A new method of fabricating electrodes for microchip devices that involves the use of Teflon molds and a commercially available epoxy to embed electrodes of various sizes and compositions is described. The resulting epoxy base can be polished to generate a fresh electrode and sealed against poly(dimethylsiloxane) (PDMS)-based fluidic structures. Microchip-based flow injection analysis was used to characterize the epoxy-embedded electrodes. It was shown that gold electrodes can be amalgamated with liquid mercury and the resulting mercury/gold electrode is used to selectively detect glutathione from lysed red blood cells. The ability to encapsulate multiple electrode materials of differing compositions enabled the integration of microchip electrophoresis with electrochemical detection. Finally, a unique feature of this approach is that the electrode connection is made from the bottom of the epoxy base. This enables the creation of three-dimensional gold pillar electrodes (65 \( \mu \text{m} \) in diameter and 27 \( \mu \text{m} \) in height) that can be integrated within a fluidic network. As compared with the use of a flat electrode of a similar diameter, the use of the pillar electrode led to improvements in both the sensitivity (72.1 pA/\( \mu \text{M} \) for the pillar versus 4.2 pA/\( \mu \text{M} \) for the flat electrode) and limit of detection (20 nM for the pillar versus 600 nM for the flat electrode), with catechol being the test analyte. These epoxy-embedded electrodes hold promise for the creation of inexpensive microfluidic devices that can be used to electrochemically detect biologically important analytes in a manner where the electrodes can be polished and a fresh electrode surface is generated as desired.

Keywords:
Electrochemical detection / Microchip electrophoresis / Pillar electrode

1 Introduction

The ability to utilize small sample volumes [1], perform fast/high-throughput analysis [2, 3], and integrate multiple processes [4–6] has made microchip-based systems a powerful analytical tool. Electrochemistry is a popular detection mode for these systems, as many small molecules (such as catecholamine neurotransmitters) can be directly detected without a derivatization step [7–9]. One common way to integrate electrochemical detection into a microfluidic network has been through the use of glass substrates that have been sputter-coated with an adhesion layer (typically \( \sim 200 \text{ Å} \) titanium) and an overlying metal layer (typically \( \sim 2000 \text{ Å} \) gold, platinum, or palladium). Electrode designs are patterned by standard lithography and either wet-etching or lift-off techniques [10], followed by bonding of the structure with another layer that contains the microfluidic flow channels. These devices have been used by our group [5, 11–15] and others [16–21] to detect many different analytes. The selectivity of these devices can be altered by modification of the electrode, control of the detection potential, or use of multiple electrodes [9, 11, 15, 19, 22]. While the patterned thin-layer plate electrodes provide many advantages, they are costly and require a specialized facility for fabrication. The sputtering process also takes a significant amount of time (from pump-down and controlled deposition rates) and multiple sputtering/patterning steps are required if different electrode materials are desired on one plate. If the electrode material is modified with a layer such as Hg, the surface is limited to one use and cannot be polished [11]. Finally, everything within the device, including the connections and the thin layer of electrode material, is planar. It has been our experience that this limits the overall electrode height to \( \sim 1 \text{ μm} \) when integration within a fluidic network is desired [15].

The conventional way to couple liquid chromatography (LC) with electrochemical detection is through the use of a...
flow cell [23–27]. These cells have electrodes embedded in polyether etherketone (PEEK) and can be easily assembled or disassembled. LC flow cells can include electrode materials, such as glassy carbon, carbon paste, copper, gold, platinum, or silver. The LC flow cells can contain one electrode or multiple electrodes running in serial or parallel mode. These electrodes can be modified to improve the selectivity [23]. The flow cells are reusable and can also be polished for everyday use, which increases the lifetime of the electrode material because it generates a fresh electrode surface. Electrochemical flow cells for LC are commercially available, but there has been no analogous work in microfluidic devices.

