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Encapsulation of Fluidic Tubing and Microelectrodes in Microfluidic Devices: Integrating Off-Chip Process and Coupling Conventional Capillary Electrophoresis with Electrochemical Detection

Vedada Becirovic, Steven R. Doonan, and R. Scott Martin*

Saint Louis University, Department of Chemistry, 3501 Laclede Avenue, St. Louis, MO 63103

Abstract

In this paper, an approach to fabricate epoxy or polystyrene microdevices with encapsulated tubing and electrodes is described. Key features of this approach include a fixed alignment between the fluidic tubing and electrodes, the ability to polish the device when desired, and the low dead volume nature of the fluidic interconnects. It is shown that a variety of tubing can be encapsulated with this approach, including fused silica capillary, polyetheretherketone (PEEK), and perfluoroalkoxy (PFA), with the resulting tubing/microchip interface not leading to significant band broadening or plug dilution. The applicability of the devices with embedded tubing is demonstrated by integrating several off-chip analytical methods to the microchip. This includes droplet transfer, droplet desegmentation, and microchip-based flow injection analysis. Off-chip generated droplets can be transferred to the microchip with minimal coalescence, while flow injection studies showed improved peak shape and sensitivity when compared to the use of fluidic interconnects with an appreciable dead volume. Importantly, it is shown that this low dead volume approach can be extended to also enable the integration of conventional capillary electrophoresis (CE) with electrochemical detection. This is accomplished by embedding fused silica capillary along with palladium (for grounding the electrophoresis voltage) and platinum (for detection) electrodes. With this approach, up to 128,000 theoretical plates for dopamine was possible. In all cases, the tubing and electrodes are housed in a rigid base; this results in extremely robust devices that will be of interest to researchers wanting to develop microchips for use by non-experts.

1. Introduction

Microchip-based systems are becoming widely used analytical tools for a variety of applications.^{1–3} There are numerous advantages of utilizing these systems, one of which is the integration of multiple processes. This can include coupling off-chip processes to the microchip as well as integrating multiple processes on-chip. Ideally, using the microchip for these types of integration results in minimal dilution, fast analysis, and improved temporal resolution so that changes in analyte concentration can be monitored in close to real-time. There are numerous examples of integrating multiple processes with a microchip, including coupling conventional capillary electrophoresis (CE) with microfabricated cell traps for single cell analysis,⁴ combining capillary liquid chromatography with on-chip CE and electrospray ionization,⁵ integrating microdialysis sampling with microchip-based analysis,^{6–10} using multi-layer microfluidic devices for integrating cell culture with analysis,^{11–13} integrating flowing red blood cells, cultured endothelial cells, and electrodes

*Corresponding author: phone: 314-977-2836, fax: 314-977-2521, martinrs@slu.edu.

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for measuring transendothelial electrical resistance,¹⁴ and coupling of digital microfluidic devices with mass spectrometry detection.¹⁵

A key aspect of integrating off-chip processes to the microchip is the manner with which the fluidic tubing is interfaced. For continuous flow processes the dead volume associated with this connection may not be crucial, but, in cases where dilution effects are an issue, the analyte concentration changes rapidly as a function of time, high temporal measurements are desired, or the sample volume is limited, the fluidic connection becomes a factor. The most common method of coupling fluidic tubing with a microchip involves inserting the tubing into or onto the device outer surface. The amount of dead volume with this type of connection can vary depending upon how far the tubing is pressed in the device and/or the size of the hole used to interface with the microchannel. Another method that has been used is inserting tubing into the side of the microchip, butted flush with the microchannel. While this has been shown to result in low dead volume interconnects, the device fabrication is not trivial. For both methods, there are often issues with the stability of the tubing/chip connection, and the device complexity is increased by having multiple interconnects.

