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Prototyping of Poly(dimethylsiloxane) Interfaces for Flow Gating, Reagent Mixing, and Tubing Connection in Capillary Electrophoresis

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Abstract

Integrated microfluidic systems coupled with electrophoretic separations have broad application in biological and chemical analysis. Interfaces for the connection of various functional parts play a major role in the performance of a system. Here we developed a rapid prototyping method to fabricate monolithic poly(dimethylsiloxane) (PDMS) Interfaces for flow-gated injection, online reagent mixing, and tube-to-tube connection in an integrated capillary electrophoresis (CE) system. The basic idea was based on the properties of PDMS: elasticity, transparency, and suitability for prototyping. The molds for these interfaces were prepared by using commercially available stainless steel wires and nylon lines or silica capillaries. A steel wire was inserted through the diameter of a nylon line and a cross format was obtained as the mold for PDMS casting of flow gates and 4-way mixers. These interfaces accommodated tubing connection through PDMS elasticity and provided easy visual trouble shooting. The flow gate used smaller channel diameters thus reducing flow rate by 25 fold for effective gating compared with mechanically machined counterparts. Both PDMS mixers and the tube-to-tube connectors could minimize the sample dead volume by using an appropriate capillary configuration. As a whole, the prototyped PDMS interfaces are reusable, inexpensive, convenient for connection, and robust when integrated with the CE detection system. Therefore, these interfaces could see potential applications in CE and CE-coupled systems.

Keywords

Flow gate; Flow-gated injection; PDMS; Prototyping; Interconnect; Capillary electrophoresis

1. Introduction

Capillary electrophoresis (CE), a powerful chemical separation technique, has attracted wide attention in scientific community and industry since its renaissance especially attributed to Jorgenson's pioneering work in 1980s [9, 10]. Advantages of CE over high performance liquid chromatography (HPLC) include reduced reagent consumption, increased separation speed, and improved resolving power. Therefore, CE techniques have been broadly employed in genomics, proteomics, metabolomics, drug discovery, and other challenging separation-related tasks [1, 20, 26].

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Basically, a sample is introduced into one end of a separation capillary by immersing the capillary tip in the sample solution. To rapidly and repeatedly inject samples for successive separations or to couple CE with an LC column, however, a flow-gated injection strategy has been developed [8, 13, 31] by using flow gates. These flow gates are mechanically machined to form a cross configuration in which the opposite branches serve as sample supply and the separation capillaries while the other opposite two branches conduct flow gating and waste straining. By adjusting the relative rates of the gating and sample flows, sample is deflected from the inlet of the separation capillary. To inject a sample, the gating flow is briefly swerved and the sample filled the cross section and meanwhile, an injection voltage is applied to electrokinetically introduce a sample plug into the capillary. By swerving the gating flow back, flow gating is re-established and separation buffer is brought to contact with the separation capillary. Therefore, the gating flow interface is a major part for the decent flow-gated injection procedure. Moreover, this flow gating configuration provided a convenient way to couple a LC microcolumn or another CE capillary for multi-dimensional separations [24, 29].

Although machined interfaces for flow-gated injection work well, they have numerous disadvantages. First, large inner diameters (typical 1/16 inches, i.e. 1.59 mm) over the outer diameter of silica capillaries (typically 360 μm) require a great gating flow rate to yield effective gating when the two opposite capillary tips are close (such as 40–100 μm) thus, in a given analysis time, consuming a large volume of buffer solution, which will increase the cost of waste disposal; moreover, the buffer is usually supplied through a syringe, and refilling of the syringe will interrupt the experiment during long-term monitoring of a biological or chemical process, while a large-volume syringe (e.g. 500 mL) and a related syringe pump are considerably expensive in price. Second, appropriate screw nuts and sleeves are required to stabilize the sample and separation capillaries, while the tightening process of the screws carries both capillaries forward, which poses difficulty in exactly setting the distance between the two capillary tips; so it takes time and is discouraging. Third, the 1/16" holes and the sleeves may fail to exactly match, which would not align the two capillary tips in a line thus taking longer time for the sample flow to fill the gap between the two capillaries before injection. To solve these problems, novel flow gates with improved performance are desired but currently unavailable.

