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Application of Micro-Fluidic Devices for Biomarker Analysis in Human Biological Fluids

Heather Kalish
National Institute of Biomedical Imaging and Bioengineering,
National Institutes of Health
USA

1. Introduction

The current interest in microanalysis has heightened over the past years with the development of capillary electrophoresis (CE) followed by the development of commercially available micro-fluidic devices such as micro -mixers, CE chips and micro-reaction vessels or plates. This has made basic micro-fluidic analysis more readily available and has extended their use to biomedical analysis, especially clinically relevant biomarkers and field studies. Here the advantages of such devices are their relative speed of analysis, lower reagent costs, smaller sample requirements, and the potential for high-throughput. These factors become important when special situations arise such as the analysis of precious, archival, or field samples, monitoring surgical procedures, assessing newborns, analyzing specific areas from biopsy materials, measuring the functional aspects of single cells isolated from biological fluids or monitoring contamination of environmental factors.

The combination of antibody-mediated isolation techniques with micro - and nano-scale electrokinetic separations has great potential for analyzing defined analytes in complex biological matrices. In chip-based formats, such systems can recover and measure up to 25 analytes in a reasonably rapid time frame. Further, such devices require sub-microlitre amounts of sample to perform the analyses. Coupling these devices to laser-induced fluorescence greatly enhances the sensitivity of the analysis allowing certain analytes such as protein, peptides, and toxins to be measured in the sub-picogram/mL range. Further, coupling micro-analysis to mass spectrometry adds in the characterization of many significant biomarkers. The combination of fast binding, bio-engineered antibodies requiring relatively short reaction time with rapid desorption and electrophoretic separation with on-line detection can make the analysis almost "real-time".

Today, chip-based analyses are performed on a variety of devices ranging from simple micro-sample plates, to micro-mixers, and chip-based CE, many of which are commercially available. However, more complicated devices such as the "lab-on-a-chip" still require intricate design and specialized facilities. These latter devices hold the potential for automation and involve procedures that utilize both chromatographic and electrophoretic driving mechanisms. Additionally, a lab-on-a-chip can involve the integration of hyphenated techniques in order to achieve the desired analysis, including the integration of a highly sensitive detection system capable of measurement in the femtomolar or attomolar range. The need for such sensitivity often arises from the extremely small sample size obtained for the analysis.

Current work in the literature has focused on the development of micro -fluidic devices for measuring important biomarkers in a number of bio-medically important areas, ranging from the assessment of head trauma patients, assessing the immune status of newborns, especially those at risk from intra-uterine infections and inflammation to exposure to toxic or environmental factors. A biomarker may be defined as "a characteristic, which is objectively measured and evaluated as an indicator of a normal or a pathogenic biological process or even a pharmacological response to a therapeutic intervention." (Atkinson, et al. 2001) This chapter will be a review of current technologies and methodologies in the field of micro-fluidic devices, their application to biomarker analysis and current challenges facing the development of new technologies.

2. Capillary electrophoresis

Capillary electrophoresis is considered one of the analytical tools that started the field of microfluidics. There have been several recent reviews written on the technique and it's applications in numerous research areas. (Siminonato et al., 2010; Ryan et al., 2010; El Rassi, 2010; Mikus & Maráková, 2009) The term capillary electrophoresis is a broad term used to refer to a variety of techniques that exploit the application of a voltage across a capillary to achieve separation of analytes. CE systems are both lab built and commercially available from numerous companies and can be coupled to a wide variety of detectors, such as mass spectrometry (MS), UV/Vis and laser induced fluorescence (LIF). However, improvements in separation media, sample preparation and detection still need to be overcome if CE is to realize its full potential in analytical research. (El Rassi, 2010)

The simplest form of CE is capillary zone electrophoresis, CZE, which separates analytes based on their charge-to-size ratio. (Kalish & Phillips, 2009) Traditional gel electrophoresis has been modified and adapted to a capillary in capillary gel electrophoresis (CGE). This technique is used when analytes that have similar charge to mass ratios need to be separated, based on just their size. (Holovics et al., 2010) The technique of choice when studying protein mixtures is capillary isoelectric focusing (CIEF). Over the past 20 years CIEF has proven to be a fast, high resolution, pI-based technique for the separation of amphoteric compounds, e.g. proteins and peptides. (Silvertand et al., 2009) Micellar microemulsion electrokinetic chromatography (MEEKC) is a mode of CE, which utilizes microemulsions as separation media and allows for separation of neutral as well as charged analytes. (Ryan et al., 2010) Capillary electrochromatography is a hybrid of CE and high performance liquid chromatography, which provides both selectivity and efficiency. (Suntornsuk, 2010) Using capillary isotachophoresis (cITP), sample components are separated based on their electrophoretic mobilities and can be concentrated 2-3 orders of magnitude. (Korir et al., 2006)

CE can be used with numerous different detection instruments, each with their advantages and disadvantages. Laser induced fluorescence (LIF), UV/Vis and mass spectrometry (MS) are the three most common instruments used for detection; however, electrochemical detection, nuclear magnetic resonance (NMR) and conductivity has been used as well. LIF is a very common method of detecting analytes separated by CE. It has a large range of sensitivity and is fairly easy to operate. However analytes need to be pre-labeled either before injection or on the column. UV/Vis requires no prior workup of the compounds of interest and is one of the most popular and useful detectors. (Olędzka et al., 2009) However, analytes must possess natural chromophores that absorb in the UV/VIS region and the limits of detection for UV/Vis are often higher than other detection methods. Both LIF and

UV/Vis allow for identification of compounds only if standards of each analyte have been previously characterized individually. Electrochemical detection offers excellent selectivity and sensitivity and the ability to modify microelectrodes to gain further selectivity for targeted analysis. (Mukherjee & Kirchhoff, 2009) Capacitively coupled contactless conductivity detection (C^4D) offers further acceleration and simplification of CE analyses. It detects analytes in their native state and does not require time-consuming sample derivatization. (Tuma, et al. 2010)

In order to identify new compounds or elucidate structural information, MS or NMR detectors must be used in conjunction with CE separation. MS is the detector of choice when trying to identify unknown compounds. CE-MS can be used as a fully automated high-throughput, high–resolution, and highly reproducible system for the analysis of clinical samples. (Kaiser et al. 2004) A detection method that is rarely used in combination with CE but offers superb specificity is NMR, as seen in Figure 1. Besides providing powerful structural information, NMR has the capability to reveal dynamic information useful in understanding various processes such as diffusion and binding. (Korir et al., 2006) All of these detection methods offer a range of sensitivity, and can be used in conjunction with both traditional bench top CE and microchip CE systems. The challenge to overcome with any of the detectors is effectively coupling the detector to the CE system.