Several years ago, our group reported a new method to fabricate microchip devices with integrated electrodes.^{16,17} This involves encapsulating electrodes in epoxy, where the resulting electrode base can be polished and PDMS channels reversibly sealed against the encapsulated electrode. It was shown that 3-dimensional electrodes can easily be created in such a device,¹⁷ and multiple electrode materials can be used to integrate microchip electrophoresis with electrochemical detection.¹⁶ We recently extended this work to show a similar approach can be used to result in electrodes encapsulated in polystyrene, with this substrate leading to much improved cell adhesion.^{18,19}

In this paper, we expand upon our previous work on fabricating epoxy or polystyrene microdevices with encapsulated electrodes by also integrating additional analytical methodology to the devices through low dead volume fluidic interconnects. A key aspect of this approach is that the alignment between the fluidic tubing and electrodes is fixed and robust. The final device can also be polished when desired and sealed against PDMS-based flow channels. It is shown that a variety of tubing can be encapsulated with this approach and the resulting tubing/microchip interface does not lead to significant band broadening or plug dilution. The applicability of the devices with embedded tubing is demonstrated by integrating several off-chip analytical methods to the microchip. This includes droplet transfer, droplet desegmentation, microchip-based flow injection analysis, and microdialysis sampling. In all cases, the new embedded approach, which may also include integrated electrodes, leads to an improved analytical performance when compared to the use of fluidic interconnects with an appreciable dead volume. Interestingly, it is shown that this approach can be extended to also enable the coupling of conventional CE with electrochemical detection. This is accomplished by embedding fused silica capillary along with palladium (for grounding the electrophoresis voltage) and platinum (for detection) electrodes. With this approach, up to 128,000 theoretical plates was possible for dopamine.

2. Materials and Methods

2.1 Microchip Fabrication

For PDMS channels negative masters were fabricated using standard SU-8 50 photoresist as previously described.^{20,21} The structure heights were measured with a profilometer (Dektak ST, Veeco Instruments, Woodbury, NY, USA). Various channel dimensions were utilized for integrating the different analytical methods and in all cases a 20:1 PDMS:curing agent ratio was used. The microchannel dimensions for each were: the droplet transfer studies = 200 μm (in width) $\times$ 105 μm ; the desegmentation experiments = 40 μm (in width) $\times$ 15 μm ;

and the flow analysis studies = 100 μm (in width) $\times$ 65 μm . For the conventional CE with electrochemical detection experiments, the microchannel used to integrate the capillary with the decoupler was either 100 μm (in width) $\times$ 65 μm or 75 μm (in width) $\times$ 25 μm . For the inserted method, microchips contained an inlet hole that was created using a 20-gauge Luer stub adapter (Becton Dickinson, Sparks, MD, USA). The diameter of the inlet hole was measured by microscopy to be 830 μm . For consistency purposes, the tubing was always inserted approximately half-way into the inlet hole. With the ~6 mm thick chips used in these studies, this led to a dead volume for the inlet hole of ~1.6 μL . Information about the imaging aspects of this work including plots involving the monitoring fluorescence over time can be found in the supporting information.

2.2. Encapsulation of tubing and electrodes

Epoxy and polystyrene devices were fabricated in a similar manner as described previously^{16–19} with a few changes specific to the encapsulation of tubing. The tubing utilized in this work included fused silica capillary, perfluoroalkoxyalkane (PFA), fluorinated ethylene propylene (FEP), and polyetheretherketone (PEEK). Incorporating microfluidic tubing into these devices involved preparing the desired type and length of tubing, punching a hole into an aluminum weigh boat, aligning the tubing with the hole, and pouring the epoxy or polystyrene into the weigh boat. The tubing was allowed to protrude past the aluminum weigh boat to avoid clogging. The epoxy devices were allowed to cure at room temperature for about 2 hours. For polystyrene-devices, polystyrene powder (250 μm particle size, Goodfellow, Oakdale, PA, USA) was poured into the aluminum weigh boat and heated at 250° C for approximately 2 hours. The weigh boat was then covered for a more uniform heating until the powder was melted, with additional powder being added to the boat as needed throughout the heating process. The substrate was then allowed to cool to room temperature. Epoxy/polystyrene devices were wet polished using a range of grits (200–1200, Buehler, Lake Bluff, IL, USA) to achieve a smooth surface for incorporation with microchips. Polishing was achieved by using a variable speed grinder-polisher (MetaServ 3000, Buehler, Lake Bluff, IL, USA). If the tubing became clogged after polishing, the device was sonicated in water for ~10 min to dislodge any material. Electrodes were prepared as previously described^{16–19} and incorporated into these devices in a similar manner. A linear alignment of the tubing i.d. and the electrode was essential in order to ensure proper integration with the microfluidic channels post fabrication. For the droplet transfer and desegmentation studies, 150 μm -i.d. PFA tubing was embedded into a polystyrene device. For all the flow analysis studies, an epoxy device that incorporated 50 μm -i.d. fused silica capillary and a glassy carbon detection electrode (1 mm dia.) was utilized. For conventional CE studies, an epoxy device was used with a 50 μm -i.d. fused silica capillary that was aligned with a palladium decoupler (1 mm and 2 mm in dia.) and a platinum electrode (0.5 mm dia.).