PDMS is an elastic and transparent polymer which has been commonly used for prototyping of microfluidic chips [15, 16, 25]. Numerous articles have reported PDMS casting techniques for fabrication of interconnects as linear connectors for tube-to-tube or tube-to-chip connection [5, 21]. Also, Bergstrom et al. developed PDMS interfaces for coupling HPLC to CE in 2D separations [3]; however, the two channels lie on different levels which cannot effect efficient flow gating. Therefore, we developed a simple procedure to prepare intersecting cross lines as molds for PDMS flow gate casting.

Additionally, we prototyped reagent mixers and tube-to-tube connectors, both based on PDMS casting. Laser-induced fluorescence (LIF) detection is sensitive and thus commonly used in CE and microchip CE. However, most of analytes lack native fluorescence and require derivatization with fluorogenic reagents including naphthalene-2,3-dicarboxaldehyde (NDA) [19], o-phthalaldehyde (OPA) [12], fluorescamine (FC) [2], 3-(4-

carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) [27], 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) [17], 7-Nitrobenz-2-Oxa-1,3-Diazole (NBD) [6], and numerous other reagents [22, 30]. Derivatization is performed either offline or online by mixing numerous reagents to start the reaction. Online derivatization is often preferred to dynamically monitor the concentration variation. Similarly, any analyses involving two or more reagents to form detectable species such as antibody-antigen binding studies and enzyme kinetics analyses also prefer online pre-column mixing and curing before being injected for electrophoretic separations [11]. Therefore, reagents mixers and tube-to-tube connectors are essential to interconnect multiple flow modules.

In this paper, we report the method of rapidly prototyping PDMS interfaces for an integrated CE system. These interfaces have smaller channel diameters, offer easy capillary alignment and stabilization, and are reusable, inexpensive, and transparent. To demonstrate the performance of the PDMS-interconnected system, amino acid neurotransmitters were derivatized online with NDA at the presence of cyanide and then electrophoretically separated.

2. Experimental

2.1. Materials and reagents

Miniature stainless steel wires and nylon fishing lines were purchased from McMaster-Carr (Chicago, IL). Sodium tetraborate, ethylenediaminetetraacetic tetrasodium (EDTA), and all amino acids were obtained from Sigma (St. Louis, MO). Potassium cyanide, dimethylsulfoxide (DMSO), and salts (NaCl, KCl, MgSO₄, CaCl₂, Na₂HPO₄, and NaH₂PO₄) for the preparation of artificial cerebral spinal fluid (aCSF) were purchased from Fisher Scientific (Chicago, IL). NDA was ordered from Invitrogen (Eugene, OR). Aqueous Solutions were prepared in DI water, and NDA stock solution was in DMSO. Fused silica capillaries with 360 μ m OD and various IDs were purchased from Polymicro Technologies (Phoenix, AZ).

2.2. Preparation of molds

Flow gate and 4-way mixer—Both molds were prepared by using a similar procedure. A stainless steel wire with a diameter of 305 μ m (0.012") or 230 μ m was tapered and pressed hard (but avoid breaking) in the middle by using a plier cutting part to produce slots on both sides (Fig. 1a); then, both edges along the slots were sanded flat with a Dremel sander (Fig. 1c). A nylon line with an appropriate diameter (230 μ m, 430 μ m, or 560 μ m) was first gently pressed by using the plier cutting part to produce a flat section, and then a hole at the center of the pressed area was punched with a sewing needle on an inverted microscope (Fig. 1d). Then, the tapered and slotted wire was inserted through the hole until reaching the slots, and the cross section was then gently pressed with the flat part of a plier (Fig. 1e). The assembly of the cross was placed in a 1-inch (diameter) plastic petri dish through 4 uniformly distributed holes on the side wall (Fig. 1f). The nylon line was tightened and stabilized with a sticky tape.

