



Poster Abstracts

(in alphabetical order by presenting author's last name)

1. Separation of opioid peptides by open-tubular capillary electrochromatography using a gold nanoparticle coated capillary

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Capillary electrophoresis is a powerful separation technique that can achieve high efficiency separations of charged analytes. However, one limitation of capillary electrophoresis is the adsorption of cationic proteins and peptides onto the inner surface of the capillary wall, due to the negatively charged silanol groups on the bare fused silica capillaries. This can lead to poor separation efficiencies and band broadening. Gold nanoparticles have been known to exhibit unique interactions with biomolecules, which has led to an increased interest in these particles for pharmaceutical applications and separation science. Our group is attempting to investigate two different approaches for the separation a number of opioid peptides using gold nanoparticles. Approach 1: Using a recently reported method for the preparation of a gold nanoparticle modified capillaries⁽¹⁾. The open tubular capillary column is produced by treating the inner surface of a fused silica capillaries with 3-triethoxysilylpropylamine, followed by the introduction of freshly prepared citrate stabilized gold nanoparticles (particle size≈25nm). The electroosmotic flow, apparent electrophoretic mobilities, resolution and plate numbers for the analytes of interest are compared to those obtained by conventional capillary zone electrophoresis on bare silica. Approach 2: We are also attempting to separate opioid peptides using the same citrate stabilized gold nanoparticles using capillaries treated with a layer of a positively charged poly(diallyldimethylammonium) chloride, that would adsorb the negatively charged gold nanoparticles, and compared it to conventional capillary zone electrophoresis on bare silica⁽²⁾.

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2. Effect of Channel Width on Human Umbilical Vein Endothelial Cell (HUVEC) Culture in Microfluidic Channels

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Endothelial cells (EC) line all blood vessels and play a crucial role in trafficking molecules between the blood stream and tissues. Microchannels mimic the in vivo environment of ECs better than well plates, and due to matching dimensions and the possibility of flow application are thus well suited to endothelial studies.

Our area of interest was the development of EC cultivation devices with different channel widths (60 to 360 μm). Crucial features of the devices include even cell distribution along the channel, seeding producibility, and compatibility with microscopy and flow application.

Hybrid polydimethylsiloxane (PDMS)-glass devices were used in the experiments, with microchannels replicated in PDMS and sealed irreversibly with glass using oxygen plasma exposure. Microchannels were coated with gelatin as substitute for extra cellular matrix, which promotes EC attachment in the microchannels. ECs were injected into the microchannels with a micropipette with a concentration of 5000 cells/ μL ; the devices were then placed in a cell incubator (37°C, 95% air, 5% CO₂). ECs were fixed with formalin and stained with 4',6-diamidino-2-phenylindole (DAPI) for observation of cell number and their distribution in the microchannels.

Tested designs of the devices allowed the development of an optimal microchannel configuration for cell culture in different channel widths. The developed device is compatible with all kinds of microscopy techniques.

ECs can be cultured in channels as narrow as 60 μm , allowing insight into the processes which occur in human microvasculature.

3. Optimization of a Microchip Electrophoresis Method for Separation and Amperometric Detection of Adenosine and Its Metabolites

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Adenosine is an important biomolecule involved in energy regulation in the brain during conditions such as traumatic brain injury (TBI) and ischemia. Inosine and hypoxanthine, two metabolites of adenosine, have also been recognized as markers of ischemia and energy failure. Under pathological conditions such as ischemia and TBI, the extracellular concentrations of adenosine, inosine and hypoxanthine in the brain are known to increase. Simultaneous and continuous monitoring of these biomarkers is extremely important to better understand neurological disorders and also for evaluating potential drug candidates for treatment of ischemia and TBI.

However, clinical instrumentation for simultaneous online monitoring of these biomarkers is currently unavailable. Microdialysis coupled with microchip electrophoresis with amperometric detection is a powerful technique that can be used for fast and reliable monitoring of biological samples. Microchips electrophoresis with chronoamperometric detection was successfully employed for the separation and detection of all three biomarkers as well as guanosine (another common nucleoside in the brain). The chip was fabricated from PDMS and had a 5 cm long separation channel and a carbon fiber working electrode.

The run buffer consisted of pH 10 boric acid with 10% (v/v) dimethylsulfoxide and field strength of 222V/cm was applied. Under these conditions, all four compounds could be separated in less than 80s. Ultimately, this method will be coupled on-line to microdialysis sampling to monitor these biomarkers in extracellular fluid of the rat brain during ischemial reperfusion and TBI.