2.3 Droplet Transfer Studies

For the droplet transfer studies as well as the microchip-based flow studies, a comparison was made between the more traditional inserted tubing method and this new embedded tubing method (refer to Figure 1 for a depiction of each). For the inserted method, the tubing was typically inserted ~half-way into the chip (See Figure 1A). Droplet studies using an embedded 150 μm -i.d. PFA tubing in a polystyrene device required sealing a 100 μm $\times$ 65 μm microchannel over the tubing i.d. Droplets were generated as described previously²² using a 150 μm -i.d. T-junction (Valco, Houston, TX), with perfluorodecalin (PFD) being used as the carrier phase and fluorescein in water being the aqueous phase. For volume studies two videos were recorded (see imaging section for more details), one of the droplets intubing and one of droplets after they had been transferred on-chip. Images from each video were then used to determine the droplet size. Desegmentation studies were based upon

previous work where an air/water interface was created at a channel intersection.²² To create the air/water interface, the tubing was inserted into a vial that was connected to a water aspirator to create a vacuum (see Figure 3E). Water was inserted into one chip reservoir, with the other remaining open to air. A corona discharge unit fitted with a fine tip electrode (model ETP BD-20, Electrotechnic Products, Inc., Chicago, IL) was used to treat the air channel, making it hydrophilic (with the water-containing channel remaining hydrophobic). PFD/fluorescein droplets created off-chip in the 150 μm -i.d. T-junction were then introduced *via* the tubing, with droplet desegmentation being achieved at the interface.

2.4 Microchip-based Flow Analysis

Microchip-based flow injection analysis was used to compare the embedded and inserted tubing methods, both with integrated electrodes. For off-chip injections, a 4- port injection valve fitted with a 200 nL rotor was used (Vici Rotor, Valco Instruments, Houston, TX). A TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid, Sigma-Aldrich, St. Louis, MO) buffer flow stream was continuously pumped (with a PHD 2000 syringe pump, Harvard Apparatus, Holliston, MA) through the valve (at 2.0 $\mu\text{L}/\text{min}$) to the flow channel sealed over the electrode. The analyte syringe was connected to the 4-port valve with a 75 μm i.d. capillary tubing using a finger tight PEEK fitting and a luer adapter (Upchurch Scientific, Oak Harbor, WA). For the inserted method studies (Figure 1A), the same connectors and a 50 μm i.d. capillary fitted with a 794 μm o.d. microtight sleeve (Upchurch Scientific, Oak Harbor, WA, USA) were used to transition from the 4-port injection valve to the microchip. For the embedded method studies (Figure 1B), the embedded capillary was connected directly to the 4-port injection valve. Amperometric detection was achieved using a CH Instruments poten-tiostat (812B, Austin, TX, USA). The working electrode was a 1 mm dia. glassy carbon electrode and a platinum wire inserted into the waste reservoir served as the counter electrode.

On-capillary hydrodynamic injections were made by pressurizing a vial (with helium) containing the analyte solution for a predetermined time. Two different pressure/injection time setups were used for these studies, with both using helium gas controlled by a regulator. One involved a solenoid valve (MAC Fluid Power Engineering, St. Louis, MO) that was controlled with an analog power supply and timer unit (Instrument Design Lab, University of Kansas, Lawrence, KS). The other method utilized a Lee valve (LHDA053115H, The Lee Co, Essex CO) that was integrated with a National Instruments Data Acquisition Board (National Instruments, Austin, TX). The acquisition board was controlled by LabView with in-house code that enabled the desired injection and run times. For either setup, a vial cap with a rubber septum as well as two vials were utilized, one with buffer and one with desired sample. The tubing from the solenoid valve was inserted through the rubber septum as was the capillary leading to the microchip. The capillary/chip was first flushed with buffer for a given time by continuously pressurizing the buffer vial. An injection was achieved by pressurizing the sample vial for a given time, with this plug being moved through the system by then pressuring the buffer vial. A typical injection pressure was 10 psi. With a 1 sec injection and a 25 cm (in length), 50 μm i.d. capillary at 20°C, this led to an injection volume of ~40 nL. Amperometric detection was carried out as described in the previous section.

