Microfluidic Devices on Polymer Substrates for Bioanalytical Applications

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1. INTRODUCTION

Development of capabilities to miniaturize analytical devices and components offers a number of potential benefits. Among these are the ability to reduce sample sizes, development of low cost, single-use disposable devices, and improved device portability. Extensive work has been done on producing such microanalytical systems on silicon or glass substrates using processes commonly employed in electronic chip manufacturing [1-2]. For many analytical applications, however, common polymeric materials provide acceptable substrates from which to produce components or complete analytical systems [3-4]. The low flow rate characteristic of these microfluidic devices is compatible with the electrospray ionization/mass spectrometry (ESI-MS) [5-7]. In this paper, development of microfluidic analytical devices fabricated on polymer substrates using excimer laser micromachining technology will be described. These include a microfluidic motherboard, dual-stage microdialysis chip, and a micro capillary isoelectric focusing device. The applications of these microdevices for cleanup, fractionation, and separation of biological samples are discussed.

EXPERIMENTAL

2.1. Fabrication Methods

UV excimer laser direct-write micromachining systems (Potomac LMT-4000 and LMT-5000) were used to produce microchannels of various geometries in polymeric materials. The systems contained Potomac TGX-1000 KrF (248 nm) excimer lasers configured for focused (direct write) operation at the machining surface. The minimum spot size of an unapertured laser beam at the work surface was approximately 10 microns. The maximum pulse repetition rate was 2 KHz with a maximum pulse energy of 45 μJ. Machining was performed on set of 4-inch (LMT-4000) or 12-inch (LMT-5000) computer-controlled X-Y stages.

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Microchannels in polymer devices were produced using two distinct methods. One approach was to use the focused excimer laser beam to directly mill channels into bulk polycarbonate or polyimide substrates. Because the unapertured laser beam width at the substrate was approximately 10 microns, multiple parallel passes were required to produce typical channels having widths of 50 or 100 microns. The depths of channels produced by this method, commonly 50 to 150 microns, were controlled by adjusting the feed rate of the part being machined or by changing the repetition rate of the laser. Microchannels produced by this method were sealed by applying a thin polyethylene terephthalate (PET) sheet directly to the face of the part with a heat activated adhesive, or by using a gasket compressed between the machined face and a second piece of flat polymer. Fluid connections to the microchannel were commonly made by mechanically machining the female part of a small chromatographic fitting (e.g., Upchurch Scientific) through the part from the back, and connecting to the microchannel with a small drilled hole.

A second approach to the formation of microchannels involved use of a polymer (e.g., polyimide) gasket with the channel machined through the thickness of the material. This method was used to produce microchannels up to 500 microns wide and 225 microns deep. For this approach, only the perimeter of the channel needed to be machined, with the remaining center portion of the channel being stripped out and discarded. When sandwiched between two solid pieces of polymer, the gasket formed a well-defined microchannel whose depth was determined by the thickness of the gasket material. These gaskets could be produced from sheet material having adhesive on one or both sides to improve sealing capabilities.

When feasible, devices were designed such that they could be assembled in a stacked geometry. In doing so, fluid flows were directed through a series of layers in the device, each layer having a distinct functionality. Minimum dead volume in the device was achieved by directing the fluid flow from one layer to the next using small diameter drilled vias.

2.2. Mass Spectrometric Analysis

A Finnigan LCQ (San Jose, CA) quadrupole ion trap mass spectrometer with a modified micro-ESI source was used for all experiments. The heated inlet capillary was maintained at 160^0C. For on-line microdialysis/ESI-MS experiment, the high voltage for ESI was applied to the metal syringe needle used for the buffer delivery and was carried to the sample side through the electrical conductivity of the buffer solution. Details of the membranes and flow rates used in specific experiments are discussed in the following section. A typical electrospray voltage was 2.2 kV. The maximum sample injection time was 40 msec, and 3 microscans were summed for each scan with a m/z range of 150 to 2,000.