4. Evaluation of Pulmonary Surfactant Properties for the Treatment of Respiratory Distress Using a Microfluidic Lung-On-Chip Device

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The overall goal of this work is the evaluation of surfactants for use in the treatment of respiratory disorders. The lack of or dysfunction of surfactant is implicated in Adult Respiratory Distress Syndrome (ARDS) and is the cause of progressive lung failure in premature infants with Neonatal Respiratory Distress Syndrome (NRDS). Treatment via surfactant replacement therapy has focused on the use of animal derived surfactants which possess properties close to that of human lung surfactant. However, the proper lipid and cholesterol concentrations necessary to achieve the ideal surface tension and viscosity remain unknown.

The evaluation of surfactant properties in an *in vitro* biological system can be challenging. In the case of lung tissue however, these challenges are further complicated by the fact that the tissue must 'breathe' in order to properly approximate the living system. Additionally, it is during these periods of inhalation and exhalation that viscosity and surface tension become most vital. Upon inhalation, surfactant must be viscous enough to spread over the expanding alveoli and upon expiration the surfactant film must form a monolayer able to reach near zero surface tension to avoid collapsing. A multilayer poly-dimethylsiloxane (PDMS) microfluidic device has been constructed to study the properties of surfactant with varying lipid and cholesterol compositions. This device provides not only a substrate upon which to grow viable lung cells, but, by creating a vacuum in the neighboring side channels, can simulate breathing.

5. Integration of Whispering Gallery Mode Detectors into Microfluidic Platforms for Clinical Viable Diagnostic Platform

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Whispering gallery mode (WGM) resonators enable the label-free detection of analytes based on refractive index sensing. We recently demonstrated a large scale multiplexed imaging platform where hundreds of resonators are simultaneously characterized by coupling a fluorescent dye to the resonator surface. This scheme was used to quantify several biomarkers of ovarian cancer with detection limits comparable to ELISA. Recently, we extended this technique by developing an evanescent scattering approach for characterizing the WGM resonances. Unlike the fluorescence imaging method, there is no photobleaching to decrease signal levels or limit assay times. Moreover, since signal levels scale with excitation power, measurements can be done with high temporal resolution using less expensive imaging equipment. This approach, therefore, offers promise for developing fast, inexpensive, and sensitive screens for the multiplexed detection of disease biomarkers. The development of a WGM imaging platform using evanescently scattered excitation will be discussed.

This platform is coupled with a measurement and analysis program that automates the multiplexed quantification of biomarkers and progress towards integrating the fluidics necessary for large scale screening will be presented.

6. Integration of Nanostructured Dielectrophoretic Device and Surface-Enhanced Raman Probe for Highly Sensitive Rapid Bacteria Detection

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Rapid detection of pathogens like bacteria and viruses is of great importance for monitoring water and food quality, the early detection of diseases, countering bioterrorism attacks, and other applications. Successful detection requires the manipulation and capture pathogenic particles for further analysis. This work reports a synergistic approach for the concentration, detection and kinetic monitoring of pathogens through the integration of nanostructured dielectrophoresis (DEP) with nanotag-labelled Surface Enhanced Raman Scattering (SERS). Vertically Aligned Carbon Nanofibers (VACNFs) at the bottom of a microfluidic chip was used to effectively capture and concentrate nanotag-labelled *E. coli* cells onto an active area at which the Raman laser probe was focused. The SERS nanotags were based on iron oxide-gold (IO-Au) core-shell nanoovals were coated with QSY21 Raman reporters and attached to *E. coli* cells through specific immunochemistry. The SERS signal was measured with both a confocal Raman microscope and a portable Raman probe during DEP capture, and was fully validated with fluorescence microscopy measurements under all DEP conditions. The combination of the greatly enhanced Raman signal by the SERS nanotags and the effective DEP concentration significantly improved the detection limit as low as 210 cfu/mL and speed of detection of only ~50 s. The SERS measurements were sensitive enough to detect a single bacterium. These results demonstrate the potential to develop a compact portable system for rapid and highly sensitive detection of specific pathogens. Such system is reusable, requires minimum sample preparation, and is ideal for field applications.