In this paper, we sought to develop a method to integrate electrochemical detection with microchip-based analysis systems in a manner that is analogous to the use of an electrochemical flow cell in LC. The approach that is described involves the use of Teflon molds and a commercially available epoxy to embed electrodes of various sizes and compositions. It is shown that the resulting epoxy-embedded electrodes offer many of the same advantages as the conventional LC-electrochemical flow cells and can be used as detectors for microchip-based flow injection analysis and electrophoresis. One major advantage of the epoxy-embedded electrodes is that they can be polished and a fresh electrode surface is generated as desired, giving the electrodes a longer lifetime. These electrodes, as compared to sputtered plates, are also inexpensive and easy to fabricate. Microchip-based flow injection analysis using single and dual gold detection electrodes was used to initially characterize the epoxy-embedded electrodes. Planar gold electrodes were amalgamated with liquid mercury and the resulting Hg/Au electrode used to selectively detect intracellular glutathione (GSH) from red blood cells (RBCs). It was also demonstrated that multiple electrode materials of differing compositions can easily be incorporated into the epoxy base. This enabled the integration of microchip electrophoresis with electrochemical detection through the use of a palladium electrode that functions as a decoupler and a downstream gold detection electrode. Finally, a unique feature of this approach is that the electrode connections are made from the bottom of the epoxy base. It is shown that this allows the creation of three-dimensional gold pillar electrodes that protrude into the microfluidic network. These gold pillars greatly increased both the electrode surface area and the device performance, with a 30 times improvement in the limit of detection (LOD), as compared with the use of a planar gold electrode.

2 Materials and methods

2.1 Materials

The following chemicals and materials were used as received: Nano SU-8 developer, SU-8 50, and SU-8 10 photoresist (Microchem, Newton, MA, USA); catechol, dopamine, epinephrine, boric acid, TES sodium salt, MES sodium salt, sodium dodecyl sulfate, potassium dichromate (I), sodium carbonate, mercuric, L-glutathione reduced, tris(hydroxymethylaminomethane), Ringers, potassium ferri cyanide, and potassium nitrate (Sigma Aldrich, St. Louis, MO, USA); Armstrong C-7 resin, Activator A, and Sylgard 184 (Ellsworth Adhesives, Germantown, WI, USA); 1 mm and 25 μm gold wire and 1 mm Palladium wire (Alfa Aesar, Ward Hill, MA, USA); soldering wire and heat shrink tubes (Radioshack); isopropanol and acetone (Fisher Scientific, Springfield, NJ, USA); colloidal silver (Ted Pella, Redding, CA, USA); BSA electrode polishing kit (Bioanalytical Systems, West Lafayette IN, USA); 8.89 cm diameter Teflon PTFE rod (McMaster-Carr, Chicago, IL, USA).

2.2 Fabrication

A schematic representation demonstrating the fabrication of these epoxy-embedded electrodes is shown in Fig. 1. A two-part Teflon mold was machined by traditional lathe-based turning. The base mold was turned to an od of 8.69 cm and bored to an id of 7.87 cm, with a 0.76 cm inner depth. The first electrode insertion hole was drilled with a 1 mm diameter bit located 2.74 cm away from the bottom of the base mold. The second hole was drilled 1 mm away from the first. The outer mold was bored to an od of 7.87 cm and an id of 6.86 cm, with the outer mold matching the id of the base mold for assembly. For fabrication of the epoxy-embedded electrode base, the desired wire (gold of 1 mm or 25 μm diameter; Palladium of 1 mm diameter) was affixed (soldered or connected with colloidal silver) to an extending copper wire to provide the electrical connection. Heat shrink tubing was used to insulate the connection before insertion into the mold. The electrode was inserted through the hole in the Teflon mold in a vertical orientation. Following the assembly of the mold and electrode, a thoroughly mixed combination of 57.5 g Armstrong C-7 adhesive (resin) and 4.8 mL Armstrong Activator A was poured into the mold (Fig. 1A). The mixture was cured for at least 2 h (Fig. 1B) and was removed from the mold. The base mold was removed first (as shown in Fig. 1C), followed by the removal of the outer mold. After removal from the two Teflon molds, the epoxy was ready for shaping by wet polishing. Wet polishing was achieved first with a rough grit of 200 sandpaper (Buehler, Lake Bluff, IL, USA) for 5 min, followed with a 2 min (each) polishing with higher grits (400, 800, and 1200).

