clinical laboratory. Microfluidics are thus appealing for nucleic acid based pathogen detection at the point of patient care. Finally, the possibility of cell capture and analysis using microfluidic technology could provide a cheap and disposable diagnostic for diseases such as cancer.

3. MICROCHIP ELECTROPHORESIS ANALYSIS

Microchip electrophoresis is a promising technology that is being developed and tested for biomarker detection of clinical samples such as urine, serum, cerebrospinal fluid, and saliva. Application of different separation principles, such as isotachopheresis, capillary zone electrophoresis and micellar electrokinetic chromatography in microchip electrophoresis has been explored and resulted in improved separations. Use of different detection methods such as UV absorption, fluorescence, chemiluminescence, and electrochemistry allows analyses of different biomarkers. This section of the review aims to discuss various clinical biomarkers being tested using microchip electrophoresis including lipids, carbohydrates, nucleic acids, and proteins.

3.1 LIPIDS

In this section we discuss microchip electrophoresis methods for assaying different classes of lipids including lipoproteins, cholesterol and phospholipid biomarkers. Lipoproteins are macromolecular complexes of lipids and globular proteins held together by hydrophobic and electrostatic interactions. Lipoproteins are involved in transport of lipids; and atherosclerosis, coronary heart disease, liver dysfunction and cancer are all associated with lipoproteins [26-29]. These complexes can be classified as high density lipoprotein (HDL), low density lipoprotein (LDL) or very low density lipoprotein (VLDL). Common methods of lipoprotein analysis are ultracentrifugation, specific enzyme assay, nuclear magnetic resonance, and size exclusion chromatography [30-32].

Cholesterol is an important marker for assessing risk for cardiovascular conditions such as atherosclerosis and hypertension. Total cholesterol detection using microchip electrophoresis in PDMS devices was reported by Ruecha et al. [33] Amperometric detection was used to quantify the total cholesterol by monitoring the reaction of cholesterol with ChOx. Separation and detection of cholesterol and an interfering species (ascorbic acid) using microchip electrophoresis resulted in reliable quantitation. The detection limit for cholesterol in serum without sample preparation was 1 nM, well below clinical levels, and the limit of quantitation was 1 μ M. Sample stacking was achieved by diluting serum in water instead of separation buffer, and analysis took less than 100 seconds.

Fractionation into subclasses of lipoproteins can give a better understanding of the physiological condition of a patient. Wang et al. [34] described microchip electrophoresis separation of two subclasses (large buoyant LDL and small, dense LDL) using a PDMS/glass microfluidic device. Optimum separation was achieved by modifying the channel surface with β -D-maltoside and adding hydroxypropyl cellulose in the running buffer. This combination of surface modification and sieving matrix enabled separation of fluorescently labeled HDL and the two LDL subclasses within 3 minutes. The optimized separation conditions were tested for the analysis of serum of patient before and after treatment with atorvastatin. The results showed a significant decrease in the quantity of short, dense LDL after treatment, indicating a possible prognostic application of microchip electrophoresis.

Phospholipids are susceptible to reactive oxygen species (ROS); indeed, peroxidation of phospholipid arachidonyl residues by ROS generates prostaglandins, a complex group of biomarkers found in various biofluids [35]. Isoprostanes, a subset of prostaglandins, are a ROS indicator in cardiovascular disease [36]. Because lipids tend to aggregate in aqueous solutions typically used for electrophoretic separations, Gibson and Bohn [37] described a method for characterization of lipids using non-aqueous microchip electrophoresis that could be applied to lipid biomarker detection. Figure 5A shows fluorescence images of the different voltage configurations of the device. They observed that the separation of 3 model lipids depended on the voltage and timing of the injection pulse, the background electrolyte concentration, and the electric field strength. Separation of lipid mixtures was achieved with high resolution (>5) and theoretical plate counts in excess of 7.7×10^6 plates/m (Figure 5B).