7. Development of a Separation-Based Method for Monitoring Excitatory Amino Acids in Patients with Traumatic Brain Injury Using Microdialysis coupled to Microchip Electrophoresis with Fluorescence Detection

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The overall goal of this work is to develop an integrated device that can be employed for bedside monitoring of biomarkers present following traumatic brain injury (TBI) in a hospital setting. These biomarkers include excitatory amino acids such as glutamate and aspartate, which can be found in abnormal levels within the extracellular fluid of the brain following TBI. While methods exist currently to monitor many of these biomarkers *in vivo*, they typically lack temporal resolution due to the sample sizes needed combined with the slow speed at which samples can be collected. In an effort to decrease sample size, on-line microdialysis sampling coupled to microchip electrophoresis and fluorescence detection will be employed.

Microchip electrophoresis (ME) allows for the miniaturization of separation-based sensors. This miniaturization leads to decreased reagent volumes, increased separation efficiency and decreased costs. Additionally, the microchip platform allows for integration of multiple processes on chip, including sampling and derivatization. However, while the separation process itself has decreased in size, most microfluidic systems still rely on large, expensive, benchtop equipment to operate the microchip and detect analytes of interest. Therefore, in an effort to decrease the footprint of the associated high voltage power supply and detection equipment allowing their placement in a hospital setting, this work has focused in the near term on constructing an inexpensive, portable, all-in-one fluorescence detection system. This system is capable of detecting nanomolar concentrations of fluorescently derivatized peptides and amino acids.

8. Designing and Characterization of Multilayer Microchip for Single Cell Analysis

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In single cell analysis, developing high throughput microfluidic devices is essential for fast and efficient cell analysis. Many fluid handling techniques allows transporting 25-40 cells/h. Traditional fluid handling techniques such as Vacuum/pressure driven flow always suffering with low through puts. In these techniques, flow rates are less controllable and small adjustments cannot be made. We introduced novel fluid handling technique in order to increase the analysis rate up to ~1000 cells/h. On-chip integration of miniaturized peristaltic pumps are used to control the fluid flow throughout the microfluidic channel networks. Set of micro pumps that are fabricated with poly dimethyl siloxane (PDMS) were used as peristaltic pumps.

These pumps are simple, low cost, durable, easy to fabricate and bio-compatible. They provide precise control of the flow rate, and produce continuous flows up to 9.2nL/s. Two-layer microfluidic chip was fabricated using multilayer soft lithographic technique. Jurkat cells were labeled with Oregon green (OG) and 6-carboxyfluorescein(6-CFDA). Two fluorescent dyes were electrophoretically separated using microchip electrophoresis(ME) coupled with laser induced fluorescent(LIF) detection.

9. Separation and detection of superoxide from RAW 264.7 macrophages using a commercial fluorescent probe in ME-LIF system

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Reactive oxygen species (ROS) are part of the natural aerobic metabolism of cells and are involved in various signaling and regulation processes. However, ROS can also cause oxidative stress when antioxidant defenses of the cell fail to regulate their production. Specifically, superoxide can cause cellular oxidative stress through its dismutation to hydrogen peroxide and molecular oxygen as well as by reacting with nitric oxide to produce peroxynitrite, an extremely toxic molecule. However, due to its high reactive nature, superoxide can be difficult to detect and quantitate. Therefore, the present work focuses on a method for the detection of superoxide produced inside RAW 264.7 macrophage cells before and after external stimuli.

MitoSOX is a mitochondria targeted fluorescent probe that produces a specific one-electron oxidation product (2-OH-MitoE⁺) upon reaction with superoxide and was used in these studies. The reaction of MitoSOX with other intracellular hydride acceptors can generate a different two-electron oxidation product. In order to separate the different reaction products, microchip electrophoresis was elected since it has been used to analyze bulk cell lysates and can be configured later for single cell analysis studies on cell heterogeneity. The 2-OH-MitoE⁺ standard was obtained by the reaction of the probe with nitrosodisulfonate (NSD). The identity of the peak was confirmed by comparing migration times with the product formed in a xanthine/xanthine oxidase system. Finally, macrophages were incubated with the probe after stimulation and the results compared to non-stimulated samples.

