製藥與生物技術國家型計畫年度研究成果報告

【快速蛋白質分析之微流體晶片系統之研發－III. 整合微流體晶片與微電纜離子化法質譜儀之微裝置供作蛋白質的快速鑑別】

計畫類別：□個別型計畫     □整合型計畫
計畫編號：NSC89－2323－B－006－010－
執行期間：八十九年八月一日至九十年四月三十日

執行單位：國立成功大學環境醫學研究所
計畫主持人：廖寶琦

中華民國九十年五月十日
年度研究成果報告內容

一、計劃緣起。

This three-year project is a sub-project of four grouped projects that aim to develop high throughput microfluidic chips for protein analysis. The goal of this sub-project is to design, fabricate, and validate the usefulness of an integrated chip-based protein-identification-microfluidic-module (PIMM). The proposed PIMM is composed of an immobilized trypsin reactor, a separation channel, and a μ-ESI spraying nozzle. When used with mass spectrometer, the PIMM can perform rapid and sensitive protein identification. The feasibility of the proposed PIMM is based on two emerging technologies, chip-based micro-electrospray ionization mass spectrometry (μ-ESI-MS) and search algorithms to map mass spectral data to protein and DNA sequence databases. In addition to simple protein identification, the PIMM can also be integrated with other microfluidic components into a so-called μ-TAS (micro total analytical system) to achieve sophisticated analysis of proteins for various biomedical and diagnostic applications.

The feasibility of the proposed work

The feasibility of the proposed protein-identification-microfluidic-module (PIMM) is based on two emerging technologies, chip-based micro-electrospray ionization mass spectrometry (μ-ESI-MS) and search algorithms to map mass spectral data to protein and DNA sequence databases. To utilize mass spectral data to search sequence databases for protein identification, a protein must be cleaved into small peptides and the peptides analyzed by mass spectrometry. This makes integration of a chip-based immobilized enzyme reactor with a chip-based micro-electrosprayer attractive because the integration reduces sample loss due to sample transferring steps. Integration also reduces time of analysis. Since enzymatic digestion of a protein may result in a complex mixture of peptides, separation may be necessary before the peptides are introduced into ESI-MS. Therefore, the proposed PIMM will be composed of an immobilized trypsin reactor, a separation channel, and a micro-ESI spraying nozzle.

Electrospray ionization mass spectrometry

The study of electrospray phenomena dates back many years. In 1970s, Dole and coworkers did extensive investigation into electrospray process and use it to produce gas phase macro ions (Hop and Bakhtiar, 1997). The ions were measured by ion retardation or ion mobility techniques but not by modern mass spectrometers. In 1984, the combination of electrospray ionization and mass spectrometry was reported simultaneously by both Yamashita and Fenn, and Aleksandrov and coworkers. In 1988, Fenn and his research group reported the successful use of electrospray to produce large protein ions (Smith et al., 1991).

In a simplified picture, ESI is a way to produce gas phase ions by removing solvent molecules from solvated ions in a solution. In ESI, a spray is produced by applying a high electric potential (typically 3-6 kV) to a small flow (1-100 μL/min) of liquid containing analytes (e.g. proteins in water/acetonitrile) from a capillary tube (Bruins, 1994). The liquid surface is highly charged by the
electric potential and a fine spray of charged liquid droplets is formed when the liquid leaves the end of the tube. The "spray" phenomena can take place by the action of the electric potential alone, so it is named "electrospray".

**Miniaturized and chip-based electrospray nozzles**

Wilm and Mann (1996) reported first that a miniaturized ESI source (sub µL/min flow rate, using a 1-20 µm spray orifice, in contrast to the conventional ESI that has an orifice of several hundred microns), offers several analytical advantages. These include the consumption of sample solution is very limited, a few microliters of sample solution can provide hour-long mass spectral signals, and the tolerance to salt contamination, wide range of solvent composition and pH. The miniaturized ESI sources with tiny spray nozzles that are made of pulled silica capillary are referred as nano-electrospray or micro-electrospray (µ-ESI) ion sources in the literature (Gatlina et al., 1998; Krishnamurthy et al., 1999).