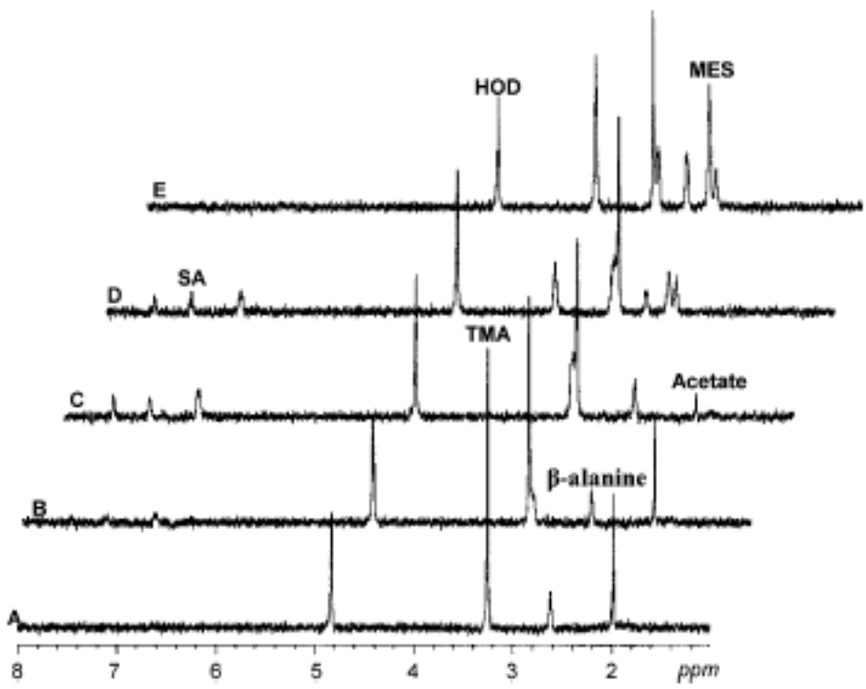

Fig. 1. Profile of the migration of ions in the course of an anionic cITP-NMR experiment. The buffers used were 160 mM DCl/80 mM β-alanine/20 mM TMA acetate (LE) and 160 mM MES (TE). The analyte is 250 μM salicylate. Spectrum A contains the resonances of the LE only (TMA acetate and β-alanine). In spectrum B, the salicylate resonances (SA) begin to emerge in the aromatic region of the spectrum and become more intense in spectra C and D. The TE resonances (MES) begin to emerge in spectrum D, becoming more intense in E. Note that the acetate resonances are detected only up to spectrum C. Reprinted with permission from *Analytical Chemistry,* 2006, 78, 7078-7087. Copyright 2006 American Chemical Society.

3. Microchip Capillary Electrophoresis

Further miniaturization of CE has placed the entire process on a microchip. Micro-CE has all the benefits of traditional CE and further lends itself towards portability and automation. Microchips for CE have been made out of glass, PDMS, polymers and even plastic, which means they are disposable. One dilemma to overcome is that chips need to be onetime use only, but at the same time have to provide all the steps necessary for complex analysis. Analysis of physiological fluids and tissues using microfluidic devices presents a special challenge, both in terms of sensitivity and fouling of microchannels by matrix components. (Coyler et al., 1997)

Microchips have been made with various configurations of channels, allowing for mixing, labeling, separation and detection all within the chip. Perhaps one of the most important factors in the successful resolution of any compound mixture is the design of the chip. (Kalish & Phillips, 2009) Obstacles to overcome in chip design include reproducibility of injection volume, separation length which can be increased by moving from straight channels to meandering ones as seen in Figure 2, and delivery of the analytes to the detector. Sample volume on a microchip ranges in the order of nanoliters to picoliters, so the successful resolution and detection of the isolated compounds remains one of the largest obstacles in the field of micro- CE.

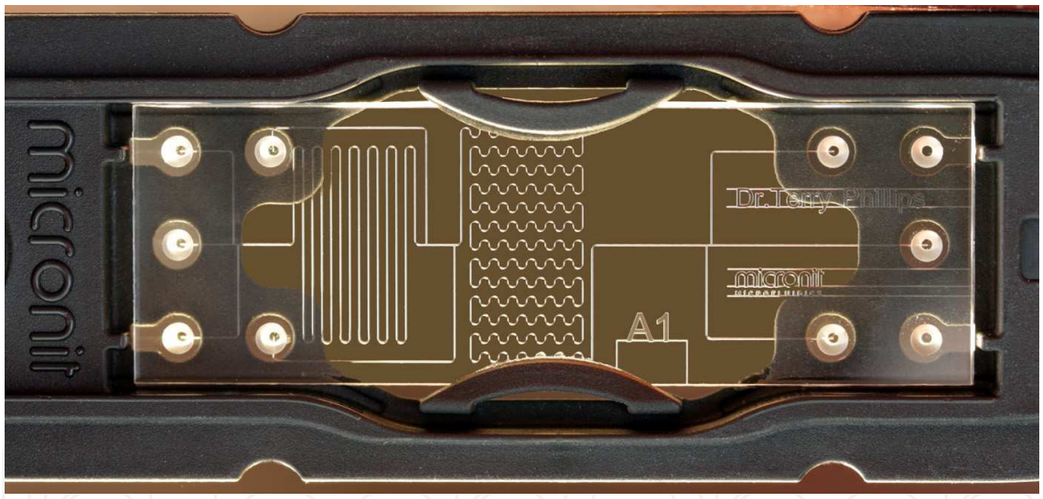

Fig. 2. A Micronit microchip (Netherlands) with a serpentine channel for longer separation and a wavy channel to allow for sample mixing.