Microdialysis sampling of a concentration change was done for both embedded and inserted methods. A commercially available 4-mm membrane brain microdialysis probe (BASi Instruments, West Lafayette, IN) was prepared by placing two tubing connectors (Flanged Tubing Connectors, BASi Instruments) to both inlet and outlet sides (o.d. 635 μm and i.d. 254 μm). The probe was rinsed with water for at least 24 hours prior to the experiment. The inlet side of the probe was connected to a syringe (via a 50 μm i.d. capillary), and the outlet side was either connected to the capillary that was inserted into a PDMS microchip or to a

capillary that was already encapsulated into the epoxy. In both cases, the capillary that was to be connected to the probe was fitted with a 794 μm o.d. microtight sleeve (Upchurch Scientific). This made a snug connection with the flanged tubing connector. A perfusate flow rate of 0.8 $\mu\text{L}/\text{min}$ was used for these studies. To stimulate a concentration change, the probe was first placed in a vial containing 1 mM fluorescein and allowed to equilibrate by reaching a steady fluorescence intensity (as measured by a fluorescence microscope described in the imaging section). The concentration change was initiated by moving the probe to a vial containing 5 mM fluorescein for 10 sec, after which time the probe was re-inserted into the 1 mM fluorescein vial.

2.5 Conventional Capillary Electrophoresis

For the CE experiments, on-capillary hydrodynamic injections were made as described above with a solenoid valve, an analog power supply, and timer unit (Instrument Design Lab, University of Kansas, Lawrence, KS) controlled the solenoid valve. The only difference was that the vial cap had a platinum wire inserted through the septum. This wire was connected to a 30 kV power supply (Glassman High Voltage, Inc., Whitehouse Station NJ). All CE experiments used a 50 μm i.d. fused silica capillary. While the injection volume was not the same in all experiments, a typical injection pressure was 2.5 psi. With a 50 cm capillary and a 0.5 sec injection (at 20 $^{\circ}\text{C}$) this led to an injection volume of ~ 3 nL. This volume could be tuned by changing the injection pressure or time. For all experiments, the palladium decoupler was the electrophoretic ground (cathode). Amperometric detection was done using a CH Instruments potentiostat. The working electrode was platinum, and a platinum wire served as the counter electrode. The buffer used was 25 mM boric acid (pH 9.2). Samples were prepared by dissolving analytes in water followed by dilution to the desired concentration in electrophoresis buffer. Between each electrophoresis run, the capillary was flushed for 4 min with buffer. Prior to use, the buffer was degassed for 10 min with helium gas at 2.5 psi.

For studies involving Ohm's Law plots (data in the supplemental information), a comparison was made between an encapsulated capillary coupled with an on-chip palladium decoupler and a straight capillary that was simply grounded in a buffer vial. For the palladium microchip studies, an 8-cm capillary (50 μm i.d.) encapsulated in epoxy was used. A PDMS microchannel was sealed over the capillary i.d. and a 1 mm palladium decoupler. For the capillary comparison studies, the capillary outlet was inserted into a separate buffer vial, with a platinum wire (inserted through the vial septum) being connected to ground. To measure the electrophoretic current as a function of the applied separation voltage, a 1 M resistor was connected in series between the ground and either the palladium decoupler (for chip studies) or the platinum wire (for straight capillary studies). A voltmeter was used to measure the voltage drop across the resistor and the current was calculated from this value. The high voltage was increased in 800 V increments. The electrophoresis buffer was 25 mM boric acid (pH 9.2).

3. Results and discussion

3.1 Encapsulation of Fluidic Tubing and Electrodes in Microchip Devices

The overall goal of this work was to build upon our previous work on fabricating epoxy or polystyrene microdevices with encapsulated electrodes by integrating additional analytical methodology to the devices through low dead volume fluidic interconnects.^{16–19} Several different methods have been used to integrate off-chip process to the microchip through fluidic tubing. The most common method involves inserting the tubing into or onto the surface of the device, with the example shown in Figure 1A being for a PDMS microchip. The amount of dead volume with this type of connection is dependent upon how far the

tubing is pressed in the device and/or the size of the connecting hole. In addition, the exact dead volume of this type interconnect can vary by device. For continuous flow analysis this dead volume may not be critical, but for off-chip methods, where concentration changes are important, it can be substantial. In this work, we compared this new embedded approach with the more traditional method of inserting the tubing directly into the PDMS microchip (Figure 1). For consistency reasons and to make a comparison with fluidic interconnects that have an appreciable dead volume, in this work the tubing was inserted half-way into the device. As shown in the figure, this leads to a relatively large amount of dead volume (with these devices, $\sim 1.6 \mu\text{L}$). In addition, this type of inserted connection is not very stable, with any perturbation to the tubing possibly causing leakage or causing the chip to no longer be sealed.