3.2 CARBOHYDRATES

Glycosylation, the addition of a carbohydrate moiety, is a post-translational modification that affects the function of proteins; for example, carbohydrates expressed on the surface of cells vary in structural complexity. Understanding the significance and the function of these modifications requires robust methods for their characterization and quantitation. The most common method of analysis of glycoproteins is by mass spectrometry. Glycoproteins are often treated enzymatically to release the polysaccharides, which can be further cleaved into monosaccharides [38,39]. Mass spectrometry is a powerful tool for carbohydrate analysis but is less well suited for point-of-care diagnostics. Microchip electrophoresis offers simplified instrumentation for analysis of carbohydrates, making it an excellent fit with point-of-care assays.

Mitra et al. [40] described electrophoretic analysis of N-glycans from RNase B and glycoproteins in the blood sera of an ovarian cancer patient and a disease-free individual using glass microchips with asymmetrically tapered turns that reduced dispersion (Figure 6A). The plate count was 940,000 for a 36-cm channel and 600,000 for a 22-cm channel. The microchip devices were used to analyze both native and desialylated N-glycans. Isomer separation of mannose 7 and mannose 8 was observed in the longer (36 cm) but not in the shorter (22 cm) channels (Figure 6B). The asymmetrically tapered turn geometry improved over the spiral geometry reported earlier by Zhuang et al. [41] for separation of N-glycans that resulted in 400,000 to 655,000 plates. In both designs, increasing the electric field from 75 V/cm to 1250 V/cm resulted in an increase in resolution, as well as a reduction of the analysis time.

Monosaccharides with similar structure, molecular weight and electric charge are difficult to separate. Nagata et al. [42] demonstrated the use of a discontinuous pH buffer system for the separation of neutral and amino monosaccharides. The sensitivity was improved by a factor of 65 compared to results in a continuous buffer system.

3.3 NUCLEIC ACIDS

DNA and RNA analyses are important in disease diagnosis, forensics, and drug screening. Traditionally, fragments of nucleic acids are amplified through PCR and then analyzed. Reagents used in these processes can be expensive; therefore miniaturization of nucleic acid analysis is beneficial for clinical diagnostics. Sample preparation, PCR and electrophoresis can also be integrated using microfluidics resulting in a real lab on a chip.

Clinical genotyping is becoming important especially with the paradigm shift toward personalized medicine [43]. For example, warfarin is a common oral anticoagulant medication that is one of the top 20 most commonly prescribed medications in the US; however, the dosage of this drug to obtain a therapeutic effect varies among individuals [44]. Proper dosage is vital because an incorrect dosage can have adverse effects and in extreme cases be fatal. Genotyping for warfarin dosage using a single PCR reaction and subsequent microchip electrophoresis was reported by Poe et al. [45] By using microchip PCR, the amplification time was reduced to 30 minutes from 45 minutes with benchtop PCR, and the complete assay, combining PCR with microchip electrophoresis, took only 60 minutes. The assay determined the patient's genotype using a tetraprimer amplification refractory mutation system in a single reaction (Figure 7A-B) and allowed rapid typing of over 30 samples (Figure 7C). This reduction in reagent and sample consumption using microfluidics could enable low-cost genotyping for warfarin and other genotype-sensitive medications.

Multiplex PCR is commonly used to quickly detect aberrations in DNA sequences of large genes, such as short tandem repeat DNA sequences that have a repetitive unit of 1-6 base pairs and can be used to study polymorphisms or mutation rates important for forensic analysis and identification of genetic diseases [46]. Conventionally, PCR products from multiplex PCR are probed using a Southern blot that can take several hours to yield results. Also, in some cases quantitative analysis of the PCR products is necessary for a diagnosis, which is difficult to achieve via Southern blotting. Le Roux et al. [47] developed a prototype

of an integrated microchip for multiplex PCR amplification and electrophoresis. They showed baseline separation of a 10 allele ladder with comparable separation results to a conventional benchtop PCR system. The valveless plastic microchip devices they used had an effective separation length of 7 cm. Under optimized separation conditions they were able to discriminate short tandem repeats having two-base or single-base differences. They also showed consistency of results over time and even when devices were transferred from one laboratory to another. These results suggest the possibility of using portable integrated PCR-microchip electrophoresis devices without the need to calibrate before use. These integrated microchips for multiplex PCR could be used in clinical analysis or personalized medicine.