Microchips which can incorporate the purification and pre-concentration of samples are becoming more common place as the move towards systems that can be used in point-of-care settings gains momentum. Many processing steps including desalting, labeling and extraction have been successfully performed in microchip systems. (Yang et al., 2010) The integration of an affinity column and capillary electrophoresis channels within a microdevice for the isolation and quantitation of a panel of proteins has been demonstrated by Yang et al. (Yang et al., 2010) Using this microdevice, it was possible to selectively extract and analyze four proteins in spiked human blood and the system has the potential to be expanded to 30 biomarkers using additional antibodies in the affinity columns. This device is but one example of numerous new micro-fluidic devices that incorporate multiple analytical steps onto a microchip platform.

Improvements will be required in detectability, reproducibility, and ease of fabrication, together with integration of different functional operations, to enable Micro-CE for protein separation to provide comprehensive solutions for applications in the fields of proteomics, glycomics, and biomarker detection for diagnosis. (Tran et al., 2010)

4. Immunoaffinity Capillary Electrophoresis (ICE)

Immunoaffinity techniques use immune complexes to capture specific analytes from complex samples, such as human blood or serum, and then use CE to separate and detect the analytes. Sensitivity is greatly enhanced by this technique as the signal to noise ratio for the analytes is greater. Additionally small samples can be reused over and over as analytes of interest are withdrawn and the remaining sample can be recycled. Derivatizing the capillary with antibodies to allow for the selective capture and analysis of specific analytes makes ICE a very practical analytical technique. (Kalish & Phillips, (b) 2009) This technique can allow for the simultaneous measurement of numerous analytes with little sample pre-treatment and fairly small increases in overall analysis time.

Antibodies can also be immobilized to substances other than capillary walls to carry out immunoaffinity capture. Using immobilized recombinant cytokine receptors, Phillips modified the ICE technique to measure only bioactive cytokines in skin biopsies. (Phillips et al., 2009) The immobilized cytokine receptors were bound to a silanized glass filter and were employed as pre-separation affinity selectors in order to capture only those cytokines that were bioactive at the time of biopsy. By comparing cytokines present in normal skin biopsies to cytokines in lesions in the same skin biopsies, the severity and outcome of inflammatory episodes was predicted. Magnetic beads are another solid support to which antibodies can be bound easily and used for immunoaffinity capture.

Chen and co workers covalently bound antibodies to magnetic beads and then held them in place within the capillary walls by two magnets positioned outside the capillary walls. (Chen et el., 2008)

Caulum and co-workers present an immunoaffinity- based CE assay referred to as the cleavable tag immunoassay (CTI). (Caulum et al., 2007) The technique used is similar to ICE, but rather than measure the analytes released by the antibody, a fluorescent tag is cleaved from the detection antibody and imaged, as shown in Figure 3. This technique offers an improvement in resolution over traditional ICE as the cleaved tags can be altered if resolution improvement is necessary, whereas ICE is limited to the structures of the captured analytes.

5. Sample analysis

Human biofluids that can be analyzed by CE, micro-CE and ICE include blood/plasma/serum/dried blood spots, urine, sweat, amniotic fluid, cerebral spinal fluid (CSF), saliva, and vitreous and aqueous fluids. Many biological matrices contain high concentrations of salts and proteins, both of which can cause problems in CE analysis. Thus the composition of any biological sample plays a significant role in determining the choice of which CE analytical approach to take. (Lloyd, 2008)

A. Urine

Urine is a human fluid that is non-invasive to obtain. Samples can be easily collected and usually there is an abundance of sample available. However, samples may be so dilute that preconcentration or other preparation steps may be necessary to observe analytes present in small quantities.

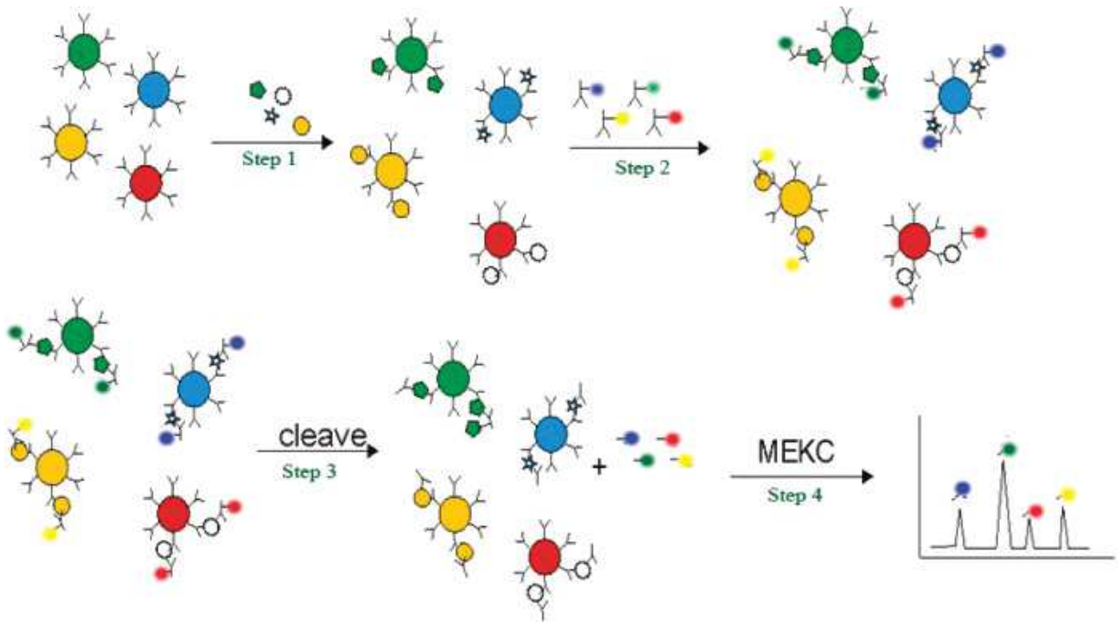

Fig. 3. CTI chemistry. Step 1: sample is added and biomarkers bind to capture antibodies immobilized on the particle surface. Step 2: detection antibodies are added. Step 3: tags are cleaved from immobilized assay. Step 4: separation and detection using MEKC with fluorescence. Reprinted with permission from (2007) *Analytical Chemistry*, 79, 14, 5249-5256. Copyright 2009 American Chemical Society.