10. A 3D-Printed Miniaturized Ion Source for Mass Spectrometry Based on Paper Spray Ionization with Integrated, Passive Fluid Control

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Ambient ionization, *i.e.* the generation of gas-phase ions for separation and detection with a mass spectrometer (MS) under atmospheric pressure without sample preparation, is a hot topic in the scientific world. Although many fascinating developments have been reported over the last few years in this area, a particularly interesting technique that has emerged is *paper spray ionization* (PSI). In this approach, sample is deposited on a piece of paper; when this paper is cut to a sharp tip, placed in front of the MS and subsequently exposed to the application of solvent and a high potential, ion spray is generated. Although cheap, small and advantageous in many other ways, PSI has some disadvantages as well, especially in light of possibilities for point-of-care applications: (1) it takes experience to properly handle and position the paper tip; (2) due to the nature of paper, these 'ion sources' are very fragile; (3) spray can be generated for only about one minute when solvent is applied manually.

In this work, we have developed a 3D-printed, polylactic acid (PLA) cartridge for PSI, to overcome the above-mentioned problems, by (1) shielding the tip, thus providing physical protection, (2) 3D-printing a click-in holder for the cartridge, for easy interfacing with the MS and (3) designing solvent features in the cartridge for immediate tip wetting (approx. 1 min.) and continuous solvent supply (tens of minutes). These solvent features are based on the movement of solvent not only through paper, by capillary action, but also between paper and PLA, which is additionally governed by the wettability of the polymer.

11. On-Line Monitoring of Catecholamines *in vivo* Using Microdialysis Coupled to Microchip Electrophoresis with Electrochemical Detection

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Microdialysis is a powerful separation technique capable of monitoring the concentration changes of multiple analytes in the extracellular fluid of the brain. This technique generates small sample volumes in a continuous flow stream. Traditional (LC) methods used for sample analysis forfeit temporal information regarding dynamic processes due to the larger volumes necessary for analysis. Additionally, sample acquisition methods traditionally involve some form of tethering or anesthetizing the animal under study, greatly reducing the available behavioral information. In order to preserve both temporal resolution and behavioral information, the ideal analysis system is one that can be employed on-line, has fast analysis times of small sample volumes, and can be placed on a freely-roaming animal. In this study, we present an approach for coupling microdialysis sampling to microchip electrophoresis and electrochemical detection at a carbon electrode for monitoring neurochemicals in the dopamine metabolic pathway. The device is comprised of a double T PDMS/glass hybrid microchip with detection at two pyrolyzed photoresist film carbon electrodes in series, and was used to separate five analytes in the dopamine metabolic pathway in under 100 s. The developed method was used to monitor the dopamine metabolic pathway *in vivo* in rats after the administration of L-DOPA. The complete device and associated instrumentation can be used remotely and on-animal, for near-real time *in vivo* monitoring.

12. Microfluidic Lectin Barcode Array for High-Throughput Glycomic Profiling

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Protein glycosylation has been associated with almost every aspect of malignancy and thus holds the key to understanding the molecular mechanisms of cancer and to developing new biomarkers [1]. Despite its biomedical significance, progress in glycomics has considerably lagged behind genomics and proteomics. While MS-based analyses have shed light on the biological and clinical implications of glycans, low-throughput MS methods have had difficulties in correlating glycosylation aberrations with the pathological status. Similar to DNA and protein microarrays, lectin array offers a simple and high-throughput tool that is complementary to MS, which enables the whole tissue-level studies of human plasma glycome [2]. However, lectin-based assays suffer from an intrinsic limitation of the lectin-glycan interactions that are much weaker than the antibody-antigen affinity. Microfluidics offers a unique engineering solution to address this limitation because of its advantages in leveraging assay efficiency, speed, sensitivity, and throughput for genomic and proteomic analyses [3]. Surprisingly, very limited progress has been made to develop novel microfluidics-based glycomics analysis techniques. Here we report on a microfluidic lectin-barcode chip for high-throughput glycan profiling of cancer biomarkers. We have investigated two formats of lectin arrays using a panel of 16 lectins. Various blocking methods have been studied to suppress the interferences caused by non-specific lectin-glycan interactions and the glycans on the detection antibodies, significantly improving the performance of the antibody-overlay-lectin array. Using a biomarker for ovarian cancer, CA125, we demonstrated the feasibility of the microfluidic lectin barcode assay for glycan profiling of disease biomarkers.