3.4 PROTEINS

Enzyme linked immunosorbent assay and western blot analysis are commonly used methods as serological tests for different diseases such as HIV and hepatitis B. However, these methods take hours and require multiple processing steps. Furthermore, traditional on-plate assay or membrane analysis requires reagents that increase the cost. In western blot analysis, membrane processing is a slow step that may take at least one hour. An integrated microchip electrophoresis system with western blotting developed by Jin et al. [48] reduced the time to 22-32 min for a complete immunoassay. Figure 8A shows a schematic of the microchip electrophoresis device interfaced with a blotting membrane. They tested this system on a number of proteins, including actin, carbonic anhydrase and lysozyme. Proteins were detected at concentrations as low as 2.4 nM (Figure 8B). Furthermore, with an increase to 9 samples in one membrane, the analysis time decreased to 6 min/sample. Integrating multiple channels in the microfluidic devices could result in still higher throughput analyses.

Single-cell protein analysis is an important step to elucidate cell signaling and determine cell heterogeneity, for example in drug discovery and cancer studies [49]. Hughes et al. [50] demonstrated single-cell western blotting in microfluidic devices made from a microscope slide coated with a thin, photoactive polyacrylamide gel. Cell lysis and western blotting were performed in situ. Proteins released from lysed neural stem cells were electrophoresed and immobilized on the polyacrylamide gel before probing with fluorescent antibodies for multiple differentiation markers. Kang et al. [51] further tested the application of these devices to study the heterogeneity of cells within a tumor and assess the response of tumor cells to a chemotherapeutic drug. The whole system consisted of single cell imaging, cell lysis, polyacrylamide gel electrophoresis, UV crosslinking of proteins onto the gel and target probing with fluorescent antibodies. This system allowed correlation of cell phenotype to proteomic profile. They tested this set-up to study drug resistance of a glioblastoma cell line and were able to analyze up to 30 cells/min. This approach may become a valuable tool to identify small populations of drug resistant cells within a tumor that are currently difficult to identify using conventional methods.

One of the agreed upon hallmarks of etiology of Alzheimer's disease is the accumulation of β -amyloid peptides in amyloid plaques found in cerebrospinal fluid (CSF), blood and urine [52]. However, detection of these peptides in blood and urine is more difficult because their concentrations are 10-100 times smaller than in CSF (1 to 10 ng/mL). Mohamadi et al. [53]

described a two-step microchip analysis of five different β -amyloid peptides from CSF in PDMS devices. To enrich the target analytes, they performed immunocapture in a microchip and labeled the eluted analytes with fluorescent dye. They found that β -amyloid 42 could be detected by microchip electrophoresis, but baseline separation of the five β -amyloids was not successfully achieved in purified samples or CSF samples. However, analysis of markers in CSF was obtained within 150 seconds. They also found that preconcentration of CSF was a necessary step to be able to detect the amyloid peptides. This detection method shows a promising application of microchip electrophoresis in detecting Alzheimer's disease.

Haptoglobin (Hp) binds to hemoglobin released by dead red blood cells, and this complex is then transported to the liver for recycling. Thus, Hp testing is often done to determine how fast red blood cells are destroyed. Common methods for detecting haptoglobin phenotypes are based on electrophoretic separation using a polyacrylamide gel, but this method requires both separation and staining for detection [54]. Different Hp phenotypes have recently been shown to have association with major diseases: the Hp 2-2 phenotype is correlated with higher risk of developing refractory hypertension and schizophrenia [55,56]; in contrast, Hp 2-2 appears to have protective benefit while Hp 1-1 increases the risk for cervical cancer. Huang et al. [57] tested microchip electrophoresis for Hp phenotyping and showed that fluorescently labeled Hp from a liver cancer patient's serum had lower Hp 2-2 levels compared to normal serum. Hp was only clearly detected in serum upon depletion of the abundant human serum albumin and immunoglobulins. The analysis of processed serum took only 150 s by microchip electrophoresis, while 400 s were needed for conventional capillary electrophoresis. Low cost, short separation time and sufficient sensitivity are the advantages of microchip electrophoresis for the detection of diseases associated with haptoglobin.