Human urine samples from both healthy individuals and patients with various chronic kidney diseases were analyzed by CE-MS by Good, et al. to produce a peptidome analysis of naturally occurring human urinary peptides and proteins. (Good et al. 2010) The advantages of using CE-MS as a proteomic tool for profiling the peptides/proteins include the insensitivity of CE towards interfering compounds, the ability to detect both large and small highly charged molecules, and the lack of interference by precipitates.

The identification and validation of urinary biomarkers as an indicator of patients suffering from anti-neutrophil cytoplasmic antibody associated vasculitis was carried out by Haubitz and co-workers. (Haubitz et al., 2009) Using CE- coupled with MS, the group was able to identify 113 potential biomarkers and changes in these biomarkers could be observed during periods when the patients were undergoing immunosuppressive therapy. This allowed for a non-invasive kidney monitoring and potentially non-invasive diagnosis of patients with anti-neutrophil cytoplasmic antibody associated vasculitis.

Liu and co-workers were able to increase the limits of detection for human urinary proteins by tagging the proteins with gold nanoparticles, which amplifies the mass spectrometry signal, and increase the techniques overall sensitivity. (Liu et al., 2010) Changes in the cholinergic system may be indicative of neuronal degradation in diseases like Alzheimer's and related dementia.

Biomarkers of the cholinergic system are choline and acetylcholine, which Mukherjee and Kirchhoff detected and quantified using CE coupled with electrochemical detection. (Mukherjee & Kirchhoff, 2009) This sensitive system was able to detect biomarkers in the range of fmol to atmol, which far exceeds previous detection limits and makes the system particularly applicable to the detection of these neuronal biomarkers in human samples.

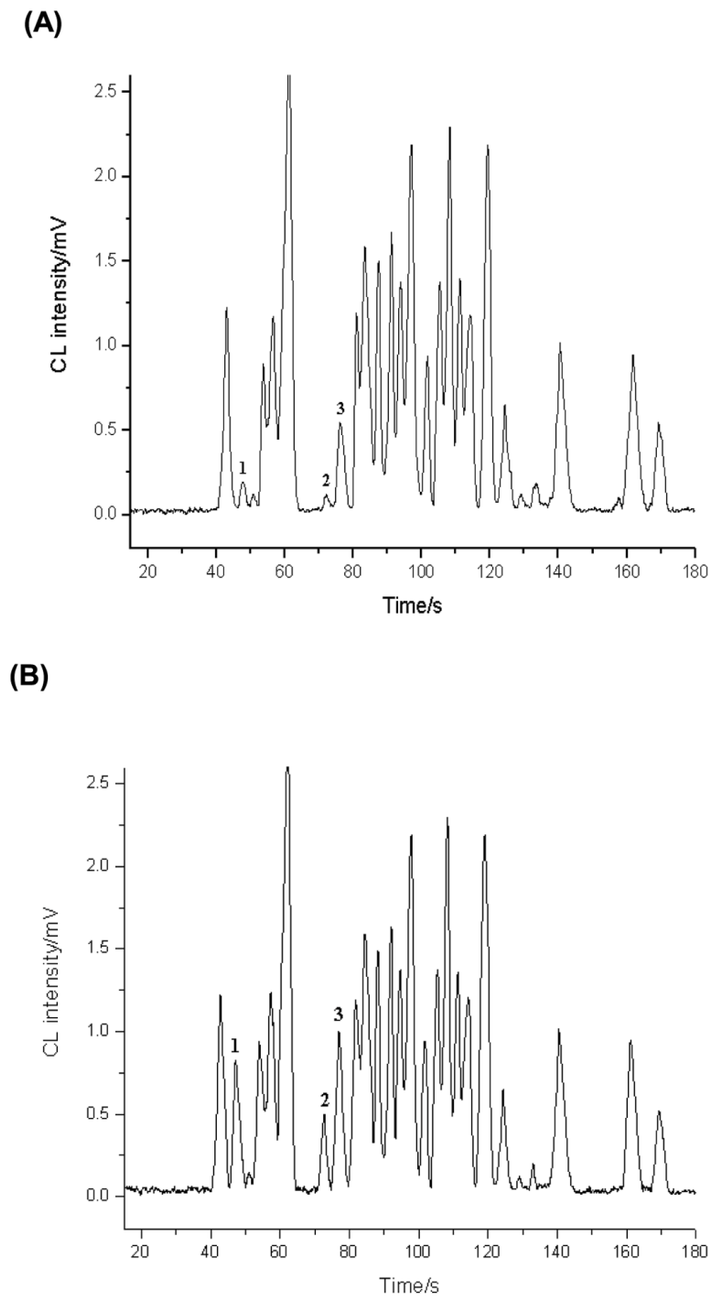

Fig. 4. Electropherograms obtained from the separation of a human urine sample (A) and the sample spiked with Agm, E and DA at 3.5 x 10^{-6} M each (B). The experimental conditions were: Electrophoretic electrolyte was 20 mM phosphate buffer (pH 10.0) containing 10 µM HRP and 25 mM SDS. The oxidizer solution was 20 mm phosphate buffer (pH 11.0) containing 110 mM H_2O_2. Peaks: 1. Agm(Agmatine); 2. E (epinephrine); 3. DA (dopamine). 'Reprinted from Journal of Chromatography A, 1216, Zhao, S.; Huang, Y.; Shi, M. & Liu, Y.M. Quantification of biogenic amines by microchip electrophoresis with chemiluminescence detection. 5155-5159, Copyright 2009, with permission from Elsevier.