3. RESULTS AND DISCUSSION

3.1. System Integration Platform - Microfluidic Motherboard

Dramatic gains in detector system performance can be expected through the seamless integration of miniature and microfluidics sample handling components with microscale chemical and biosensors through a novel motherboard technology: the microfluidics system integration motherboard. The objective of this work was to develop a suitcase-sized portable standard platform which integrates microfluidics sample handling components and standard connectors to interface the other miniturized biosensors or pathogen detectors based on "plug-and-play" modules. All components including micropumps, microvalves, microfluid channels and electronics were tightly integrated in the same microfluidic motherboard.

The microfluidic motherboard borrowed the "functionality on a chip" concept from the electronics industry and was intended to be the heart of a complex microfluidic device. The motherboard platform was designed to be tightly integrated and solid-state (i.e., liquid flows were all confined within machined microchannels), reducing the need for tubing for fluid distribution and connectivity. This concept greatly facilitated system integration and miniaturization. The first-generation motherboard consisted of three fluid reservoirs connected to three embedded piezoelectric micropumps (IMM) by microchannels fabricated from adhesive-backed polyimide [8]. The fluids could either be pumped independently or mixed in microchannels before being directed to exterior analytical components via outlet ports. Acrylic was used for all polymer parts of the fluidic motherboard except the laser-machined polyimide gaskets used to form the microchannels.

A second-generation microfluidic motherboard was designed and fabricated that incorporated valves in addition to the pumps (Figures 1-2). Initial tests indicate that the second-generation microfluidic motherboard is much more convenient to use compared with the previous version. Because all components in motherboard are based on "plug-and play" design, it is much easier for part replacement and repair.

It has been demonstrated that the microfluidic motherboard can deliver sample solutions and reagents to other devices (e.g., chemical and biosensors) at flow rates of between 10 and 300 μl/min. Integration of microfluidic motherboard with other micro biosensors and detectors is currently being explored in our lab.