Linear connector—A 200- μm (ID) silica capillary was smoothened on one end with sand paper. A 230- μm wire was tapered and inserted into the smoothened side of the capillary until it stopped. The linear assembly was placed in a 1-inch petri dish through two opposite holes on the side wall. For capillary-to-capillary connectors, 230- μm wires were directly used as molds.

2.3. Prototyping

PDMS prepolymer and curing reagent at the mass ratio of 10/1 were thoroughly mixed, degassed under a vacuum, and poured into a petri dish with the mold suspended. The mixture was cured for >30 minutes at 80 °C. The wire was pulled out followed by the nylon line. The solidified PDMS block was taken out of the petri dish and then was placed back to the oven and cured at 110 °C for > 1 hour to enhance PDMS rigidity. Finally, the PDMS blocks were cut into cubic shapes and were ready to use (see Fig. 2b).

2.4. Instrumentation

As schematically shown in Fig. 3, the lab-built CE detection system consisted of a Zeiss Axioskop 20 microscope, a 442-nm laser (Laserglow Technologies, Toronto, ON, Canada), a set of filters (Edmund Optics Inc., Barrington, NJ): a bandpass excitation filter (442 ± 5 nm), a fluorescence dichroic filter (458 nm cut-on), and a bandpass emission filter (482 ± 17 nm), a 40 $\times$ oil-immersing objective (Carl Zeiss Microscopy, Thornwood, NY) and a PMT module (H10722-210, Hamamatsu Photonics, Japan). A high-voltage power supply (Model CZE1000R) was purchased from Spellman High Voltage Electronics Corporation (Hauppauge, NY). A 3-way solenoid valve (Cole-Parmer, Vernon Hills, IL) was used for gating flow switching. The system was controlled via custom-programmed LabVIEW software (National Instruments, Austin, TX). A single-syringe pump (Harvard Apparatus, Holliston, MA) was used to deliver separation buffer also serving as the gating flow. A 4-syringe pump (Chemyx Inc., Stafford, TX) was used to supply sample and derivatization reagents through individual gas-tight Hamilton syringes (Reno, Nevada). Capillaries were tapered by using circularly-cut sand paper mounted on the Dremel rotor. The capillary was held and rotated using two fingers with one end against the rotating sand paper at an angle of about 30–45°.

2.5. Amino acid separation

Amino acid solutions were diluted to expected concentrations with aCSF from stock solutions prepared in DI water. NDA in water/DMSO (50/50 in volume) was diluted to 5 mM with DI water and 10 mM tetraborate buffer at pH 9.2 from NDA stock solution in DMSO. 10-mM KCN in 20-mM tetraborate buffer (pH 9.2) and 50-mM EDTA was also prepared from a 100-mM stock solution in DI water. Separation was performed by using a 10- μm (ID) capillary at an effective length of 10 cm and 16 cm in total. Amino acids were pumped through a syringe (sample in the syringe) or dialyzed through a side-by-side dialysis probe[28] (aCSF in the syringe) which was manually inserted to standard sample solutions. Flow-gated injection was performed at -5 kV (0.5 s) applied at the capillary outlet through a centrifuge tube filled with separation buffer, 20-mM tetraborate at pH 9.2, and the buffer

waste was grounded through a stainless steel tube inserted in the flow gate (Fig. 3). Separation was performed under -25-kV high-voltage ($-\text{HV}$, Fig. 3).

3. Results and discussion

3.1. Interface fabrication and capillary arrangement

Basically, the molds were assembled by using commercially available stainless steel wires and nylon lines. These wires can spring back when bended, and nylon lines with various diameters ($230\text{ }\mu\text{m}$, $430\text{ }\mu\text{m}$, and $560\text{ }\mu\text{m}$) have high strength. The pressed flat part of the nylon line simplified the punching procedure with a sewing needle. A tapered wire was inserted and had tight contact with nylon lines, which prevented flowing PDMS from filling gaps. The cross section should have small dimensions to reduce dead volumes, especially the 4-way mixing interface (3 to 1). The pressed slots on the wire accommodated the thickness of nylon lines on both sides (see Fig. 1a) so the total thickness of the cross after final pressing was reduced to the same as the wire diameters ($230\text{ }\mu\text{m}$ or $305\text{ }\mu\text{m}$). The cross section of either the flow gate or the mixer might have tiny PDMS pieces (see Fig. 2d) due to the roughness coming from the inherent nature of the assembled molds; however, this roughness rarely affected the function of interfaces based on visual observation of flows in interfaces and the reproducible peak height as shown in Fig. 8c.