In clinical diagnostics, selective detection of target biomarkers in complex biological samples is necessary for an accurate diagnosis. Often, immunoaffinity columns are utilized to purify and concentrate biomarkers, especially for low abundance ones [58]. Biological samples such as CSF and tissue biopsies are typically obtained only in limited amounts and also require sample preparation before analysis. Thus, developing microfluidic analysis of quantity-limited samples would be advantageous.

As one example, dermatological diseases like atopic dermatitis may require lesion biopsy and immunochemical analysis to determine if the lesion is caused by infiltrating neutrophils leading to clinical inflammation or by infiltrating T-cells suggesting prolonged associated inflammation. Kalish et al. [59] analyzed 6 μ m diameter frozen biopsy sections from atopic dermatitis lesions for the presence of 5 chemokines by using an integrated microchip immunoaffinity capillary electrophoresis system. Only 200 nL of tissue homogenate with a total protein concentration of 1 μ g/mL was used for analysis. The immunoaffinity disks allowed specific extraction of biomarkers and the succeeding microchip electrophoresis separation enabled quantification and confirmation of specificity. Quantifying the chemokines from the biopsy allowed the two types of clinically relevant allergic skin lesions to be distinguished. This method is advantageous over traditional immunoassays, because it uses a small sample volume so it is well suited for detection of disease biomarkers from skin

biopsies. The integrated device demonstrates the promise of analyzing complex tissue homogenates.

3.5 CONCLUSIONS

Recent advances in microchip electrophoresis showcase exciting results in the analysis of various biomolecules with potential clinical application. Advantages such as fast analysis, low sample consumption, sufficient sensitivity for on-site analysis and low cost make microchip electrophoresis a promising platform for point-of-care diagnostics. Microchip electrophoresis is an attractive alternative to traditional methods such as ultracentrifugation for lipid determination. Furthermore, microchip electrophoresis of carbohydrates is enabling for the study of disease-linked glycosylation, with use of simplified instrumentation that is applicable for the point of care. Integration of microchip electrophoresis technology with on-chip PCR shows great potential for genotyping applications in the emerging field of personalized medicine. Finally, microchip electrophoresis has been combined with several common biochemical analysis methods such as western blot and immunoaffinity. Protein biomarkers from varied and complex specimens can be analyzed, with reduced sample and reagent consumption, which should streamline clinical sample analyses.

4. PROSPECTS

Microfluidics as a clinical diagnostic tool has great potential to improve healthcare by providing fast, small-volume, and sensitive bio-analysis at the point of care. This accurate and timely information can help to monitor disease onset and prognosis, thus facilitating therapeutic interventions. Microfluidics can also be used in developing and testing therapeutic molecules such as disease-related antibodies that can also help in patient treatment.

Microchip electrophoresis has been demonstrated to be applicable for analysis of different biomarkers including lipids, carbohydrates, nucleic acids and proteins. The various materials available for fabricating microfluidic devices make it possible to use aqueous or non-aqueous buffers suitable for analysis of different biomarkers. Interfacing with different detection systems also expands the versatility and sensitivity of microchip electrophoresis for various biomarkers. Finally, the relatively simple fabrication of these devices makes it easy to design layouts that can be adapted to various analyses.

Integration of multiple processes in a single microfluidic device offers totally automated sample analysis that may be applicable for clinical laboratories and point-of-care diagnostics. Recognition of the benefits offered by this technology no doubt played a key role in the development of commercial microchip electrophoresis platforms offered by companies such as Agilent, BioRad and Bio-Techne. However, these commercial systems are still expensive and benchtop, rather than point of care. Furthermore, validation of assays for clinically relevant biomarkers is needed for this technology to be widely used in clinical laboratories or point-of-care diagnostics.

The study and modification of the surface chemistry of materials used for microfluidic devices should improve control of the physical phenomena that affect fluidic interactions

and lead to more reproducible and predictable results. Further characterization of the effects of device layouts and engineering designs should shed additional light on the effects of channel dimensions and geometry on fluid dynamics and assays. Advances in fabrication methods can further decrease the channel dimensions and ease the integration of processes and electronics needed for automated analysis. Miniaturization and improved packaging of detection systems such as optical and electrochemical, along with data analysis techniques, would move a step closer to a complete lab-on-a-chip system for in-field applications. The implementation of 3D printing techniques for microfluidic systems and packaging should further reduce device cost and manufacturing time. Such advances should lead to low-cost, portable, sensitive and fast tools for analyzing clinical biomarkers from biological fluids or biopsies.