Another type of miniaturized ESI source was firstly proposed in several papers published in 1997. Xue and his co-workers described that a microfabricated multi-channel glass chip were successfully integrated to an ESI-MS. The microchip device was fabricated by photolithographic, wet chemical etching, and thermal bonding procedures. The detection limit of the device for myoglobin was found to be lower than 60 nM (Xue et al., 1997b). The device was also applied successfully to monitor tryptic digestion products (Xue et al., 1997a). Ramsey and Ramsey described a similar device that generated electrospray from microchip using electroosmotic pumping (Ramsey and Ramsey, 1997).

Figeys and his co-workers also described a microfabricated device to deliver µ-ESI (Figeys et al., 1997; 1998). However, the electrospray nozzle was not built on the microchip. Instead, a fused silica capillary was used for the spray nozzle and connected to a microfluidic chip. The device was proposed to be used for rapid protein identification by searching sequence databases with the information contained in the collision-induced spectra of selected peptides.

Desai and his co-workers proposed based on their observation that a “hanging out” micro nozzle provided better performance (Desai et al., 1997). In 1998, Foret and his co-workers used glass as substrate and added a micromachined pneumatic nebulizer to assist the ESI (Foret et al., 1998). Xu and his co-workers also reported a microfabricated dialysis device for sample cleanups. This is especially useful for samples in a salted solution (Xu et al., 1998). Figeys and Aebersold (1998d) utilized electro-osmotic pressure to make a solvent gradient delivery system to be used with µ-ESI for protein analysis. Wang and his co-workers reported the use of a Parylene polymer material to make micro electrospray nozzles. They considered the flexible polymer is suitable for the purpose because the nozzle will not be too fragile (Wang et al., 1999). Xiang and his co-workers reported the use of polycarbonate material. Their device can perform dual microdialysis and to be used for the analysis of complicated mixtures.

The growing interest in the analysis of small amount of samples makes µ-ESI device an ideal ion source for the situations when the amount of available sample is limited.
Large-scale nucleotide sequence projects are beginning to produce complete sequence information for many species. Many protein/nucleotide sequence databases are becoming complete and available. A powerful approach for utilizing such sequence information is through the data produced by mass spectrometry. Mass spectrometry provides a rapid and sensitive experimental method to map proteins of interest to the database (Yates et al., 1995; Yates, 1998).

One of such algorithms involves with peptide mass fingerprinting (peptide mass map, or peptide map) obtained from proteolytic digests of proteins analyzed by mass spectrometry (Esoubas et al., 1998; Scheler et al., 1998).

Another type of searching algorithm is based on the data generated from tandem mass spectrometry (MS/MS). Data generated from tandem mass spectrometry experiment can also be used for searching protein sequence database without interpreting the data for a well-defined amino acid sequence. Yates and his coworkers have shown that ‘uninterpreted’ data generated from tandem mass spectrometry experiments, such as LC-ESI-low energy collision-induced dissociation (CID), contain sufficient information to uniquely identify the correct amino acid sequence from large protein databases (~160000 entries). Mann and Wilm (1994) also demonstrated that a very short series of sequence ions (‘sequence tag’) generated by ESI-CID tandem mass spectrometry experiments combined with the molecular weight information of the peptide can be used for database searching.

There are distinct strengths and weaknesses for both of the search algorithms. It does not require a tandem mass spectrometer to generate peptide maps. A simple MALDI-TOF or LC-MS can provide peptide maps for tryptic digests of proteins. One advantage that MS/MS approach may have over peptide map approach is that MS/MS database search algorithm can be used for protein mixtures, since MS/MS approach utilizes data from peptides rather than proteins. Other the other hand, peptide map approach will fail to identify any protein correctly if a tryptic digest of mixed proteins is analyzed. Besides, MS/MS database search algorithm has a better chance than peptide map approach to work when the protein is post-translationally modified. Under certain circumstances, MS/MS database search algorithm may even identify the protein and identify the post-translational modification at the same time.