After shaping the electrode base by wet polishing, the epoxy-embedded electrodes were polished for everyday use with a BAS electrode polishing kit (Fig. 1D). The electrode was first rinsed with water and a fine grit pad was placed with the adhesive side attached to the provided glass plate. An alumina suspension was added to the polishing pad before placing the electrode face down. A smooth, circular motion was applied for ~1 min with uniform pressure to generate a fresh electrode surface. After polishing, the
electrode was thoroughly rinsed with deionized water before use.

### 2.3 Flow analysis and electrophoresis

Negative masters for the PDMS structures were fabricated as previously described [19, 28] using SU-8 50 photoresist for PDMS-based flow injection channels and SU-8 10 for the electrophoresis chip design. The structure heights were measured with a profilometer (Dektak® ST, Veeco Instruments, Woodbury, NY, USA). For these studies, PDMS structures were reversibly sealed over the electrode surface (as shown in Fig. 2), although it was also found that PDMS can be irreversibly sealed to the epoxy electrodes following treatment of both layers in a plasma cleaner (Harrick Scientific, Pleasantville, NY, USA). The setup used for microchip-based flow analysis has been previously reported [11, 29, 30]. A PDMS flow channel (100 μm width, 100 μm height, and 3 cm length) was reversibly sealed on the epoxy surface over the electrode. A hole punch was used to make the outlet reservoir and the inlet hole was created using a 20-gauge Luer stub adapter (Becton Dickinson, Sparks, MD, USA). A buffer flow stream was continuously pumped at 3.0 mL/min to the flow channel via a 500 mL syringe (SGE Analytical Science) and a syringe pump (Harvard 11 Plus, Harvard Apparatus, Holliston, MA, USA). The syringe was connected to 150 μm id capillary tubing using a finger-tight PEEK fitting and a Luer adapter (Upchurch Scientific, Oak Harbor, WA, USA). The same connectors and a 150 μm id capillary fitted with a 794 μm microtight sleeve (Upchurch Scientific) were used for transition from a four-port rotary injection valve (Vici Rotor, Valco Instruments, Houston, TX, USA) to the microchip (see picture in Fig. 2A). The four-port injection valve enabled reproducible 200 nL injections to the PDMS-based flow channel. Amperometric detection was performed with a three-electrode system using a CH Instruments potentiostat (Austin, TX, USA). Gold was used as the working electrode; platinum wire was used as the auxiliary and Ag/AgCl was used as the reference.

Cell studies were carried out on human RBCs from a healthy subject. The cells were graciously isolated and donated by Dr. Randy Sprague’s Laboratory in the Department of Pharmacological and Physiological Sciences at the Saint Louis University Medical School, as previously reported [31, 32]. Human blood was centrifuged at 500 × g at 4°C for 10 min and the plasma, Buffy coat, and the upper layer of RBCs were removed by aspiration. The packed RBCs were resuspended and washed three times in a Tris/Ringers wash buffer (4.7 mM KCl, 2.0 mM CaCl₂, 140.5 mM NaCl, 1.2 mM MgSO₄, 21.0 mM tris(hydroxymethylaminomethane) and 5.5 mM glucose with 0.5% bovine serum albumin). RBCs were diluted from an 86% hematocrit to a 1% hematocrit with Tris/Ringers buffer that did not contain glucose or bovine serum albumin. For experiments where intracellular glutathione was determined, lysis buffer (same as dilution buffer with the addition of 30 mM SDS, pH 7.4) was loaded into the syringe pump and continuously pumped to the microchip at 3.0 μL/min. The cell solution was injected onto the microchip via the four-port injection valve. The cells lysed as they transversed the fluidic network and the intracellular GSH was detected at the Hg/Au amalgam electrode (preparation described below). Amperometric detection at the Hg/Au electrode was carried out at +0.023 V versus a saturated calomel electrode (SCE). Electrophoresis separations were performed with a gated injection scheme using PDMS flow channels (24 μm tall and 40 μm wide) and a buffer of 10 mM boric acid with 25 mM SDS (pH of 9.2). The gated injections were carried
out by applying a high voltage (HV) (+1200 V) to the buffer reservoir, a fraction of the HV (+960 V) to the sample reservoir, with the sample waste reservoir and decoupler being grounded (field strength of 240 V/cm). Injections were achieved by floating the HV for 500 ms. A LabSmith HVS448 3000 V High Voltage Sequencer with eight independent HV channels (LabSmith, Livermore, CA, USA) was used as the electrophoresis voltage source. Amperometric detection was performed with gold as the working electrode and a platinum wire served as the auxiliary and reference (quasi-reference) electrodes.