Another method that has been used with PDMS and glass devices to make low dead volume interconnects is to have the tubing inserted into the side of the microchip, butted flush with the microchannel. With glass devices, this requires fabrication of channels on both sides of the substrate so that that tubing i.d. is perfectly matched in the microchip.¹⁰ When using PDMS devices, the tubing of interest can be laid alongside the microchannel structure and the PDMS poured over both.²² While both approaches do result in low dead volume connections, the fabrication is not trivial, and there can still be issues with the stability of the tubing/chip connection. In this work, we show that various types of tubing can be encapsulated in both epoxy and polystyrene at the same time and manner as other functionalities, such as electrodes for electrochemical detection (see Figure 1B). The resulting epoxy or polystyrene base can be polished to result in a smooth surface that can be sealed against PDMS microchannels. A variety of different tubing can be encapsulated with this approach including fused silica capillary ($50\text{--}150 \mu\text{m}$ i.d.), PEEK ($150 \mu\text{m}$ i.d.), FEP ($795 \mu\text{m}$ i.d.), and PFA ($150 \mu\text{m}$ i.d.). The tubing encapsulation is rugged, with the tubing and electrode alignment being fixed in the epoxy or polystyrene so that a microchannel can be reversibly sealed over both for integrated detection. The devices can be disassembled, polished as desired (to create a fresh electrode surface), and reassembled. Devices with encapsulated tubing and electrodes have been used for up to 1 year, demonstrating the robust nature of this approach. In this paper, we wanted to investigate the use of this embedded approach to integrate off-chip functions such as droplet transfer, droplet desegmentation, microchip-based flow analysis, and conventional CE with a microchip device.

3.2 Evaluation of Capillary to Chip Transfer

A key feature of these embedded tubing interconnects is the possibility for a low dead volume interface. To first investigate the amount of band broadening that arises from transferring an analyte band from tubing onto a microchip with the embedded approach, a flow injection comparison study was made between a $50 \mu\text{m}$ i.d. capillary and a $50 \mu\text{m}$ i.d. capillary embedded in epoxy. For the capillary study, a detection window was made 25 cm from the inlet. With the embedded capillary, a microchip was sealed over the encapsulated capillary i.d. (see Figure 1C for a micrograph of the capillary/chip interface), and detection was made in the microchip after the plug had been transferred (length to the detection zone was also 25 cm). For both, a 40 nL fluorescein plug was hydrodynamically injected into the capillary inlet. After injection, buffer was used to pump this plug through the capillary at 10 psi. The amount of fluorescence was monitored as a function of time at the detection window with a fluorescence microscope. The results of this experiment are seen in Figure 2, with an overlay of 5 injection and detection events for each method. As can be seen in the figure, very little band broadening occurs during the transfer from the capillary to the microchip. Analyses of these peaks (Figure 2C) show no statistical difference (as tested by a t-test at the 95% confidence level) between the peak width at half-height for the capillary and the on-chip measurements. There is a statistical difference (as tested by a t-test at the

95% confidence level) between the average peak skew values (measured at 10% of the peak height) (p -value = 0.02); however, there is just a very slight increase in the peak skew (≈ 0.14). This study shows that very little band broadening occurs during the transfer of analyte bands from the capillary onto the microchip using this embedded tubing approach.