Significance of the proposed work

The proposed project intends to design and fabricate a microfabricated device for protein identification by integrating a μ-ESI spraying nozzle with an immobilized trypsin reactor and a separation channel into a single chip module. The function of such a device, as well as the combination of these microfluidic components, has not been demonstrated in the literature before. Two novel designs of on-chip μ-ESI spraying nozzle will also be introduced in this proposal. The proposed PIMM can be used as a standalone device for the identification of unknown proteins that will only requires little amount of sample. The PIMM can also be integrated easily with other microfluidic devices to make a μ-TAS for protein analysis.
References:


二、計画目的。

The major goal of the proposed work is to design, fabricate, and validate the usefulness of an integrated chip-based microfluidic module that is capable of identifying unknown proteins in a rapid and sensitive fashion. This integrated chip-based microfluidic module will be referred as protein-identification-microfluidic-module (PIMM). The proposed PIMM will be composed of a micro-electrospray ionization (μ-ESI) device, an immobilized trypsin reactor, and a separation channel between the previous two components. All three components will be integrated on a single microfluidic chip. When used with mass spectrometers, the PIMM will be able to perform identification of proteins of interest by consuming just a few microliters of solution containing minute amount (low picomoles to femtomoles) of protein sample. The PIMM can also be integrated with other microfluidic components into a so-called μ-TAS (micro total analytical system) to
achieve sophisticated analysis of proteins for various biomedical and diagnostic applications.

The specific aims of the proposed work are:

(1) To design and fabricate a chip-based μ-ESI device that can be interfaced to a commercially available mass spectrometer and produce meaningful mass spectral data from known proteins or peptides;

(2) To use peptide maps and tandem mass spectrometry (MS/MS) data generated by the μ-ESI device to evaluate various database search algorithms to determine the most suitable one for the device;

(3) To integrate the μ-ESI device with an immobilized trypsin reactor (to cleave a protein into small peptides enzymatically) and a separation channel (to fractionate the peptide digest mixture) into a chip-based PIMM that can be used for rapid and sensitive protein identification;

(4) To validate the usefulness of the PIMM using a known protein and/or a mixture containing several known proteins;

(5) To integrate the PIMM with modules developed by sub-project 1 into a μ-TAS for protein analysis; and

(6) To demonstrate the usefulness of the μ-TAS by applying it to study the intra-cellular signaling pathways of vascular endothelial growth factor receptors (VEGFRs).

三、執行進度，包括：

1. 研究成果。

According to the schedule set in the proposal, the proposed work is to be completed in three years. Specific aims (1) and (2) are expected to be completed by the end of the first one and half years, (3) and (4) by the end of the second year, and (5) and (6) in the third year. Followed are the specific aims (1) and (2) of the proposed work:

(1) To design and fabricate a chip-based μ-ESI device that can be interfaced to a commercially available mass spectrometer and produce meaningful mass spectral data from known proteins or peptides.

(2) To use peptide maps and tandem mass spectrometry (MS/MS) data generated by the μ-ESI device to evaluate various database search algorithms to determine the most suitable one for the device.

Although specific aims (1) and (2) are expected to be completed by the end of the first one and half years, they have been principally accomplished by now. The accomplished work has been described in a manuscript submitted recently to a peer-reviewed journal for publication. The manuscript is modified slightly and attached below, as a part of this progress report.
Title

A disposable poly(methylmethacrylate)-based microfluidic module for protein identification by nano-electrospray ionization tandem mass spectrometry

Abstract

The design, fabrication, and its analytical utility of a poly(methylmethacrylate) (PMMA)-based microfluidic module for nano-electrospray tandem mass spectrometry (nano-ESI-MS/MS) were described. The microfluidic module can be mass-produced at low costs and used as a disposable device to generate nano-ESI-MS/MS signals for protein identification from low amount of protein samples. Compared with commercially available nanospray capillary tips, the module gave comparable signal quality and also offered advantages in convenience and easiness in operation, permitting repeated usages, and disposability.

Introduction

The advance of human genome project has shown many promises in biomedical research field. Within a few years, complete human genome sequence databases will be available to scientists for understanding disease mechanism, diagnosis, and new drug development [1]. While how the genomic sequence information will be exploited is still under intensive consideration, it is obvious now that, by using state-of-the-art mass spectrometry (MS) methods, proteins of interest to biomedical researchers can be mapped to sequence databases to find their identities [2]. This opens a possibility for systematic identification of the complete set of proteins that is expressed by the entire genome by a group of cells, that is, proteome. Proteome is a linguistic equivalent to the concept of genome. Proteomic research, or proteomics, refers to the studies of the proteome itself and the functional analysis of the proteins using technologies of large-scale protein identification [3-5].