### 2.4 Electrode modification

For the selective detection of thiols, gold electrodes were amalgamated using elemental mercury (Sigma Aldrich). Physical depositions of mercury on gold, ranging from 1 s to 10 min, were investigated. The optimized conditions consisted of suspending 20 μL of elemental mercury over the gold electrode for 30 s, scraping off any excess mercury left on the electrode with the edge of an index card, and letting the fresh amalgam sit for at least 1 h. Before use, the amalgam electrodes were polished with the BAS (Bioanalytical Systems) electrode polishing kit as described above.

Electrodepositions were used to create gold pillar electrodes that protrude into the channel. Electrodepositions were carried out by filling a PDMS reservoir with a solution of 50 mM dicyanoaurate (I) (in 0.1 M Na2CO3) and applying a potential of −1.2 V to the gold electrode (versus Ag/AgCl). The current produced during the electrodeposition was monitored using a CH Instruments potentiostat. A 30 min deposition time was used to create the pillars in this study. Cyclic voltammetry (using a CH Instruments potentiostat) and ferricyanide (diffusion coefficient of 7.6 × 10⁻⁶ cm²/s [33]) were used to determine the pillar surface area. The scan rate was 0.1 V/s and the scan was performed from 0 to 0.6 V versus an Ag/AgCl reference electrode. The concentration of ferricyanide (in a 0.5 M KNO₃ solution) was 10 mM.

### 2.5 Imaging

Fluorescent images (shown in Fig. 2) were obtained using fluorescein (Sigma Aldrich) and an upright fluorescence microscope (Olympus EX 60) equipped with a 100 W Hg Arc lamp and a cooled 12-bit monochrome Qicam Fast digital CCD camera (QImaging, Montreal, Canada). Images were captured with Streampix Digital Video Recording software (Norpix, Montreal, Canada) and Image Pro express software (Media Cybernetics, Silver Spring, MD) was used to measure channel dimensions. Non-fluorescent images (Figs. 3–5) were captured from a stereoscope (Olympus SXZ12) operating in bright-field mode using a Sony 3CCD color camera (Leeds Precision Instruments, Minneapolis, MN, USA). The other bright-field image (Fig. 6) was captured with the upright microscope (Olympus EX 60) and the Sony color camera. Confocal images of the gold pillar electrodes were imaged with a Keyence VK-9170 Violet Laser Scanning Confocal Microscope (Keyence). The accompanying VK Viewer software allowed collection of color 3-D image data over the entire electrode surface as well as a line scan to obtain a profile of the entire electrode. Contact angle measurements were obtained by taking an image (10 × ) of a 0.5 μL water droplet on the epoxy base with a Digital Blue microscope (Model 24221, Intel). The contact angle was calculated directly from this image, as previously described [34].

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3 Results and discussion

3.1 Fabrication and assembly

The use of glass substrates that have been sputter-coated with an electrode material is a popular method of performing amperometric detection in microfluidic devices, as various electrode designs can be created through lithography and either wet-etching or lift-off steps. These types of electrode plates have been used for a variety of applications including the development of palladium decouplers for integration of microchip electrophoresis with electrochemical detection [12, 15]. While these plates offer many advantages and are relatively well established, their fabrication is costly and time consuming. One of the major disadvantages of these thin-layer electrodes is their inability to be polished. It is standard practice when using either conventional electrodes or LC-electrochemical flow cells to polish the electrode before use. This provides a well-defined and clean electrode surface. Iron, steel, and nickel electrode materials have been previously encapsulated in an epoxy base, such as the ones used here, for electrochemical corrosion and dissolution studies [35–37]. The purpose of these studies was to investigate whether this type of approach can be used to integrate electrochemical detection with microfluidic devices in a manner, where the cost and time of the electrode fabrication are reduced and the resulting electrode is polishable.