The measurement of free cortisol in urine was carried out by Olędzka et al. (Olędzka et al., 2010) Using a solid phase extraction (SPE)- coupled MEKC with UV detection, free cortisol was detected and quantified with a limit of quantification in the 5 ng/mL range. This non-invasive measurement of cortisol was fast, precise and detected changes due to stress situations.
Biogenic amines are naturally formed by the enzymatic decarboxylation of natural amino acids, however certain levels have been shown to promote adverse effects on human health. Using micro- CE coupled with chemiluminescence detection, Zhao et al. were able to quantify biogenic amines in human urine samples, as seen in Figure 4. (Zhao et al., 2009) By pre-labeling the samples, the assay sensitivity was increased and three biogenic amines were able to be identified in human urine samples.

B. Saliva

Saliva is another non-invasive biofluid that can be used to investigate biomarkers. It is readily obtained, constantly reproduced by patients and produced in sufficient quantities for analysis. For patients, the non-invasive collection method of oral fluid sampling reduces anxiety and discomfort. However, the sample matrix is more heterogeneous, and because of the low levels of salivary biomarkers, it sometimes becomes difficult to distinguish between background and target- specific signal in these low concentration samples. (Jokerst et al., 2009)
Saliva from both healthy controls and patients suffering from oral, breast and pancreatic cancers were collected by Sugimoto et al. (Sugimoto et al. 2010) and analyzed by CE-MS to develop a metabolic profile specific to each of the diseases. The samples were used without pretreatment other than centrifugation to remove any solid particles and dilution of the cancer patient samples, due to high electrolyte content.
A panel of 28 biogenic amino acids (AA) were separated and identified by Tůma and co-workers. (Tůma et al., 2010) Using a minimum capillary length on a bench top CE with C^4D detection, a decrease in analysis time and an increase in sensitivity resulted in the identification of 23 of the 28 amino acids in saliva. The decreased separation times and low limits of detection make it applicable to analysis of a variety of human biofluids.
Amino acids were also separated and analyzed from human saliva by Jiang et al. (Jiang et al., 2009) Using copper ions in the running media and an online sweeping enrichment technique, pictured in Figure 5, two of the most prevalent amino acids in human saliva were separated and identified using a CE with UV detection and no sample pretreatment.
A third examination of amino acid neurotransmitters present in human saliva samples was done by Deng and co workers. (Deng et al., 2008) N-Hydroxysuccinimidyl fluorescein-O-acetate, a fluorescein-based dye, was used to derivatize saliva that was centrifuged and diluted with water. Six analytes were recovered from native and spiked saliva samples using CE-LIF to perform the separation and detection steps.
Bradykinin, a vasoactive nonapeptide, and its metabolites were identified using CE-LIF by Chen et al. (Chen et al., 2009) Using transient isotachophoresis preconcentration, 3 bradykinin metabolites were recovered with close to 90% recovery rates from saliva of both a healthy female and a male suffering from hypertension and coronary disease. The method was also applied to human plasma, which showed similar recovery rates of 2 bradykinin metabolites.
The presence of four hormones in saliva was evaluated by Wellner and Kalish using a standard double T microchip. (Wellner & Kalish, 2008) By placing disposable immunoaffinity disc into the sample port, 4 hormones were removed and concentrated from human urine. Samples were compared with no pretreatment and pretreatment cleanup and results indicated that urine analysis yielded false positives when no pretreatment was preformed.

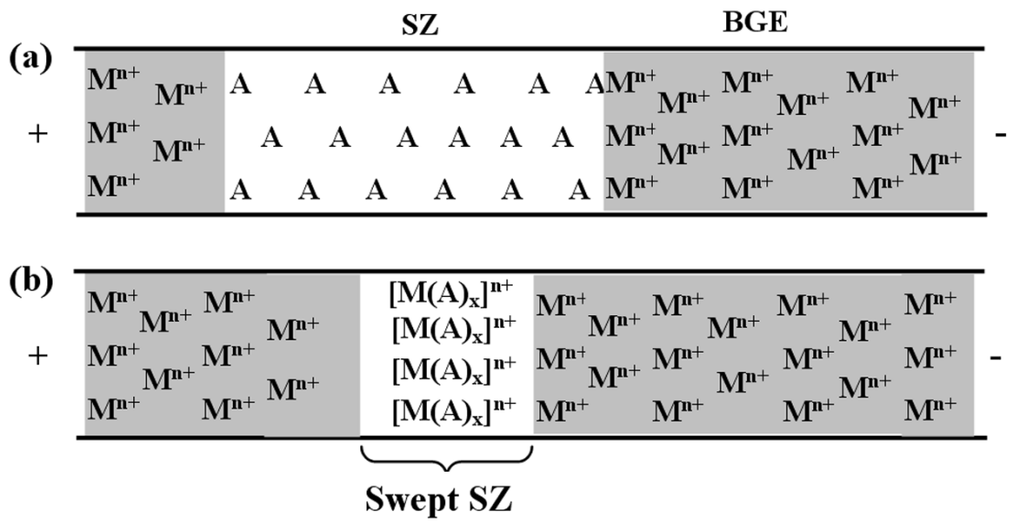

Fig. 5. Schematic diagram of coordination sweeping. (a) is the situation before voltage applied, and (b) is the situation of sweeping after voltage applied. SZ, sample zone; BGS, background solution. "A" represents the free analytes, amino acids, and "M^{n+}" represents transition ions contained in the CE running medium, copper ions. Jiang, X.; Xia, Z.; Wei, W. & Gou, Q. (2009) Direct UV detection of underivatized amino acids using capillary electrophoresis with online sweeping enrichment. *Journal of Separation Science,* 32, 11, 1927-1933. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

C. Cerebrospinal fluid

CSF is contained primarily in the spinal canal, the ventricles of the brain and in the subarachnoid space at a total volume of about 125-150 mL. With a low protein concentration compared to other biofluids, CSF may be considered more analysis friendly matrix than plasma. (Lloyd, 2008) However, due to the invasive nature of sampling this fluid, analyses are limited.