To achieve large-scale protein identification, it has been speculated that microfabricated devices may provide an effective alternative solution to other means [6]. Microfabrication offers at least two major noticeable advantages, miniaturization and integration, and is therefore considered to be able to mass-produce microdevices at low costs to perform large-scale chemical analysis [7]. Microfabricated microfluidic chips have been proposed to carry out chemical reaction, separation, and identification, and “lab-on-a-chip” is termed to describe such a microfluidic system [6]. Recently, it is also noticed that microfluidics for manipulating liquid solution is highly compatible with the contemporary efforts to fabricate miniaturized elecrospay (ESI), or nano-electrospray (nano-ESI), ion source to be used for MS [8]. A few dozens of papers have been published [9-30] and reviewed [6] to describe various microfluidic devices in conjunction with ESI-MS or nano-ESI-MS systems.

Most of the microfluidic devices for ESI-MS reported to date used glass or quartz substrates [9-22]. The microfluidic channels of the glass/quartz microdevices were typically fabricated by photolithographic, wet chemical etching, and cover bonding procedures [11]. There have been a few reports described the application of polymer materials, such as parylene [23], polycarbonate [24-26], poly(dimethylsiloxane) (PDMS) [27, 28], and poly(methylmethacrylate) (PMMA) [29, 30], to microfabricated ESI-MS devices. The microfabrication methods for the polymer substrates varied,
ranging from simple knife cutting [30], replica molding [27, 28], UV laser machining [24-26], X-ray lithography [29], to a complicated micromachining process on a silicon/parylene composite substrate [23]. In this report, we describe a PMMA-based microfluidic module fabricated by hot-embossing process using a quartz master template. The microfluidic module can be mass-produced at low costs and used as a disposable device to generate nano-ESI-MS signals for protein identification from low amount of protein samples.

Material and methods
--- Chemicals and Materials

Methanol (HPLC grade) was purchased from Mallinckrodt (Phillipsburg, NJ, USA). Formic acid was purchased from Riedel-de Haeh (Seelze, Germany). Bovine serum albumin (BSA), fibrinopeptide A (M.W. 1536.6 Da), osteocalcin fragment 7-19 (M.W. 1407.6 Da), bradykinin (M.W. 1060.2 Da), LMPTYLYLK (M.W. 1028.3 Da), and VGGYGYGAK (M.W. 871.0 Da) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All reagents were of the highest grade available and were used directly without further purification. CE water was deionized distilled water that was filtered through a Barnstead E-pure system. The resistance of the water was more than 18.0 MΩ/cm². Both the buffer and sample solutions were filtered through 0.22 μm membrane before chip electrophoresis. NanoES sparying capillaries (catalog# ES381) were purchased from Protana (Odense, Denmark).