The fabrication strategy outlined in Figs. 1 and 2 was used to construct epoxy-embedded electrodes for use with microchip-based analysis. The process started with the machining of a double Teflon mold, a base mold that contained two electrode insertion holes (1 mm in diameter with a 1 mm spacing) and an outer mold, into which the base mold could be fitted. In this study, either a 1 mm gold wire, a 1 mm palladium wire, or a 25 μm gold wire electrode was inserted into the electrode insertion hole(s) and placed in a vertical orientation. Following the assembly of the mold and electrode, a thoroughly mixed mixture of Armstrong C-7 adhesive (resin) and 0.8% of Armstrong activator A was poured into the mold as shown in Fig. 1A. The mixture was left to cure for at least 2 h (Fig. 1B), after which the epoxy-encapsulated electrodes were ready to be taken out of the mold. First, the base mold was removed (Fig. 1C), followed by the removal of the second mold. Several wet polishing steps were utilized to shape and finely polish the epoxy base. Before assembly with a PDMS structure (described below), a BAS fine grit polishing pad with deionized H₂O or alumina suspension was used to generate a fresh electrode surface (Fig. 1D). The cost of each epoxy-encapsulated electrode base varied depending upon the desired electrode material. For studies using a single gold 1 mm diameter electrode the cost of each base was ~$38, molds that contained both a 1 mm palladium and 1 mm gold electrode cost ~$50 to produce, and studies involving the use of a 25 μm diameter gold wire resulted in an electrode base that contained only ~$7 of material. It is important to note that the lifetime of each electrode has yet to be reached in our laboratory. The polishing steps result in the loss of very little material and we have used electrode bases for over 1 yr without any significant loss in the dimensions or electrode performance. In addition, the entire electrode fabrication was carried out in ambient laboratory conditions, so a clean room is not necessary for these studies.

The microchip-based flow analysis system was created by the assembly process shown in Fig. 2A, with reversible sealing of PDMS-based fluidic structures over the epoxy-encapsulated electrodes. Multiple micrographs were taken of fluorescein pumping at 3 μl/min through reversibly sealed microfluidic channels, with no observed leakage around the channels or electrodes (Fig. 2C). Similar to what is done with an LC-electrochemical flow cell, a reversibly sealed device can easily be disassembled, the electrode polished, and the device reassembled. If irreversible sealing is desired, it was found that this can be achieved through the use of the oxygen plasma cleaner [28]. Before plasma treatment, the contact angle of the epoxy base was 95° and after the plasma treatment the epoxy base became more hydrophilic and the angle dropped to 25°.

3.2 Characterization with microchip-based flow injection analysis

This new type of electrode material was initially characterized with microchip-based flow injection analysis using a 10 mM MES buffer (pH 5.5) and a 100 × 100 μm × 3 cm flow channel. Initially, a single 1 mm diameter gold electrode was used (Fig. 3A). The oxidizing potential for catechol was optimized to be +0.9 V versus Ag/AgCl (determined by a hydrodynamic voltammogram, HDV). Seven consecutive injections of a 100 μM catechol solution demonstrated that the electrode response was reproducible, with average peak currents being 4.20 ± 0.14 nA (3.3% RSD). A calibration curve for 75 nM–50 μM catechol was determined and showed that the electrode response had a linear correlation with the concentration (r² of 0.996). Dual electrodes in a serial configuration were fabricated as shown in Fig. 1 by simply having an additional insertion hole accurately fixed relative to the first electrode hole. Experiments with dual gold electrodes in a generator/collector mode using two 1 mm diameter gold electrodes and a 1 mm spacing were carried out to determine the collection efficiency that is possible with this configuration (Fig. 3B). The first electrode was held at +0.9 V to oxidize the 300 μM catechol sample to the corresponding quinone. The second downstream electrode was operated at −0.2 V to monitor the reduction of the quinone back to catechol. The collection efficiency was calculated by dividing the oxidation peak current by the reduction peak current. This experiment was conducted for three consecutive days, with each experiment having seven injections of a 300 μM catechol sample, and resulted in an average collection efficiency of 90.0 ± 1.9%.
3.3 Hg/Au electrode for thiol detection