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13. Detection of Peroxynitrite in Single Macrophage Cells Using Microchip Electrophoresis with Laser Induced Fluorescence

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Peroxynitrite (ONOO⁻) is produced in cells by the reaction of excess nitric oxide and superoxide. ONOO⁻ causes cytotoxicity through reactions with important intercellular biomolecules, such as proteins, lipids, and DNA, thereby inhibiting their function. Macrophages are immune cells that can be differentiated into pro-inflammatory and anti-inflammatory phenotypes, which produce different amounts of peroxynitrite due to the variation in available nitric oxide. Since ONOO⁻ has a very short half-life (~1 sec under physiological conditions), its direct detection is difficult. Therefore, this research is focused on the development of a method for monitoring ONOO⁻ production in single macrophage cells due to cellular heterogeneity using a novel fluorescent probe, HKGreen-3, which has been shown to be selective towards ONOO⁻. To determine the intercellular concentration of ONOO⁻, HKGreen-3 was produced with an acetate group (HKGreen-3A), allowing it to travel across cell membranes. Once inside the cell, cellular esterases cleave off the acetate group, creating HKGreen-3, which reacts with ONOO⁻ to form a fluorescent product. Due to the presence of interferences in the system, a separation method was necessary. This was accomplished using microchip electrophoresis utilizing a 5 cm simple-t design. Single macrophage cells were transported through the microchip with a gravity flow and were lysed upon exposure to a perpendicular electric field. First, macrophages were labeled with 6-carboxyfluorescein to test the system's functionality. HKGreen-3A was then added to cells and the separation between HKGreen-3A and HKGreen-3 was observed. In the future, this system will be used to accurately measure the concentration of ONOO⁻ in single stimulated macrophage cells.

14. Simultaneous separation of Gram positive and negative bacteria and fungi using chip-based capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection

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Rapid and on-site analysis of microorganisms becomes essential for industries and public health sectors. Chip-based capillary electrophoresis (CE) can serve as a reasonable alternative because of its simplicity, portability and high-throughput. Currently, chip-based CE with laser induced fluorescence (LIF) detection for the separation of a mixture of Gram positive and negative bacteria and fungi (e.g. *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*) was investigated. Nile blue (NB), a positive charged hydrophobic fluorescent dye, at 12.5 μM was optimal to ensure complete cell staining. The overall cell surface charge was converted from the prevalently anionic membrane proteins to the cationic NB-stained particles enabling them to be detected by the highly sensitive LIF detection with excitation and emission wavelengths at 635 nm and 685 nm, respectively. The electrolyte pH and concentration of polyethylene oxide (PEO) played significant roles on the microorganism separation. Baseline separation of the mixture was achieved ($R_s > 5.3$) with excellent efficiency ($N \sim 38\ 000$) in 0.5 min on a standard DNA chip with a 14 mm separation channel. The electrolyte was a TBE buffer (pH 10.5) consisting of 3.94 mM Tris, 0.56 mM boric acid, 0.013 mM Na_2EDTA , and 0.025% PEO, with injection/separation voltages of +1000/+1000V. The developed method was also successfully applied to separate another mixture of Gram positive bacteria (*i.e.* *Bacillus subtilis*, *Micrococcus luteus*, and *S. aureus*) within 30 s. These applications demonstrate that chip-based CE-LIF can be valuable for analysis of microorganisms where speed, throughput and cost are major concerns.

15. Development of On-Column Detection with Dual Au/Hg Electrodes for Capillary Electrophoresis

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Capillary electrophoresis is a powerful separation method due to its high efficiencies and its ability to analyze nanoliter volume samples, making it ideal to couple with microdialysis sampling. Electrochemical detection (EC) offers several advantages over other detection methods, notably its subnanomolar detection limits, and biological analytes of interest already being electrochemically active. However, capillary electrophoresis produces high separation currents that can greatly affect the EC detector. A decoupler is a solution that provides a break in the capillary, which is then covered by a porous material that will allow only current to escape the capillary. This permits the separation current to exit the capillary and into ground, while analytes remain in the capillary and are pushed towards the working electrode with the assistance of electrophoretic flow. A decoupler design previously designed in the C. Lunte lab was implemented where a capillary was ablated with several holes using a CO₂ laser, and cellulose acetate was used to provide a semi-permeable membrane. This design can dissipate larger amounts of current compared to previous designs, while providing improved structural support.

Thiols and disulfides are biomarkers of oxidative stress. Thiols react with reactive oxygen species and are converted into disulfides. The ratio of thiols and disulfides can provide information on the amount of damage to DNA, proteins, and/or lipids. In particular, N-acetylcysteine, cysteine, cystine, homocysteine, homocystine, glutathione, and glutathione disulfides were detected in a parallel-opposed dual Au/Hg electrode design. This design allows for the simultaneous or individual detection of thiols or disulfides which is not possible with a single electrode.