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Figure 1. Paper microfluidics for analyte detection. (A) Schematics (left) and photograph (right) of the graphene nano-ink biosensor arrays on paper microfluidics. (B) The biosensing mechanism. Color scheme is: red - graphene; yellow - silver paste; blue - paper; grey - microfluidic channels; light green - enzyme; dark green - analyte. Adapted with permission from reference [13].

Figure 2.

Schematic for the fabrication of a paper microfluidic device. (A) Cross-sectional view of the filter paper substrate. (B) Filter paper mask for pattern defining. (C) Paper mask incubated with the trimethoxyoctadecylsilane (TMOS) solution. (D) Substrate filter paper sandwiched between glass slides and the TMOS treated paper mask. (E) Assembled structure after thermal treatment. (F) Final device with hydrophilic–hydrophobic contrast. (G) Device photograph (coated with water to distinguish channels from the background). Adapted with permission from reference [17].

Figure 3. Analysis of H1N1 virus on a single microfluidic chip platform. (A) Device with three fluid handling parts: fluidic reagent input (left), waste (center), and DNA product output (right). Sample preparation chamber performs analyte capture, RT-PCR, and ssDNA generation, and the electrochemical DNA detection cell performs the sensing and quantitation. (B) Throat swab sample is inserted in a RNA stabilizer tube and combined with virus and antibody-coated magnetic beads. (C) External magnets direct sample to the sample preparation chamber. (D) RT-PCR reagents are injected. (E) Thermal treatment is performed. (F, G) On-chip RT-PCR is performed, and then single-stranded DNA is generated. (H) The product is transported to the DNA detection cell for hybridization, and

the subsequent detection signal is measured using AC voltammetry. Adapted with permission from reference [21].

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Figure 4.

Design and operation of a micro-Hall detector (μ HD). (A) Magnetic nanoparticle labeled cells acquire a magnetic moment proportional to the expression of the targeted biomarker. This magnetic moment is measured via the Hall voltage (V_H) of the μ HD sensors. (B) Capacitively coupled amplifiers measure V_H . (C) A μ HD device contains an array of sensors on GaAs, topped by a PDMS layer with a microfluidic channel (left). Inset is a photograph of the actual device. The sensor (center) has eight staggered elements to detect passing cells in the flow channel (marked by dotted lines). Hydrodynamic focusing (right) helps in lateral positioning of cells to the middle of the channel, and the chevron pattern pushes cells vertically toward the bottom of the channel. Adapted with the permission from reference [25].

Figure 5.

Microchip electrophoresis of model lipids. (A) Device operation in a buffer system composed of a tetraalkylammonium salt in N-methylformamide. (B) Electropherograms of model lipids (1) 1-hexanoyl-2-[6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-hexanoyl]-*sn*-glycero-3-phosphate (ammonium salt), (2) 1-hexanoyl-2-[6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-hexanoyl]-*sn*-glycero-3-[phospho-*mc*-(1-glycerol)] (ammonium salt), and (3) [N-[(7-nitro-2,1,3-benzoxadiazol-4-yl)-methyl]amino-palmitoyl coenzyme A (ammonium salt). Adapted with permission from reference [37].

A

B

Figure 6. Microchip electrophoresis of N-glycans. (A) Device showing asymmetrically tapered turns. (B) Electropherogram of N-glycans from serum samples of a control individual and ovarian cancer patients. Adapted with permission from reference [40].

Figure 7. Microchip electrophoresis for genotype determination. Representative electropherograms for the (A) *1*2/AB and (B) *1*1/AB genotypes. (C) Microchip data showing the different genotypes of 35 samples that matched 100% with results obtained from other validated methods. Adapted with permission from reference [45].

Figure 8. Microchip electrophoresis integrated with protein capture membrane. (A) Schematic of microchip electrophoresis integrated with western blotting. (B) Separation and blotting results of ladder, actin, carbonic anhydrase and lysozyme. Adapted with permission from reference [48].