The initial work with LC-electrochemical flow cells showed that Au electrodes can be amalgamated with liquid mercury and the resulting Hg/Au amalgam electrode can be used for the selective detection of thiols at low potentials (relative to the potential needed for direct detection at a bare electrode) [23]. Our group has previously shown that the traditional method of using elemental mercury for the amalgamation process is not possible for thin-layer patterned gold electrodes, as the strong attraction between mercury and gold results in complete removal of the 2000 Å Au film [11]. This was rectified by the use of an electrodeposition process to generate Hg⁰ in situ in a controlled manner [11]. While this approach did allow us [11] and subsequently others [38] to selectively detect thiols at low potentials, the inability to polish the electrode surface results in electrodes that cannot be used for more than one day and the mercury could not be reapplied to the electrode. In this study it was found that the ability to encapsulate traditional metal electrode materials enabled the use of elemental liquid mercury for the creation of Hg/Au amalgams. A 1 mm diameter gold electrode was exposed to liquid mercury for an optimized time of 30 s, and excess mercury was removed with the edge of an index card. After 1 h, the Hg/Au amalgamated electrode was polished with a BAS fine grit polishing pad. Microchip-based flow injection analysis and a 10 mM MES buffer (pH 5.5) was used to characterize the Hg/Au electrode, with glutathione (GSH) being the test analyte. The optimized potential for detection of GSH was found to be +0.2 V versus Ag/AgCl (determined by a HDV), which is in good agreement with previous studies [11, 23]. Figure 4A demonstrates the reproducibility of the electrode response from five consecutive injections of a standard GSH solution. The electrode exhibited a linear response (50–400 μM GSH), with an \( r^2 \) of 0.997.

To demonstrate the ability of the epoxy-embedded electrodes to monitor analytes in a complex matrix, studies were carried out to measure the intracellular glutathione content in human RBCs. As previously described, GSH is the dominant thiol present in RBCs [39, 40] and flow injection analysis was used to characterize the epoxy-embedded electrodes. (A) Demonstrating the reproducibility with 7 consecutive injections of catechol (100 μM). (B) Dual electrode detection of catechol (300 μM), with monitoring of both oxidative (+0.9 V) and reductive (–0.2 V) electrodes.
injection analysis can be used for its quantitation [11]. The cells were received as an ~86% hematocrit solution and diluted with a Tris/Ringers buffer solution (pH 7.4) to 1% hematocrit prior to analysis. The lysis buffer used in the flow system was composed of Tris/Ringers with the addition of 30 mM SDS. Cells were injected and lysed as they transversed the fluidic network, with the released GSH being detected at the Hg/Au electrode. The working Hg/Au electrode was held at −0.023 V versus a SCE. Cell blanks were achieved by injecting a solution of RBCs at a 1% hematocrit with Tris/Ringers buffer without any SDS in the flow system (no peaks were detected). To quantify the intracellular GSH concentration, the standard addition method was employed (Fig. 4B). Standard additions of GSH (from a 10 mM GSH stock solution) were added to two separate aliquots of the RBC stock solution, so that the final added GSH concentration was 100 and 200 µM, respectively. Using the linear regression from the standard addition curve ($r^2 = 0.997$), it was found that the RBCs contained 586 attomoles of GSH per cell. This value is similar to those previously reported for GSH in human [39] or rabbit [40] RBCs using a 30–60 min derivatization step followed by fluorescence detection. Importantly, this method requires only 60 s between cell lysis and GSH detection.

3.4 Use with microchip electrophoresis

Microchip electrophoresis with electrochemical detection has been an attractive method for the separation and detection of small molecules [8, 9, 41]. It has been shown that an effective way of integrating these two techniques involves the use of a palladium decoupler electrode and a downstream detection electrode [13–15, 42]. The decoupler electrode is incorporated within the fluidic network to provide an electrophoretic ground and adsorb hydrogen produced from the reduction of water at the cathode. Band broadening is minimized because the decoupler allows the detection electrode to remain in the fluidic network. Palladium is not a widely used electrode material and depositing additional metal layers downstream from a palladium electrode would involve multiple sputtering/patterning steps adding to the time, expense, and complexity of the device fabrication. In this study, epoxy-embedded electrodes were used to integrate microchip electrophoresis with electrochemical detection. Two electrodes were embedded into the epoxy base, a 1 mm diameter palladium electrode (decoupler) and a 1 mm diameter gold detection electrode, with a 1 mm spacing between the two electrodes. A PDMS-based microchip with a standard T-configuration and 27.5 mm separation channel was reversibly sealed over the electrodes (Fig. 5A). A gated injection scheme was used to discretely inject a 70 µL plug into the separation channel, with a field strength of 240 V/cm being used to separate a mixture of dopamine, epinephrine, and catechol. As seen in Fig. 5B, injections of 200 µM of each analyte resulted in a resolution of 1.5 between dopamine and epinephrine and 4.2 between epinephrine and catechol. The electrode response was reproducible, with dopamine having an average peak height of 3.88 ± 0.10 nA (2.6% RSD).