16. Whispering Gallery Mode Imaging for the Multiplexed Detection of Ovarian Cancer Biomarkers

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When diagnosed in initial stages, ovarian cancer patients have 5-year survival rates greater than 95%. Unfortunately, less than 20% of patients receive an accurate diagnosis during stage I of the disease resulting in 5-year survival rates less than 30%. This can be improved with enhanced early detection capabilities. Current diagnostic technologies measure levels of cancer antigen 125 (CA-125) which can be elevated due to ovarian cancer as well as numerous other benign conditions. Alone, CA-125 is a poor indicator of initial disease states with only 50% of women having elevated levels during stages I and II. Ultimately, multiple markers need to be measured to achieve an accurate early diagnosis of ovarian cancer. To address this need, we are developing a sensitive and specific optical biosensor for the detection of multiple putative ovarian cancer biomarkers. Numerous publications have highlighted the ability of recirculating light within a microresonator to be effective refractive index detectors. The confinement of light, termed a whispering gallery mode (WGM), is achieved when the proper resonant conditions are met. An imaging approach has been developed utilizing WGM resonators and surface attached fluorescent dye molecules.

Additionally, biological recognition elements are attached to the resonator surface to provide biosensing capabilities. Multiplexed capabilities are incorporated through encoding the analyte identity in the microsphere size.

Furthermore, microfabrication methods can be utilized to provide enhanced multiplexed capabilities by creating individually labeled wells for each microresonator. Progress toward developing a WGM imaging approach to quantify multiple ovarian cancer biomarkers will be presented.

17. PDMS-interconnected Microfluidic Systems for Rapid Separations of Neurotransmitters

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In vivo measurement of neurotransmitters in cerebrospinal fluidic (CSF) is one of the effective methods to monitor the level variation of specific neurotransmitters that may be involved in specific neuronal activities. HPLC or capillary electrophoresis coupled with microdialysis is a powerful approach for these measurements and is widely used in pharmaceuticals and neuroscience research. HPLC usually requires relative large sample volumes (e.g. 5-15 μL), which are infeasible when limited sample is available¹; whereas CE is able to separate nano- or pico-liter samples in a shorter time such as in 20 s, which could improve the temporal resolution during in vivo measurements. We have developed an integrated CE system targeting on in vivo measurements of essential neurotransmitters including glutamate and catecholamines. This CE system employs PDMS interfaces to connect multiple flow capillaries for online sampling, mixing, derivatization, injection, and separation. Experimental results show that this system is capable of performing long-term measurements with reproducibility, accuracy, high sensitivity and robustness. An excellent reproducibility in peak height was achieved as 1.6 %RSD with the high separation efficiency of 250 k theoretical plates. Furthermore, the flow gate with smaller diameters reduced flow rate by 25 fold for effective gating flow compared with mechanically machined counterparts. It is anticipated that this PDMS-fabricated method can be adapted to CE-coupled analytical instrumentation, and thus be employed in miniaturized device and eventually decrease the cost and complexity of multifunction analytical systems.

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18. Search Ovarian Tumor Derived Circulating Exosomes using ExoSearch Chip

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Exosomes are cell-released small membrane vesicles derived from the endolysosomal pathway, and enriched with a group of tumor antigens (e.g., proteins, mRNA and miRNA). Exosomes release is exacerbated in tumor cells and increasingly presented in plasma and ascites of patients in variable cancers. Exosomal protein markers may constitute a “cancer signature” for improving the early detection of cancer. However, molecular definition of exosomes is not well established and complicated by the presence of other membrane derived subcellular structures, such as apoptotic vesicles, exosome-like vesicles, membrane particles, and ectosomes. Due to the substantial size overlap among these membrane vesicles, there is no well-defined approach for molecular analysis of exosomes. Even more, the conventional exosome isolation protocols are labor-intensive, time-consuming (>5 mL blood, > 10 h) and inefficient. To address current challenges in exosome study, we developed a microfluidic based ExoSearch platform for specific, rapid isolation of exosomes (~30 min) directly from ovarian cancer patient plasma, and downstream examination of a panel of circulating exosomal markers in ovarian cancer (CA-125, HE4, EpCAM). Ultimately, our ExoSearch Chip will provide an effective platform for blood-based, multi-marker diagnosis of ovarian cancer.