3.3 Droplet transfer studies

To further characterize the ability of embedded tubing to make low dead volume connections, droplet transfer studies were undertaken. The use of droplets generated off-chip and then transferred to the microchip for analysis has been shown by others to be very helpful in conserving concentration changes that occur in methods such as microdialysis sampling and also to couple different separation modes.^{10,23–26} It has been shown, however, that low dead volume fluid connections are needed to ensure that the droplets do not merge or coalesce during the microchip transfer.^{10,25} In this work, droplets were generated off-chip using a T-junction, PFD as the carrier fluid, and fluorescein in water as the aqueous phase. A comparison was made between the encapsulated and inserted tubing methods by analyzing the droplet volume both in tubing as well as on-chip post-transfer. The findings are shown in Figure 3. For the embedded method, the average droplet volumes were 8.3 ± 1.7 nL in-tubing and 10.4 ± 1.6 nL on-chip after transfer (Figures 3A–B). This implies on average coalescence of 1.25 droplets during the transfer from tubing to the microchip. A similar coalescence value (1.0) is seen by comparing the droplet frequency both in-tubing and on-chip (see supplemental information, Figure S1). The inserted method, however, showed a great deal of droplet coalescence. As seen in Figures 3C–D, for this study the average droplet volume in-tubing was 5.7 ± 0.8 nL, while once it was transferred on-chip many droplets converged, and the average droplet volume was 32.9 ± 7.7 . This implies that, on average, 5.8 droplets coalesce with the inserted method. It is clear that the low-dead volume nature of the encapsulated tubing enables droplets to be transferred from off-chip processes. A key advantage of this approach is that the tubing is fixed in the epoxy or polystyrene base. This results in a very robust tubing/chip interface.

One issue with using droplets is that some techniques such as electrochemistry and electrophoresis require the removal of the non-conductive carrier phase prior to the analytical measurement. We previously reported an approach for PDMS devices where an air/water interface can be created at the intersection of two channels.²² Treatment of the air-filled channel with a corona discharge results in that channel becoming hydrophilic while the water containing channel remains hydrophobic. In that study, the off-chip droplets were introduced to the microchip through tubing coming from the side of the microchip (as described in the preceding section). As shown in Figures 3E–F, by applying a vacuum to a vial in which the encapsulated PFA tubing inlet had been placed, an air/water interface can easily be created in a PDMS microchip. In this case, a polystyrene base was used, with water and air being pulled through their respective reservoirs to the chip intersection and through the tubing. By applying the corona discharge to the air reservoir, that channel was rendered hydrophilic. In Figure 3F, it can be seen that when the segmented flow encounters the hydrophobic/hydrophilic intersection only the aqueous (fluorescein) phase enters the hydrophilic channel, with the PFD remaining in the hydrophobic channel. Due to the robust nature of the encapsulated tubing, this could be used in future work for integrating electrochemical detection²² or electrophoresis for droplet analysis.^{10,22,25}

3.4 Microchip-based flow analysis

In cases where the detection mode is selective, it is common to integrate off-chip injection methods with microfluidic devices so that plugs of analyte can be introduced in a reproducible and straightforward fashion. There have been several reports of inserting the fluidic tubing into the device, with the tubing usually being connected to a 4 or 6-port

injector.^{17,27–29} In this study, microchip-based flow injection analysis using both the inserted and embedded fluidic methods was performed with both an off-chip injector (4-port rotary valve) and on-capillary hydrodynamic injection. Importantly, in these studies a glassy carbon detection electrode was also embedded in the epoxy base and amperometry was used as the detection mode. For the embedded studies, a microchannel was sealed over the capillary i.d. as well as the glassy carbon electrode. The epoxy base (that contains the tubing and electrode) can be polished each day before analysis is desired.

Catechol was used as the test analyte and an overview of the results is seen in Figure 4. In general, the most dramatic outcomes were seen with the on-capillary injection studies, where there was a much reduced injection volume (40 nL), as compared to the 4-port valve (200 nL). For both, considerable differences were seen in terms of peak size, shape, peak width at half-height, and sensitivity for the embedded approach. With on-capillary injection (Figures 4A–B), the embedded method provided a peak-width at ½ height of 4.3 sec, as compared to a 8.5 sec peak-width at ½ height for the inserted method. The embedded method also led to more symmetrical peaks and a better sensitivity (0.5813 nA/μM vs. 0.3182 nA/μM for the inserted method, see Figure S2). While less of an effect on the peak height was seen with the larger volume off-chip injection scheme, there was still a significant difference between the embedded and inserted capillary methods. With the off-chip injection, the embedded method resulted in a peak-width at ½ height of 9.0 sec while the inserted method had a much larger peak-width at ½ height of 24.7 sec.