Steinberg et al. used CE to investigate the role of nitric oxide (NO) in cluster headaches, by measuring their oxidation products, nitrite and nitrate in CSF. (Steinberg et al., 2010) The CSF samples were able to be processed with little pre analytical cleanup and provided a sample that was closer to the areas affected by the disease then previously studied plasma samples. Their results found that NO appears to be involved in pathology of cluster headaches, but increased levels of NO do not appear to directly promote them.

The use of CE to separate and quantitate five amyloid peptides considered as potential biomarkers of Alzheimer's disease was undertaken by Verpillot, et al. (Verpillot et al., 2008) A novel CZE method using a dynamic coating sufficiently separated the five amyloid peptides with minimal adsorption by the capillary wall. However the technique was not sensitive enough with UV detection to measure peptides directly from CSF without preconcentration.

Using noncovalently coated capillaries, Ramautar et al. were able to use CE-UV and CE-MS to analyze CSF for organic acids. (Ramautar et al., 2008) The CE-MS system was able to distinguish the metabolic profile of a healthy individual from the metabolic profile of an individual suffering from complex regional pain syndrome. However, a large set of samples from both groups needs to be analyzed to determine conclusive differences.

In order to establish a quantitative protein profile for CSF samples from patients suffering from traumatic brain injury, Zuberovic et al. used CE coupled with MALDI-TOF. (Zuberovic et al., 2009) To minimize protein wall interaction, capillaries were coated and samples were also prelabeled with isobaric tags. A total of 43 unique proteins were identified and their concentration levels varied over time with the progression of the brain injury.

Microchip CE lends itself to CSF analysis due to the small amount of sample necessary for analysis. CSF samples collected from patients with cephalitis, brain tumors and surgical brain damage with analyzed by CE with chemiluminescence by Zhao and co workers. (Zhao et al., 2009) Three carnosine- related peptides were separated and identified using the micro CE-CL method; however the sensitivity limits were only low enough to detect one of the analytes in actual human CSF samples.

Microchip CE with LIF was used to determine the levels of D-Asp and D-Glu in human CSF samples by Huang, Shi and Zhao. (Huang et al., 2009) Samples were prederivatized with FITC to allow for detection with LIF and analysis took place in a cross T chip. While D-Asp was detected by this method, D-Glu was not. The absence might be due to the lack of D-Glu in CSF samples or amounts that are unable to be detected by the method used.

D. Blood/ Plasma/ Serum/ Dried Blood Spots

Blood is the most accessible biofluid to analyze. It is relatively non-invasive to obtain, is not easily contaminated by external factors, like urine or saliva, and can often be used with little or no pretreatment.

The quantitative analysis of IgG in human serum can be valuable to detect disease and monitor disease progression. However the detection of IgG in human serum can be complicated by the abundant amounts of other proteins and high abundance of human serum albumin. Wu and co-workers improved upon a MEKC-UV method to determine the IgG in human serum, by using solid phase extraction for the removal of human serum albumin and on-column preconcentration to improve sensitivity. (Wu et al., 2010)

IgE in human serum was also measured by Chen and co workers. (Chen et al., 2008) While IgE has the lowest concentration its role in the development of allergy and parasitic diseases has focused attention on this immunoglobulin and driven Chen et al. to develop an immunoassay CE technique that can rapidly detect IgE from only 1 μL of human serum. This technique could be further modified by modifying the magnetic beads and determining different IgE antigens specific for certain allergens.

To evaluate the correlation between neurotrophins and clinical diagnosis of traumatic brain injury, Kalish and Phillips used ICE to measure the concentrations of five different neurotrophins from patient's sera with mild, moderate or severe traumatic brain injury,as seen in Figure 6. (Kalish & Phillips, 2010) Five neurotrophins were simultaneously identified and measured from a small sample in about 40 minutes from serum samples, which are easier to obtain in a clinical setting than samples directly at the site of the brain injury.

Increased levels of α-1-acid glycoprotein (AGP) have been related to cancer and comparing its isoforms between healthy and diseased individuals might provide valuable information about diagnosis. Ongay et al. purified AGP from human serum by different procedures and analyzed the samples by CZE-UV and CZE-ESI-TOF-MS to evaluate which purification method worked best. (Ongay et al., 2009) Overall they obtained a higher yield of AGP using a method without acidic precipitation, but neither preparation affected glycosylation of AGP.

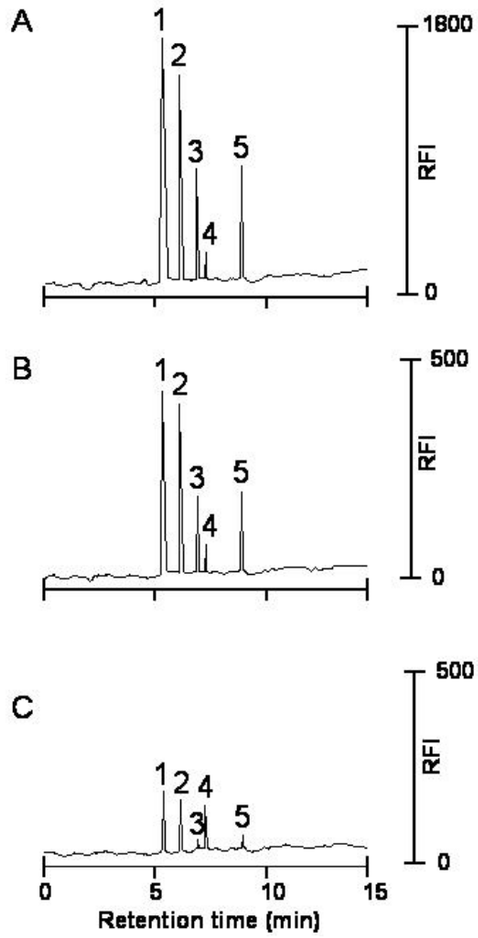

Fig. 6. Electropherograms from ICE analyses of unspiked serum samples of patients suffering from (A) mild, (B) moderate, and (C) severe head trauma. Analyses were performed under the conditions described in Section 2.6. Peak identification: 1. BDNF(brain-derived neurotrophic factor), 2. CNTF(ciliary neurotrophic factor), 3. NT 3(neurotrophin-3), 4. NT-4(Neurotrophin-4), 5. β-NGF(β-nerve growth factor). ' Reprinted from Journal of Chromatography B, 878, Kalish, H. & Phillips, T.M. Analysis of neurotrophins in human serum by immunoaffinity capillary electrophoresis following traumatic head injury, 194-200, Copyright 2010, with permission from Elsevier.