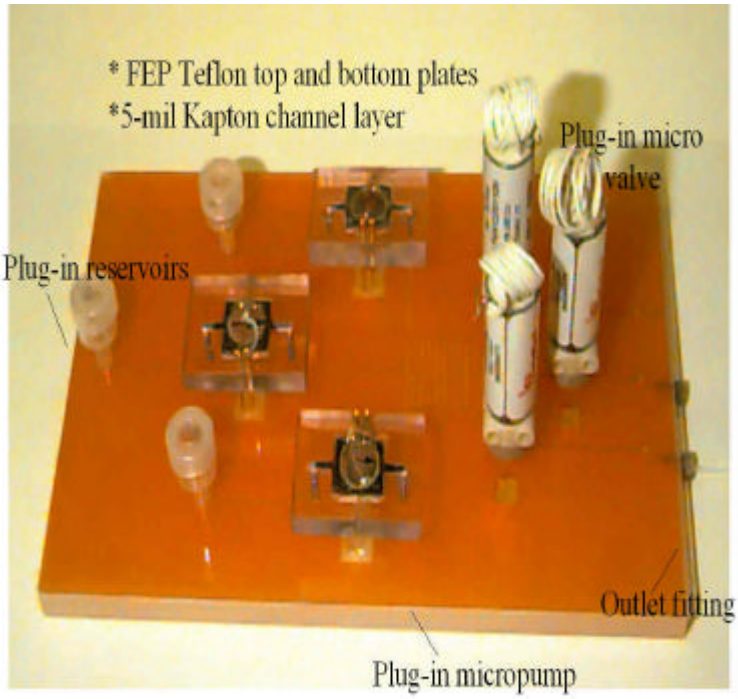

Fig. 1. Microfluidic Motherboard

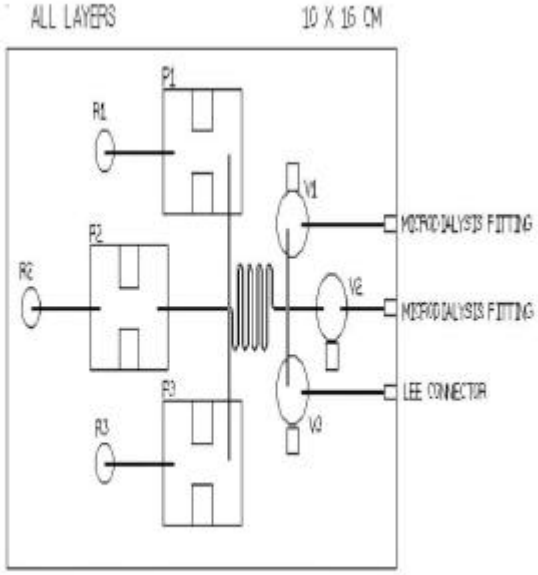

Fig.2. Microchannels in Microfluidic Motherboard

3.2 Microfabricated Dual-Stage Microdialysis Devices for On-Line Fractionation and Cleanup in ESI-MS Analysis of Proteins

A microfabricated dual-stage microdialysis device was developed for fast and efficient fractionation and cleanup for ESI-MS analysis of complex biological samples. Figure 3 shows the structure and flow diagram of the dual-microdialysis device. The sample channel (60 μm deep, 150 μm wide) was machined directly into the polymer chip (30x30x6 mm) by using multiple parallel laser passes. A 500 μm wide buffer channel was machined from 225 μm thick polyimide with a silicone adhesive on one side. The adhesive was used to

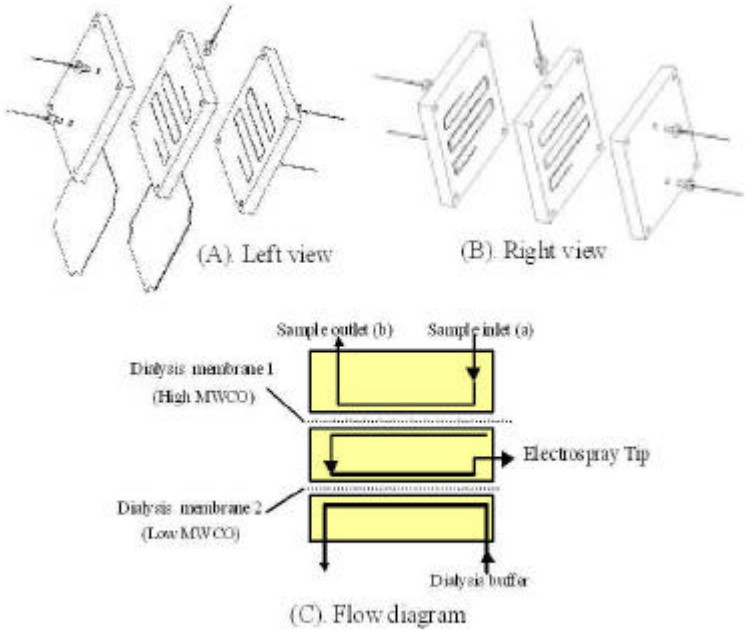

Fig. 3. Dual-Stage Microdialysis Device

attach the buffer channel to a flat polymer chip. Two microdialysis membranes are sandwiched between three polymer chips with microfabricated serpentine channels. This novel design makes the dual-microdialysis device highly compact and rugged, eliminating the tubing for connection of two stages of microdialysis units. The fluid channels were designed such that the raw sample flows in a microchannel along one side of the first stage membrane so that a portion of sample flows through the membrane and enters the second stage. This partial

flow in first stage provides a mechanism for sweeping out cellular residues in the sample channel and results in a reduced possibility of membrane clotting. Alternatively, the sample channel outlet (b) can be completely sealed if the life-time of the membrane is not of concern. The sample flow rate entering the second stage of the device can be adjusted by controlling the flow rate at outlet (b) by throttling the sample flow through fused silica tubing of various length and inner diameters. Sample entering the second stage of the device is stripped of the small molecules and salts by a dialysis buffer flowing in a microchannel on the opposite side of the second stage membrane.