The final flow analysis study involved the use of a microdialysis probe. There have been numerous studies of integrating microdialysis sampling with microchip-based analysis.^{6,8–10,26,30} For this work, a microdialysis probe was affixed to capillary that was either embedded into an epoxy base or later inserted into the microchip. To simulate a concentration change, the microdialysis probe was moved from a vial of 1 mM fluorescein to a vial containing 5 mM fluorescein for 10 sec, followed by it being moved back to the 1 mM fluorescein vial. As can be seen in Figure S3, for the embedded method the average lag time was 87.7 sec and the average peak width at 10% height was 94.7 sec, while the inserted method demonstrated an average lag time of 93.3 sec and an average peak width at 10% height of 120.7 sec. The effect of the lower dead volume encapsulated tubing connection was not as dramatic as with the microchip-based flow injection studies, primarily due to dilution effects in the relatively large volume commercially-available probe as well as the microconnectors used to transition from the probe to the tubing. However, this clearly shows that embedded approach with microdialysis sampling resulted in a more dramatic response to the concentration change.

3.5 Integrating Conventional CE with Electrochemical Detection

There have been numerous approaches for coupling conventional CE (using fused silica capillaries) with electrochemical detection.^{31–36} The most common method involves the use of a bare fracture decoupler, where a fused silica capillary is fractured and placed in a vial so that electrophoretic voltage can be grounded through the fracture but analyte bands are pushed through the remaining capillary and detected with electrodes inserted into the capillary outlet.^{32–36} While effective, these systems are homemade in nature, require a good deal of expertise to create, and, as opposed to fluorescence and absorbance detection for CE, have yet to be commercialized. Since the embedded capillary method had been seen to result in a low dead volume interconnect that can also be integrated with electrodes, we next explored using this approach to integrate conventional CE with electrochemical detection. Palladium electrodes, along with platinum detection electrodes, were embedded as well. Palladium has been shown, by others with CE³¹ and our group with microchip electrophoresis,^{11,16,37} to be an effective method of decoupling the separation voltage from

the detector by providing a path to ground before the detection electrode and absorbing the resulting hydrogen that is produced from the electrolysis of water at the cathode.

Figure 5 shows the experimental setup used for integrating conventional CE with electrochemistry in this work. The capillary was embedded in epoxy along with a palladium decoupler (1 mm dia.) and platinum detection electrode (0.5 mm). As shown in Figures 5B–C, a PDMS microchannel was used to interface the capillary with the palladium decoupler and platinum detection electrode. After a hydrodynamic injection had been made, the electrophoresis voltage was applied, and analyte bands electrophoresed through the capillary and were transferred into the microchannel where the voltage was grounded by the palladium decoupler. The analyte band then passed over the platinum electrode before exiting the microchannel to an outlet reservoir that also contained the platinum counter electrode. The effect of the PDMS interfacing microchannel size on the electrophoresis separation was first investigated. Figure 6 compares the separations of dopamine, norepinephrine, epinephrine, and catechol (100 μM each) using different two different channel dimensions and a field strength of 200 V/cm. Use of a 100 $\mu\text{m} \times 65 \mu\text{m}$ microchannel resulted in 16,500 theoretical plates for dopamine, 15,800 theoretical plates for norepinephrine, 13,600 theoretical plates for epinephrine, and 21,400 theoretical plates for catechol. A smaller interfacing microchannel (75 $\mu\text{m} \times 25 \mu\text{m}$) resulted in improved performance, with 45,000 theoretical plates for dopamine, 36,000 theoretical plates for norepinephrine, 40,700 theoretical plates for epinephrine, and 42,000 theoretical plates for catechol. This much improved performance can be explained by the cross-sectional area of the 75 $\times$ 25 μm microchannel (1875 μm^2) more closely matching the 50 μm capillary (1962.5 μm^2), with the larger channel 100 $\mu\text{m} \times 65 \mu\text{m}$ (6500 μm^2) resulting in significant band broadening after the transfer to the microchannel. An Ohm's Law study (see the supplemental information, Figure S4) was also carried out, with a straight 50 μm i.d. capillary that was grounded in a buffer vial showing similar trends as an embedded 50 μm i.d. capillary with a 75 $\times$ 25 μm interfacing microchannel and a palladium decoupler ground. In both, non-linear behavior could be seen at field strengths greater than 600 V/cm.