Newborn blood spots were analyzed for inborn errors of metabolism (IEM) by Chalcraft and McKibbin. (Chalcraft & Britz-McKibbin, 2009) Using CE-ESI-MS, dried blood spots from healthy volunteers were extracted and analyzed to determine levels of metabolites in healthy adults. Twenty underivatized metabolites associated with IEM were detected by this new method without chemical derivatization, sample desalting or complicated sample handling.
Both plasma and carotid plaque samples were analyzed by Zinellu et al. to measure thiols in patients undergoing carotid endarterectomy. (Zinellu et al., 2009) CZE-LIF was conducted on both sets of samples to determine if the distribution of thiols differed. Three thiols showed correlation between levels in plasma and plaques, while others were higher or lower in plaques than in plasma. Therefore, evaluating both the plaque and plasma might provide a more complete picture of the plaque progression and fate.

A microchip-CE based noncompetitive immunoassay technique was used for assaying a tumor marker in human serum. Ye et al. coupled LIF detection with microchip based CE to analyze the serum of normal and cancer patients for the cancer biomarker, carcinoembryonic antigen (CEA). (Ye et al., 2010) Using a double T chip and offline incubation of human serum with CEA monoclonal antibody, the CEA levels of normal patients and patients with different cancers were quantified. In all cases the cancer patients showed a higher level of CEA than normal patient levels.

E. Amniotic and follicular fluid

There are very few examples of analyses on these fluids, however they can be very valuable in assessing the health of both the mother and fetus. Amniotic fluid is accessible if the mother undergoes an amniocentesis; however, this is a single time point in the timeline of a fluid that changes on a daily basis. Examination of follicular fluid may lead to a better understanding of reproductive health in a woman.

The proteome of normal amniotic fluid (AF) and disease biomarkers, which may serve as predictions of birth outcomes, are starting to be reported in the literature. Gao and co-workers have used CE to analyze both the major components of amniotic fluid and to determine if any of the components might relate to birth outcome. The concentrations of albumin, IgG, transferrin and uric acid at 15 weeks gestation were measured by CE and it was determined that room temperature storage or multiple freeze thaw cycles revealed no detectable changes to the major components. (Gao et al., 2009)

CE was also used by Gao et al. to determine that higher levels of transferrin and uric acid in second trimester amniotic fluid correlated strongly with both birth weight and gestational age. (Gao et al., 2008)

Follicular fluid may contain biomarkers or proteins which can assist in reproductive medicine. Wen et al. used CZE coupled with UV/Vis to examine proteins found in follicular fluid of women undergoing controlled ovarian hyperstimulation. (Wen et al., 2009) Proteins from follicular fluid were resolved and all but one showed a decrease in concentration as the diameter of the follicle increased. Protein removal may assist researchers in examining follicular fluid closer and identifying smaller peaks.

A combination of analytical techniques can be used to profile the components in complex biological fluids. Hanrieder and co workers, used isoeletric focusing followed by tryptic digestion and CE coupled off line to MALDI-TOF MS/MS, as shown in Figure 7 to analyze human follicular fluid. (Hanrieder et al., 2009) This complex analysis led to the identification of 73 unique proteins, including several proteins known to be involved in human reproduction.

F. Sweat and vitreous fluid

There are very few publications dealing with analysis of these biofluids. Due to the difficulty of collecting vitreous fluid, it is not surprising that there are few publications dealing with its analysis. It is somewhat surprising that there are not more publications dealing with the analysis of sweat. Sweat is an analytically friendly biofluid, with very low protein concentrations. (Lloyd, 2008).

The analysis of sweat for cations, amines and amino acids was carried out by Hirokawa et al. (Hirokawa et al., 2007) While CE coupled with UV detection was used to detect alkali and alkaline earth cations and other target analytes in three male samples, there was variability in the results, which were dependent on the individual and the sampling spots samples were obtained from.