The motivation for use of a microfabricated dual-microdialysis approach to MS sample clean-up was an attempt to improve on the shortcoming of similar larger-scale devices described previously [9]. A protein mixture consisting of 30 *m*M BSA, 8 *m*M cytochrome *c* and 2.4 *m*M ubiquitin in 10 mM PBS was used to evaluate the performance of the dual microdialysis device. A 50 kDa MWCO membrane and a 8 kDa MWCO membrane were used in the first and the second stage microdialysis, respectively. The sample was injected at 0.5 *m*L/min, and the clean-up buffer at 10 *m*L/min. As shown in Figure 4A, direct infusion of the protein mixture produced a no useful ESI-MS spectrum. ESI-MS performance is well known to be poor for such solutions, presumably due to the excess BSA and NaCl. After the on-line dual dialysis in the microfabricated device, the spectrum in Figure 4B clearly showed peaks from cytochrome *c*, ubiquitin, and removal of the unresolved envelope of peaks at higher m/z. The improvement in spectral quality using the dual-dialysis device enabled effective peak assignments and accurate molecular weight determinations.

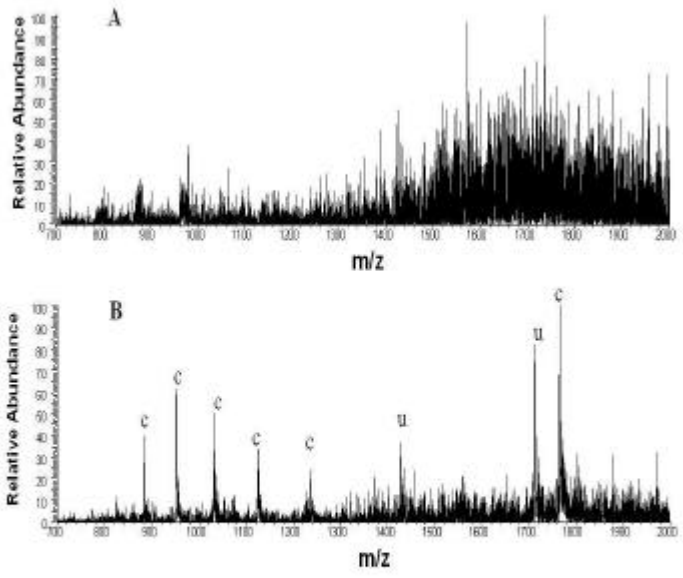

Fig. 4 Dual-Dialysis On-line ESI-MS Spectra of a Protein Mixture Consisting of 30 μM Bovine Serum Albumin (BSA), 8 μM Cytochrome *c* and 2.4 μM Ubiquitin in 0.01 M PBS. (A) 100 kDa (MWCO) membrane used on the first stage of the dual dialysis device; (B) 50 kDa (MWCO) membrane used on the first stage of the dual dialysis device.

For analyzing complex biological samples, initial sample fractionation and clenup can simplify analytical procedure. On line dual-dialysis using the microfabricated device is a fast and efficient means to achieve do this. The molecular weight range selected for analysis by dual-microdialysis can be controlled by selection of appropriate MWCO membranes at both stages. Application of dual-microdialysis/ESI/MS for the identification of “biomarks” of crude *E.coli* whole cell lysate has been successfully demonstrated in our lab [10].

3.3. Microfabricated Capillary Isoelectric Focusing (CIEF) Device for Protein Separations

CIEF is a high-resolution technique for the separation of protein mixtures based on the difference in their isoelectric points. A major drawback of conventional CIEF technique is poor reproducibility because of inconsistent sample handling and instability of the coating required on the silica capillary. Successful on-line interfacing of the microfabricated isoelectric focusing device with ESI-MS may provide a rapid and automated tool to concentrate, separate, and analyze complex protein mixtures. In this work, microfabricated CIEF chips on polymer substrates (polycarbonate, polyimide) were used for the separation of proteins. Electroosmotic flows in microchannels directly laser-machined on these polymers are typically lower than those in fused silica capillary. This suggests that isoelectric focusing experiments may be performed directly in these microfabricated CIEF chips without the need for coating of the microchannels, allowing for improved reproducibility in this microchip CIEF mode.