In the flow gate, tapered capillaries were inserted into the cross section with an appropriate distance (e.g. $40\text{ }\mu\text{m}$) apart which was much smaller than the diameter (430 or $560\text{ }\mu\text{m}$) of the gating flow channel as shown in Fig. 2c, while the gating flow was guided by using two stainless steel tubes at diameters of $0.51/0.82\text{ mm}$ (ID/OD). Considering that the backpressure in the flow gate was released through the gating flow waste branch, the channel diameter for silica capillaries was $305\text{ }\mu\text{m}$ (instead of $230\text{ }\mu\text{m}$) which reduced the elastic stress applied on the $360\text{-}\mu\text{m}$ (OD) capillaries thus permitting easier capillary insertion and distance adjustment. On the other hand, smaller PDMS channels than tubes secured tubes in position prevented liquid leaking.

In the mixing interface, the sample capillary was tapered and inserted to the center of the cross section and the other capillaries were also close to the cross section as shown in Fig. 4. The capillary configuration in Fig. 4A had the sample branch in the middle and derivatization reagents on both sides. When the three flows were on, the sample was flushed away by the other two flows thus reducing the dead volume of the sample. Higher flow rates limited the diffusion time and a clear sample flow path was observed (Fig. 4Ac). Fig. 4B demonstrates an alternative of the flow arrangement, in which the sample was positioned on one side and was inserted to the center of the cross section. This arrangement may be used when the sample and one of the reagents are incompatible due to various reasons such as precipitation formation. The mold for the mixing interface used $230\text{-}\mu\text{m}$ wires and nylon lines; the fabricated PDMS channels had tight contact with the inserted $360\text{-}\mu\text{m}$ (OD) capillaries thus tolerating high back pressure and preventing liquid leaking. For 3-way mixing (2 to 1), one branch of the 4-way mixer could be blocked by using a sealed capillary or a solid bar with an appropriate diameter.

For tube-to-tube connection between identical diameters, single wires were used as molds, while connections of 22-gauge syringe needles (150/718 μm ID/OD) to 360- μm (OD) capillaries required differing diameters; thus, a mold was prepared by using a silica capillary (200/360 μm ID/OD) and a wire (230 μm) as shown in Fig. 5a. The completed PDMS connector had one side with a larger inner diameter to accommodate the syringe needle and the other side with a smaller diameter for the capillary (Fig. 5b). The two tubes could be inserted close thus reducing the dead volume as shown in Fig. 5c. The 718- μm needle in a 360- μm PDMS channel offered stronger sealing than the 360- μm silica capillary in a 230- μm channel. The combination tolerated up to 120 psi in back pressure before leaking was observed on the capillary side. This pressure was estimated by pumping a dye solution through a PDMS connector into a 100- μm capillary filled with air and sealed with super glue on the other end. A higher back pressure (over 200 psi) tolerance was observed when the ratio of the PDMS prepolymer/curing reagent was decreased to 5/1 in mass that offered harder PDMS.