The effect of field strength on a separation was next investigated using the 75 $\times$ 25 μm interfacing microchannel (Figure 7). Using a field strength of 200 V/cm yielded 39,000 theoretical plates for dopamine and 36,600 theoretical plates for catechol. As expected, increasing the field strength resulted in improved separation performance, with 400 V/cm giving 75,000 theoretical plates for dopamine and 55,000 theoretical plates for catechol. The limit of detection for this setup was found to be 740 nM. In the above studies, a 3 nL injection volume was used. Using a smaller injection volume (0.5 nL) with a field strength of 200 V/cm also resulted in an improved separation performance (Figure S5), with the number of theoretical plates for dopamine and catechol being 128,600 and 91,000, respectively. It should be noted that this study utilized relatively large (500 μm dia.) platinum electrodes that also could be subject to electrode fouling. Future work will investigate the use of smaller electrode materials that are not as prone to fouling (such as carbon). However, this study clearly shows that the encapsulation of capillary and electrodes is an effective means to couple conventional CE with electrochemical detection. As opposed to other decoupling methods, the alignment is fixed, reproducible and reusable. The device can also be disassembled and polished when desired.

4. Conclusion

This work demonstrated that embedding fluidic tubing, along with other components such as electrodes, in epoxy or polystyrene can lead to low dead volume interconnects. Importantly, alignment between the fluidic tubing and electrodes is fixed and the substrate can also be polished when desired and sealed against PDMS-based flow channels. It was shown that this

approach, when compared to the use of fluidic interconnects with an appreciable dead volume, can result in an improved analytical performance for the integration of several off-chip analytical methods including droplet transfer and microchip-based flow analysis. This approach was extended to also integrate conventional CE with electrochemical detection, with up to 128,000 theoretical plates for dopamine being obtained. In all cases, the tubing and electrodes are housed in a rigid base; this results in extremely robust devices that will be of interest to researchers wanting to develop microchips for use by non-experts. Future work will entail further exploration of using this approach to integrate conventional CE with electrochemistry, the coupling of microchip electrophoresis with conventional CE for enhanced separation capabilities, and chip-to-chip interconnections for modular cell-culture devices.

Supplementary Material

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Figure 1. Inserted versus embedded tubing design with integrated electrodes. (A) Schematic of off-chip injection with inserted capillary; (B) Schematic of off-chip injection with embedded capillary; (C) Image of tubing/chip interface.

Figure 2.

Overlay of fluorescence signal for flow injection analysis of fluorescein (40 nL injection) with (A) on-capillary detection; (B) on-chip detection after transfer from the capillary. The length from the inlet to the detection window was 25 centimeters for each. (C) Table comparing peak width at half height and average skew at 10% height ($n = 5$ for both). A 50 μ m i.d. fused-silica capillary was used for these studies.

Figure 3.

Droplet transfer studies (A) Droplets in 150 μ m i.d. PFA tubing; (B) Droplet transfer from PFA tubing to microchip with embedded method; (C) Droplets in 150 μ m i.d. PFA tubing; (D) Droplet transfer from PFA tubing to microchip with inserted method; (E) Desegmentation design showing air/ water interface before treatment; (F) After corona treatment down air-filled channel, with droplets being desegmented at the interface.

Figure 4.

Overlay amperograms of microchip-based flow injection analysis of catechol (50 μM) using **(A)** on-capillary injection with embedded capillary; **(B)** on-capillary injection with inserted capillary; **(C)** off-chip injection with embedded capillary; **(D)** off-chip injection with inserted capillary; **(E)** Table comparing peak width at half-height for each method. In all cases a 50 μm i.d. fused silica capillary was used.

Figure 5.

(A) Setup for integrating conventional CE with electrochemical detection. A 50 µm i.d. fused-silica capillary is embedded along with a palladium decoupler and platinum working electrode. A PDMS microchip is used to interface the capillary with the decoupler and detection electrode. (B) Micrograph of the microchannel showing the 50 µm i.d. capillary, 2 mm palladium decoupler, and 500 µm platinum detection electrode; (C) Close up of the capillary-microchannel interface.

Figure 6.

(A) Effect of microchannel cross-sectional area on the separation of dopamine (DA), norepinephrine (NE), epinephrine (EPI), and catechol (CAT) (each at 100 mM) using a field strength of 200 V/cm; (B) Effect of field strength on the separation of dopamine (DA) and catechol (CAT) (100 mM each) utilizing a $75\ \mu\text{m} \times 25\ \mu\text{m}$ PDMS interfacing microchannel. In each case, a 3 nL injection was made. Experimental details: buffer = 25 mM boric acid (pH 9.2), decoupler = 2 mm palladium electrode; detection electrode = 500 μm platinum electrode.