To reduce the dead volume in the interface between microchip and the mass spectrometer and improve both speed and sensitivity, an electrospray emitter was directly fabricated into the end of the device (Figure 5). Extensive experience with ESI at PNNL has demonstrated that sharper emitter "tips" give higher electric fields at the tip and result in better electrospray performance. The laser-machined microchannel (50 μm wide, 60 μm deep, and 16 cm long) in the CIEF chip (11 x 3 x 0.5 cm) was fabricated directly on the polycarbonate substrate, similar to the sample channel on the microdialysis device described above. A

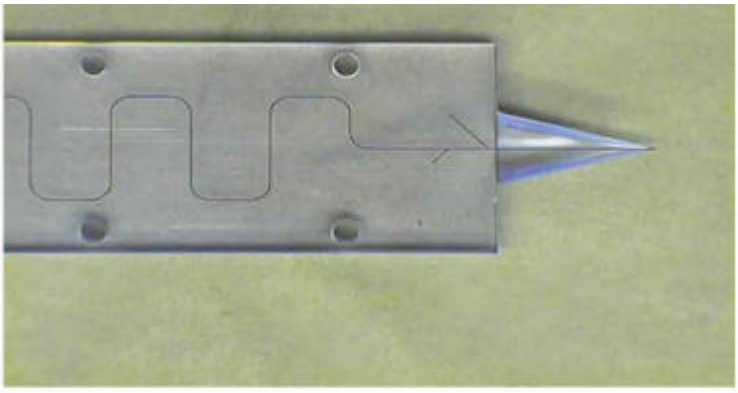

Fig. 5 CIEF microchip with a sharp electrospray emitter tip

dialysis membrane or ion exchange membrane was integrated on-chip at both the cathode and anode reservoirs, which allowed electric conduction in the microchannel but prevented the fluid from backflowing through the membranes.

CIEF separation with mass spectrometric detection was conducted in two steps as shown in Figure 6. In the first step, sample solution containing proteins and 1 5 pharmalyte (PI 3-10) was injected and filled the entire channel, across which electric field was applied. Isoelectric focusing was performed at a constant potential(-8 kV) for approximately 5-10 min. Proteins were focused on different zones based on difference in their isoelectric points (PIs**)**. In the second step, separated proteins were moved into the MS detector by applying pressure from a syringe pump at a flow rate of 0.05 μL/min. During operation, the electrospray emitter tip was positioned in front of the sampling orifice of the mass spectrometer. The applied high voltage (2 kV) and nebulizer gas (nitrogen) flowing around the electrospray emitter tip create a fine spray of charged particles for the MS analysis.