3.2. Flow-gated injection

Fig. 6 shows the flow-gated injection process. Two 40- μm (ID) capillaries were inserted into the PDMS flow gate tip-to-tip at 40 μm apart. A 0.5-s interval between swerving off the gating flow and switching on the injection voltage allowed the sample (green dye in buffer) to fill the gap thus the injected sample would closely represent the bulk sample solution (Fig. 6b). Also, a 0.3-s interval before the application of the separation voltage was maintained to clear the sample around the separation capillary (Fig. 6d). As can be seen in Fig. 6e, a clean sample plug was injected and then electrokinetically pumped downstream for separation. The gating extent of the sample is determined by the flow rate ratio of the gating flow and the sample for a specific capillary gap. Fig. 7A demonstrates the gating situation at various gating flow rates while the sample was maintained at 400 nL/min. As can be seen in Fig. 7Aa, 40 $\mu\text{L}/\text{min}$ provided ineffective gating, which would promote sample leaking into the separation capillary thus increasing the background signal, while 80 $\mu\text{L}/\text{min}$ produced decent gating as shown in Fig. 7Ab, and the flow rate ratio is 200, roughly 25-fold less than the minimum requirement (5000) in the machined flow gate as reported [8]. This gating flow reduction, on the one hand, was due to the smaller diameter (0.43 mm) of the PDMS flow gate channel than the machined one (1.59 mm); on the other hand, the tapered capillaries (360 μm OD) blocked a larger percentage of the sectional area of the PDMS channel. Apparently, a greater gap between the two capillary tips requires smaller gating flow as shown in Fig. 7B, but it takes a longer stagnant time for the sample to refill the gap before injection thus lowering sample transfer efficiency and finally lengthening total analysis time.

3.3. Performance of interconnected system

To demonstrate the performance of the CE system interconnected through PDMS interfaces (Fig. 2), NDA/cyanide/amino acids were used to perform online derivatization and separations. As reported _ENREF_16[4, 7, 23] NDA and cyanide may react when stored together and therefore were introduced to the amino acids sample separately. As can be seen in Fig. 2, three solutions were pumped through individual syringes connected to 40- μm (ID) capillaries via PDMS connectors; the other ends of the capillaries were connected to a 4-way

PDMS mixer, with amino acids in the middle; the confluence flowed through a 100- μm (ID) capillary at the length of 32 cm allowing ~ 3 minutes for reaction. For flow-gated injection, a 40- μm (ID) capillary with 10 cm in length was used to supply sample to a 10- μm (ID) separation capillary. The 100- μm ID and 40- μm ID were connected with a fabricated 230- μm PDMS connector. The performance of the PDMS-interconnected system was tested through separating amino acids. Fig. 8a shows a typical electropherogram showing well-resolved 5 standard amino acids which were directly delivered through a sample syringe followed by online mixing, derivatization, injection, separation, and detection. Overlap injection was also performed as shown in Fig. 8b which doubled the analysis throughput. As can be seen in both electropherograms (Figs. 8a and 8b), NDA-derivatization of primary amine-containing compounds produces by-products, while these by-product peaks are often covered by amino acid peaks when a complex mixture of sample is analyzed with capillary zone electrophoresis [28]. To test its long-term performance, more than 300 consecutive injections were performed and the results were plotted in Fig. 8c, and an excellent reproducibility in peak height was demonstrated by the %RSD (relative standard deviation) of 1.6; high separation efficiency was also obtained with the theoretical plates of 250 k. Further, amino acids at various concentrations of 1.0, 2.0, 4.0, and 8.0 μM were sequentially dialyzed for 5 minutes through a microdialysis probe and the results of glutamate are summarized in Fig. 8d, which suggests that the integrated system was able to dynamically monitor sample concentration variation with the response time of ~ 40 s at 90% of the maximum signal. Note that the 8.0- μM sample produced relatively greater variation, but its %RSD (4.5) is still in acceptable range in CE separations [14]; this greater variation possibly came from occasional instability of sample injection since the other analyte, aspartate, also presented a similar variation pattern (data not shown). These results demonstrate that the PDMS-interconnected system is potentially valuable for in vivo neurotransmitter monitoring [18, 23].

4. Conclusions

The developed method for fabricating PDMS interfaces used for flow-gated injection, multiple-reagent online mixing, and tube-to-tube connection involved a rapid and inexpensive procedure to produce functional parts for CE and other analytical techniques. These parts offered convenience and ease for the coupling of complex integrated systems, minimized dead volumes, and reduced buffer consumption in the gating flow. Moreover, these transparent interfaces had apparent advantages for easy trouble shooting since many problems would occur at interfaces, especially the mixer and the flow gate; also, the gating flow and mixing processes could be directly observed so that the alignment and configuration of the capillaries in the cross section could be optimized. Furthermore, the prototyped channels had smaller diameters than the inserted capillaries, which tightly stabilized these capillaries in position and tolerated a high flow pressure. It is anticipated that the developed procedure and interfaces could be adapted to CE-coupled analytical instrumentation and eventually decrease the cost and complexity of multiplexed analytical systems.