--- Fabrication of nano-ESI microfluidic module

The configuration of the microfabricated device (Figure 1) includes a cross microchannel and a fused silica tubing with a silver glue-coated nano-ESI tip. The cross microchannel was fabricated using hot embossing method [31, 32] on PMMA plexiglas substrate approximately 2 cm in width x 4 cm in length and 1.5 mm thick. Briefly, The micro channels on master templates were formed by the combination of metal etch mask and wet chemical etching. A commercial blank photo mask substrate (Nanofilm, Inc.) consisting of three layers (1 μm photoresist, 1 μm Cr, and 2.3 mm quartz, respectively) was used as a master template on which micro capillary channels were fabricated. Image on the film is first transferred on photoresist (PR) by standard lithography using transparent film as a mask. After PR is developed, the blank photomask is immersed in Cr etchant to transfer patterns on Cr layer. Last, residual PR is stripped, revealing the transferred image on Cr layer, which will be used as etch mask for further quartz etching. Microfluidic channels on the quartz substrate were formed by the combination of metal mask (Cr) and buffered oxide etchant (BOE, 6:1) at room temperature to fabricate capillary channels with a smoother appearance. Quartz was etched in all areas surrounding the channels, and the resulting structure was inverse raised three-dimensional image of the channels. The microchannels were imprinted by pressing a quartz master template onto the top of a PMMA blank substrate. The entire device was heated at 103°C for 5 minutes. Once the microchannels were formed, a Ni wire (400 μm i.d.) was placed in the end of the separation channel to imprint a fitting channel for capillary tubing (Polymicro, 365 μm o.d. and 50 μm i.d.) at the same imprinting temperature. The resulting PMMA plate with microfluidic channels and the capillary tubing was then clamped with another PMMA cover plate to form the sealed channels with the attached capillary tubing. The PMMA devices were heated at higher
module is used, analyte solution was transferred to sample reservoir I by a micro-pipette and electroosmotically pumped to the nano-ESI emitter, eliminating the tedious procedures to centrifuge, to air-pressurize, or to break the fine tip. Second, the microfluidic module was found to be useful for multiple repeated usages, more than 100 times without noticeable cross-contamination. In contrast, the commercial nanospray tips can usually be used for one sample since the tip end was "broken" and failed to load another sample by centrifugation.

--- Protein identification by searching sequence database

Figure 6 shows an MS/MS spectrum and the corresponding sequence tag generated from a SDS-PAGE-separated protein, BSA. The protein band was silver-stained, excised, and subject to trypsin digestion. The resulting mixture was desalted by ZipTip and analyzed by the microfluidic module. Although the signals were not strong, they could be used to deduce a sequence tag and the sequence tag was used to search PIR protein sequence database using software provided by PE SCIEX. The corresponding sequence tag was (592.2)(I/L)EN(951.4) and it mapped to the correct protein sequence in the database.

Concluding remarks

A PMMA-based microfluidic module fabricated by hot-embossing process using a quartz master template and by attaching a silver-coated pulled fused silica nano-ESI emitter, was described in this report. The use of quartz master template and hot-embossing, and the emitter was attached by UV-glue, made it possible to mass-produce these microfluidic modules at low costs. The analytical utility of the microfluidic module was compared with a commercially available nanospray tip, it was concluded that they gave comparable signal quality but the microfluidic module provide certain advantages such as convenience and easiness in operation, and permitting repeated usages without serious cross-contamination. Because of its potential in cutting manufacturing cost in mass production, it can be used as a disposable device after certain times of analyses, or for trace analysis where analyte carry-overs could potentially become a problem. Both the inputs and the outputs of microfluidic modules are liquids, so integration is readily achieved by modular designs and fabrication. As a goal to make the microfluidic devices to be a useful tool in proteomic research, additional investigations are undergoing in our laboratory to expand the function of the microfluidic module, including (1) an on-chip trypsin reactor module, (2) a desalting module, and (3) a frontal immunoaffinity-based extraction module.

References

Figure 1. A disposable polymer-based nano-ESI-MS microfluidic module and its utility in protein identification.
Figure 2. Full-scan mass spectra obtained for a 10 pmol/μL bradykinin standard solution using a microfluidic module fabricated in house (A) and a Protana nanospray tip (B).
Figure 3. The total ion current (A and B) and ion current at m/z 531 ([M+2H]^{++}) (C and D) were plotted against time for both the Protana nanospray tip (A and C) and the micro fluidic module (B and D), when they are tested using a 10 pmol/\textmu L bradykinin standard solution.
Figure 4. The ion current at m/z 531 ([M+2H]^{++}) resulted from five consecutive injections of 3 μL of 10 pmol/μL bradykinin standard solution.
Figure 5. Full-scan mass spectra obtained for a mixture containing four peptides. The concentration of each was 10 pmol/μL. The results were shown for the analyses done by a Protana nanospray tip (A) and the microfluidic module (B).
Sequence tag: (595.2)(I/L)EN(951.4)

Figure 6. Product ion scan mass spectrum obtained by the microfluidic module from an in-gel digestion mixture of 1.5 pmol bovine serum albumin separated on a SDS-PAGE gel.
2. 原計畫書之執行進度表。

The proposed work is to be completed in three years. Specific aims (1) and (2) are expected to be completed by the end of the first one and half years, (3) and (4) by the end of the second year, and (5) and (6) in the third year.