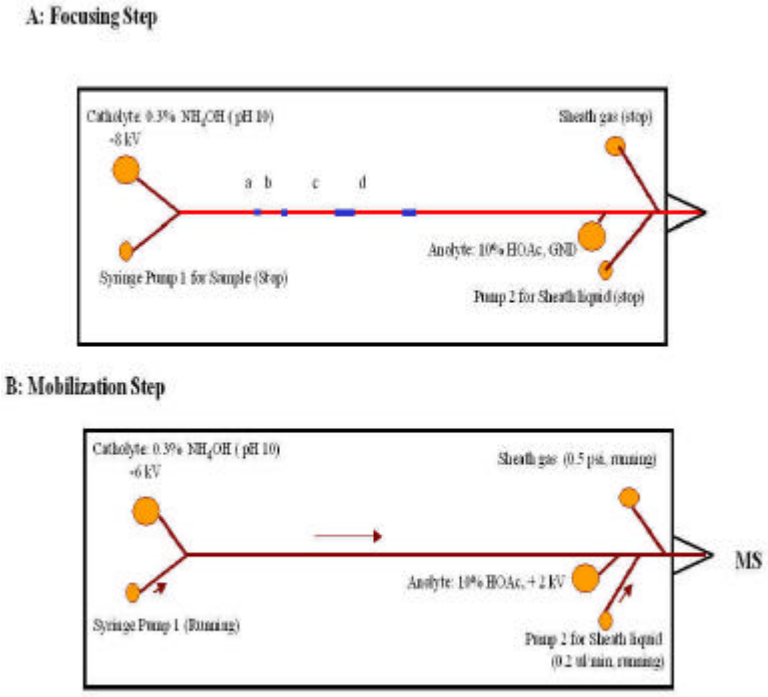

Figure 6. Two step in CIEF separation

Preliminary experiments have been conducted for the separation of a protein mixture with a concentration of 0.05 mg/mL for each protein. The separation profile is shown in Figure 7. Peak 1 and 2 in Figure 7 were from carbonic anhydrase and peak 3 from myoglobin. Figure 8 shown the positive ESI mass

spectrum of peak 2 and 3. The results indicate that electrospray directly from the sharp-edge microchip is feasible for separation and detection of proteins with microfabricated CIEF/ESI-MS.

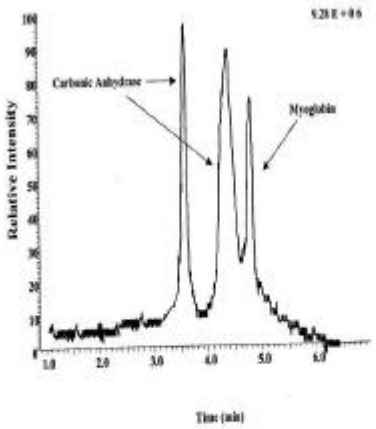

Fig.7. CIEF/ESI-MS Separation Profile on CIEF Microchip for Protein Mixture with a Concentration of 0.05 mg/ml for Each Protein. Peak 1 &2, carbonic anhydrase (pI 5.9 & 6.8); peak 3, myoglobin (pI 7.2).

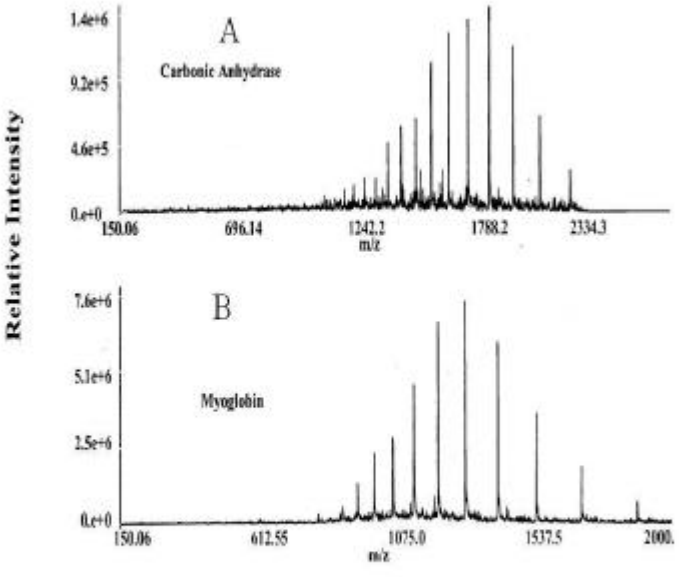

Fig.8. Positive ESI Mass Spectrum of Carbonic Anhydrase (A) and Myoglobin (B)

4. CONCLUSIONS

There are many advantages in the use of polymeric materials for producing microanalytical components and devices. We have demonstrated the fabrication of such devices using laser machining and lamination processes amenable to low cost, large-scale production. The designs used for these devices offer potential for additional size reduction with optimization of flow paths and support materials. Efficient sample cleanup, fractionation, and separation of protein samples were demonstrated using ESI with an ion trap mass spectrometer. Integration of these microfabricated devices with other nanoscale manipulations is expected to lead to more powerful, sensitive, less expensive, and rugged instrumentation for biomolecule analysis.

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