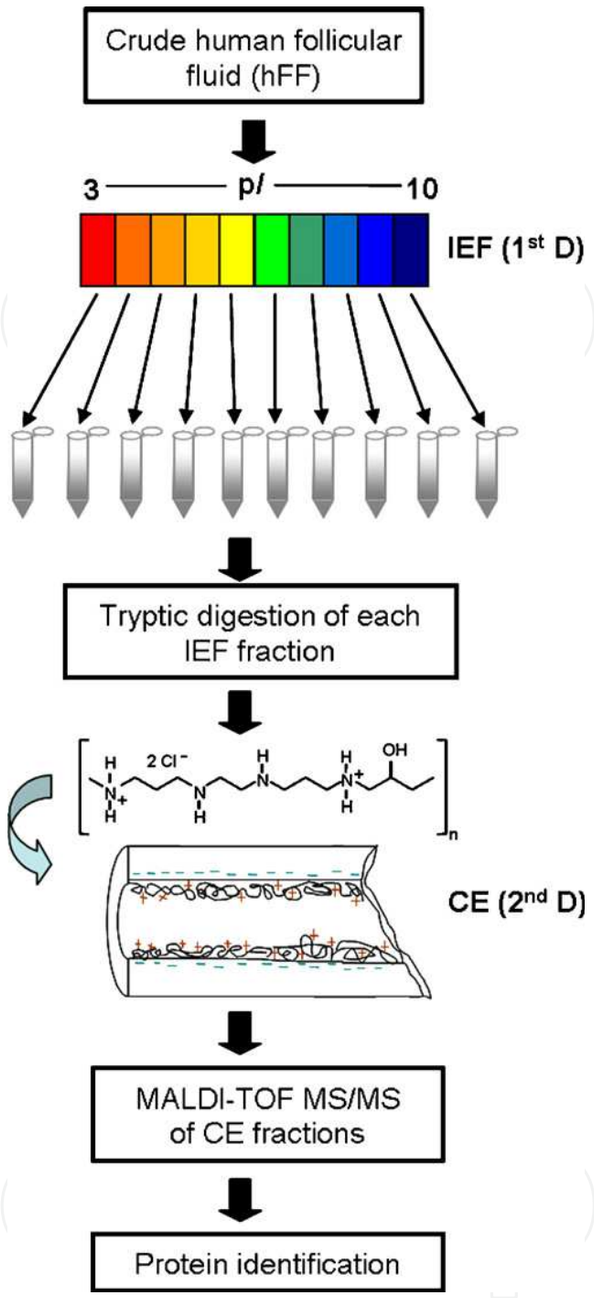

Fig. 7. Experimental overview of the liquid-phase 2D electrophoretic separation and MS profiling of the protein content in human follicular fluid, hFF. Sample prefractionation in microscale IEF was followed by separation and fractionation of tryptically digested peptides in PolyE-323 modified capillaries by CE, interfaced off-line to MALDI tandem time-of-flight MS. " Reprinted from Journal of Chromatography A, 1216,Hanrieder, J.; Zuberovic, A. & Bergquist, J. Surface modified capillary electrophoresis combined with in solution isoelectric focusing and MALDI-TOF/TOF MS: A gel-free multidimensional electrophoresis approach for proteomic profiling—Exemplified on human follicular fluid, 3621-3628., Copyright 2009, with permission from Elsevier.

Amino acid levels were also measured in vitreous fluid of patients with retinal detachment by Bertram and coworkers. (Bertram et al., 2008) Using CE coupled with LIF, patient samples were collected and analyzed for the presence of 9 amino acids. When compared to control samples, patients with retinal detachment had higher levels of glutamate than those of the controls.

Analysis of amino acids in vitreous fluid of patients with proliferative diabetic retinopathy (PDR) was also carried out by CE. Lu et al. used MEKC coupled with LIF to quantitate the differences in amino acid levels in healthy controls and patients with PDR. (Lu et al., 2007) This new method allowed for faster separation and better resolution of amino acids and showed an elevation in glutamate and arginine in PDR patient samples.

Gao et al. also used rapid CE analysis to evaluate the vitreous fluid in samples of patients suffering from PDR. (Gao et al., 2007) Using a laboratory built CE coupled to a UV/Vis detector, nitrate was measured in vitreous fluid. In PDR patients the nitrate peak is found to be significantly higher than that of the control group, suggesting NO might be involved in the disease course of PDR patients.

6. Future direction

Lab on a chip devices offer the potential of bedside analysis with very little wait time and require little to no skilled labor to operate the process. While CE lends itself to microchip format, other laboratory methods are being miniaturized as well. Sun et al. detail a miniature 96 sample ELISA- lab on a chip device that is coupled to a charge coupled device camera for detection. (Sun et al. 2010) The system is portable, sensitive enough to detect concentrations as low as 0.1 ng/mL, and flexible to adapt the system to several sample configurations.

Bed side analysis of endothelial progenitor cells detected in small volumes of white blood cells samples have been achieved using a novel microfluidic system developed by Ng and co workers. (Ng et al., 2010) The detection of clinically relevant sample volumes of CD34+ cells in a relatively short amount of time was demonstrated using label-free impedance detection on an MEA chip surface.

Slab—gel immunoblotting is an invaluable technique in immunology, however the process is extremely manual labor intensive and time consuming. He and Herr have designed, fabricated and validated an automated immunoblotting assay that relies on high-resolution polyacrylamide (PA) gel photopatterning in a two dimensional (2D) microfluidic architecture to yield polyacrylamide gel electrophoresis (PAGE), transfer, and antibody-functionalized blotting regions. (He & Herr, 2010) Using this micrfluidic immunoassay, He and Herr were able to obtain a complete native immunoblot of free prostate specific antigen (fPSA) extracted from human seminal fluid, as seen in Figure 8. This assay was carried out in fewer than 5 minutes with less consumption of antibodies, reagents and manual labor than the traditional bench-top technique.

Microfluidic devices are further being reduced in size to accommodate nanofluidic measurements. Many of these devices incorporate traditional assays such as sandwich assays on a microchip format and use new technologies to increase sensitivity. Jokerst and co workers incorporate quantum dots onto a nano-bio-chip in order to analyze serum and saliva samples. The multiplexed and programmable nano-bio-chip system has clinically demonstrated advantages including intense signal, low LODs necessary for saliva analysis and short assay times. (Jokerst et al., 2009)

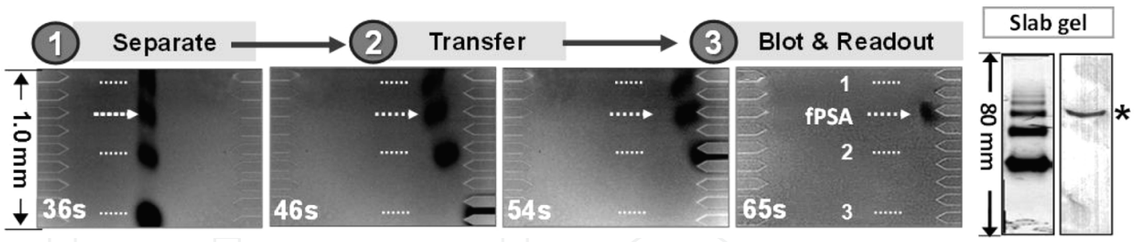

Fig. 8. On-chip immunoblotting of native fPSA extracted from human seminal fluid is rapid and automated. Fluorescence CCD images show the PA gel patterned chamber during: separation, transfer, and blot. A conventional native slab mini-gel blot allows comparison (inverted grayscale, * marks fPSA, numerals 1-3 mark non-fPSA sample peaks). Reprinted with permission from *Journal of the American Chemical Society,* 2010, 132, 8, 2512-2513.

The applications of micro- fluidic and eventually nano-fluidic devices to the analysis of human biofluids are fields that are in their infancy. In order for the fields to realize their full potential much work must be done in transferring the work out of the academic research laboratories and into the commercial sector.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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