The proposed work is expected to generate the following results (specific aims 1-6).

(1) Completion of the design and fabrication of a chip-based μ-ESI device that can be interfaced to a commercially available mass spectrometer.

(2) Determination of the most suitable database search algorithm for the μ-ESI device.

(3) Integration of the μ-ESI spraying nozzle with an immobilized trypsin reactor and a separation channel into a chip-based PIMM (protein-identification-microfluidic-module) that can be used for rapid and sensitive protein identification.

(4) Validation of the usefulness of the PIMM.

(5) Integration of the PIMM with modules developed by subproject 1 into a μ-TAS for protein analysis.

(6) Demonstration of the usefulness of the μ-TAS by applying it to study the intra-cellular signaling pathways of vascular endothelial growth factor receptors (VEGFRs).

3. 是否達預定進度？如進度落後，請說明原因。

Although specific aims (1) and (2) are expected to be completed by the end of the first one and half years, they have been principally accomplished by now. The accomplished work has been described in a manuscript submitted recently to a peer-reviewed journal for publication. That is, we are about 6 months ahead of the planned schedule. In the next 6 months, we will spend time to improve our existing chip-based μ-ESI device and search algorithms. We will also seek to patent our device as soon as possible.

4. 預算執行情形。

The budgets allocated for the first year project period are listed in the next page (研究成果報告附件). They have been executed properly without any problems.
製藥與生物技術國家型計畫年度研究成果報告

附件：赴國外出差心得報告

【快速蛋白質分析之微流體晶片系統之研發－III. 整合微流體晶片與微電漮離子化法質譜儀之微裝置供作蛋白質的快速鑑別】

計畫類別：☐個別型計畫  ☑整合型計畫
計畫編號：NSC89-2323-B-006-010-1
執行期間：八十九年八月一日至九十年四月三十日

執行單位：國立成功大學環境醫學研究所
計畫主持人：廖寶琦

中華民國九十年五月十日
1. Background information

A travel budget was requested in the proposal of this project to allow PI to gain experience in the field of microfluidics from internationally known research laboratories. The budget was approved and NT$ 110,600 was allocated for this purpose. The amount of NT$ 110,600 was intended to cover flight fares, room and board, and other miscellaneous expenses for the PI’s visit to an internationally known research laboratory. The PI decided to take this opportunity to visit Professor Fred E. Regnier’s research laboratory at Purdue University located in West Lafayette, Indiana, U.S.A. Purdue University is a renowned public university. It was founded in 1869 with an enrollment of 37,762 students in fall 1999 from 50 U.S.A. states and 127 countries.

2. Experiences gained in Professor Fred E. Regnier’s research laboratory

Professor Fred E. Regnier specialized in analytical chemistry. His research activities focused on separation science including capillary electrophoresis, analytical immunology, chromatographic sorbent development, preparative chromatography, retention mechanism and stereospecific immobilization of proteins. He has published over 210 scientific papers and received several awards, including David B. Hume Award for Achievement in Chromatography in 1982, Stephen Dal Nogare Award for Achievements in Chromatography in 1987, ACS Award in Chromatography in 1989, Martin Gold Medal Award in 1993, ISCO Award For Significant Contributions to the Area of Biochemical Analysis and/or Separations in 1995, Pierce Award in Affinity Chromatography in 1995, and The Eastern Analytical Symposium Award for Achievements in Separation Science in 1996. He had also served at the editorial boards of several prestigious peer-reviewed journals, including Analytical Biochemistry, Analytical Chemistry, Journal of Pharmaceutical and Biomedical Analysis, Analytical Methods and Instrumentation, Journal of Chromatography, Liquid Chromatography Magazine, Chimica Oggi/Chemistry Today, Journal of High Resolution Chromatography, and International Journal of Bio-Chromatography.