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Fig. 1. Schematic diagram showing the preparation procedure of molds for PDMS casting of the flow gate and the 4-way mixing interface. (a) Side view of the stainless steel wire with clamped slots; (b) top view of the clamped wire with expanded metal; (c) top view of the edge-ground wire; (d) top view of the nylon line with a needle-punched hole; (e) top view of the assembled wire and nylon line after pressing both sides of the nylon line; (f) the wire/line assembly was suspended in a petri dish. Note that schematic dimensions are not proportional.

Fig. 2.

Images of the flow gate and tubing assembly. (a) PDMS interface filled with blue dye. (b) Close look of the PDMS cross section; horizontal diameter 300 μm and vertical 430 μm . (c) Flow gate with assembled tubing. (d) Close look of the head-to-head capillaries in a flow gate.

Fig. 3.

Schematic diagram of the PDMS-interconnected CE system (see details in the text). A, pin hole filter; B, excitation filter; C, emission filter. Capillaries between syringes and the mixer have $10\text{ cm} \times 40\text{ }\mu\text{m}$ ID, the one between the mixer and the second connector had $32\text{ cm} \times 100\text{ }\mu\text{m}$ ID, and the one between the flow gate and the second connector had $10\text{ cm} \times 40\text{ }\mu\text{m}$ ID. The separation capillary was 16 cm in length $\times 10\text{ }\mu\text{m}$ ID. The gating flow was supplied through tygon tubing with 0.5 mm ID.

A. Assembled mixing interface with sample in the middle.

B. Assembled mixing with sample on the side.

Fig. 4.

Images of 4-way mixing capillary assemblies. (a) 200 nL/min each; (b) 400 nL/min each; (c) 800 nL/min each. The green dye represented the sample. The clear branches had water.

Reagent supply capillaries were 40/360 μm (ID/OD), and the confluence was in a 100/360- μm (ID/OD) capillary on the right.

Fig. 5. Images of tube-to-tube connectors. (a) Assembled mold; the capillary ID was 200 μm . (b) The interface with a needle and a capillary inserted. (c) Tight connection of two tubes to reduce the dead volume.

A. Gating by various flow rates.

B. Gating for various distances.

Fig. 7.

A. Gating by using different flow rates while the sample was maintained at 400 nL/min; 40/360- μ m (ID/OD) capillaries were 40 μ m apart. (a) 40 μ L/min; (b) 80 μ L/min; and (c) 160 μ L/min. B. Gating for various distances between the capillary tips. Gating flow rate was 80 μ L/min, and the sample at 600 nL/min. Gap distance: (a) 30 μ m; (b) 40 μ m; and (c) 60 μ m.

Fig. 6. Flow-gated injection process in the assembled PDMS interface. (a) Normal gating; (b) waiting for gap refilling with gating flow off; (c) injection; (d) Flushing with gating flow on; (e) separation voltage on.

Fig. 8.

(a) A typical electropherogram of 5 amino acids, 1.0 μM each. (b) A typical electropherogram of glutamate and aspartate at 1.0 μM each with overlap injections; Glu and Asp peaks shown are from the immediate former injection. Small peaks are derivatization by-products. (c) Glutamate peak heights of repeated overlap injections. Glutamate and aspartate at 5.0 μM each in aCSF mixed online with the derivatization reagents KCN at 10 mM and NDA at 5 mM. (d) Glutamate peak height variation at various concentrations through a microdialysis probe. The numbers above each plateau indicate glutamate concentrations in the unit of μM . Mixing ratio was 200:200:500 nL/min (NDA:KCN:sample). Separation conditions: separation buffer 20 mM tetraborate buffer at pH 9.2 and electric field 1560 V/cm.