Professor Fred E. Regnier’s research laboratory has two subgroups, Chemistry-on-a-Chip and Proteomics. Recently, Professor Fred E. Regnier had been actively involved with the research field on chromatography and electrophoresis on chips. He considered they were critical elements of future integrated, microfluidic analytical systems for life science. Liquid chromatography and electrophoresis played a major role in the life-science revolution, most strikingly in protein purification, peptide fractionation and sequencing, amino acid analysis, and DNA sequencing. The objective of his research is to examine the potential role of separation systems in the continuing evolution of biochemistry, biotechnology, and molecular biology. Very small chip-based systems may change how chemical analyses in biological, medical research and health care evolve over the next decade. Two common goals in research today are the desire to increase sample throughput and decrease the amount of sample needed and waste generated. Simultaneous pursuit of these goals has generated great interest in the area of microtechnology. Microfabricated electrophoresis chips offer not only volumetric reduction of reagents but separation times in as little as 1 s. Microchips are not limited to simple separations. The ability to design and construct complex channel systems has
facilitated the use of microchips in biochemical analyses involving postcolumn reactions, DNA sequencing, and enzyme assays.

Professor Fred E. Regnier’s research laboratory has designed and fabricated an electrophoretically mediated microanalysis system for leucine aminopeptidase using two-photon excited fluorescence detection on a microchip. In a capillary electrochromatography of peptides in a microfabricated system, reversed-phase liquid chromatography of tryptic peptides is shown in the capillary electrochromatography mode using microfabricated columns. He also pointed out that the major difference between a separation in the microfabricated CEC column and conventional separations in the HPLC mode is that separations are more readily achieved in the isocratic mode in the lower surface area microfabricated CEC columns. In his laboratory, he also demonstrated microfabricated filters for microfluidic analytical systems. Solvent and reagent filters were micromachined into quartz wafers using deep reactive ion etching to create a network of intersecting 1.5 x 10 μm channels. When placed at the bottom of reservoirs with a side exit, this channel network behaved as a lateral percolation filter composed of an array of cubelike structures one layer deep. Flow through these filters was driven by electroosmotic flow (EOF). Silanol groups at the walls of channels in the network provided the requisite charge to trigger EOF when voltage was applied laterally to the filter. Adsorption of cationic proteins in this silanol-rich matrix was controlled by the application of a polyacrylamide coating prepared by bonding N-hydroxysuccinimide (NHS)-activated poly(acrylic acid) to (ɛ-aminopropyl)silane-de-rivitized filters. Subsequent reaction of residual NHS groups in the coating with 2-(2-aminoethoxy)ethanol provided channels of low charge density and adsorptivity. These lateral percolation filters were shown to be efficacious in filtering solvents containing a variety of particulate materials, ranging from dust to cells.

His research group also developed a fabrication process for nanocolumns for liquid Chromatography. He showed that in situ micromachining can be used to simultaneously position and define (i) support particles, (ii) convective transport channels, (iii) an inlet distribution network of channels, and (iv) outlet channels in multiple chromatography columns on a single quartz wafer to the level of a few tenths of a micrometer. Stationary phases were bonded to 5 x 5 x 10-μm collocced monolith support structures separated by rectangular channels 1.5 μm wide and 10 μm deep with a low degree of deviation of channel width between the top and bottom of channels. High aspect ratio microfabrication can only be achieved with deep reactive ion etching. The volume of a 150 μm x 4.5 cm column was 18 nL. Column efficiency was evaluated in the capillary electrochromatography (CEC) mode using rhodamine 123 and a hydrocarbon stationary phase. Plate heights in these columns were typically 0.6 μm in the non-retained and 1.3 μm in the retained modes of operation. Columns were designed to have identical mobile-phase velocity in all channels in an effort to minimize out-gassing during operation. When the total lateral cross-sectional area of channels at all points along the separation axis is identical, linear velocity of the mobile phase in a CEC column should be the same. Columns were operated at atmospheric pressure.

3. Conclusions and suggestions

In the a few weeks of stay in Professor Fred E. Regnier’s research laboratory, I have gained much knowledge in the field of microfluidics. It may take me many years of efforts in my own
laboratory to build up these valuable experiences. Professor Fred E. Regnier has been particularly nice to visiting scholars and is very generous to share his research achievements with other scientists. I would strongly suggest that NSC provides funding to our scientists so they have opportunities to visit top research laboratories in the world to promote scientific excellence in Taiwan.