3.5 Formation and integration of pillar electrodes

As shown in Figs. 1 and 2, the way the epoxy-embedded electrodes are made leads to electrical connections coming from the bottom of the epoxy base. This was found to enable a simple electrodeposition approach to create pillar electrodes. Gold depositions have been employed previously on small gold wire electrodes to increase their surface area for higher loading capacity of antibodies and to study antibody–antigen interactions [43]. A similar approach is taken here to electrodeposits gold onto an embedded 25 µm diameter gold electrode. This is accomplished by sealing a PDMS reservoir around the electrode, placing an AuCN solution in the reservoir and applying a reductive potential (~1.2 V versus Ag/AgCl). If this were done with planar electrodes and connections from the side, the electrode width would be defined by the reservoir dimensions. In this case, the electrode pillar is defined by the initial gold 25 µm diameter wire, as shown in Fig. 6. Figure 6C shows a
confocal image of a pillar that was created from a 25 \( \mu \)m diameter gold electrode. The electrodeposition process is reproducible, with the average width and height of four different pillars made from four different embedded wires being 65.4 \( \pm \) 2.2 and 27.4 \( \pm \) 0.7 \( \mu \)m, respectively. The surface area of a representative pillar was determined (using cyclic voltammetry, ferricyanide, and the Randles–Sevick equation \cite{33}) and found to be \( 1.54 \times 10^{-4} \) cm\(^2\), which is significantly larger than the geometrically calculated area of a 25 \( \mu \)m flat electrode (4.9 \( \times \) \( 10^{-4} \) cm\(^2\)).

A 27 \( \mu \)m (in height) pillar was used for integration within a 100 \( \times \) 100 \( \mu \)m microfluidic channel. Pillars were easily integrated into the flow channels for characterization via microchip-based flow injection analysis with the aid of a stereoscope. Since the resulting pillar electrode may be considered as a different electrode surface than the native gold wire, a HDV comparison was performed for a 27 \( \mu \)m (in height) pillar versus a 1 mm flat gold wire electrode using catechol as the test analyte (Fig. 7A). The HDV showed that the optimized potential for

![Confocal image of a pillar](image1)

Figure 6. (A) Schematic of epoxy-embedded electrode base with pillar electrodes protruding vertically into the PDMS-based fluidic channel. (B) A micrograph of a gold pillar electrode. (C) Confocal image of the pillar electrode (height = 27 \( \mu \)m, width = 65 \( \mu \)m).

![Hydrodynamic voltammogram](image2)

Figure 7. (A) Hydrodynamic voltammogram of a flat gold electrode versus a pillar electrode. (B) Comparison of electrode response for a pillar and a flat electrode with 3 repeated injections of catechol (1 \( \mu \)M). (C) Calibration curve comparing the sensitivity and linear response for a pillar electrode versus flat gold electrode.

\[
y = 72.12x - 44.76 \\
R^2 = 0.996
\]

\[
y = 4.192x + 3.185 \\
R^2 = 0.999
\]
the pillar electrode remained the same as the original gold electrode at +0.9 V. A key feature of this pillar electrode, which protrudes into the fluidic network, is the increased performance that results from the increased electrode area. To demonstrate this, a 1 μM catechol sample was injected into a channel that contained either a gold pillar electrode (27 μm height) or a 25 μm diameter flat gold electrode (Fig. 7B). The pillar resulted in a signal increase of 121 times greater over the flat microelectrode. Calibration curves were also made for comparing a pillar (27 μm height) and a 25 μm diameter flat gold electrode (Fig. 7C). A similar enhancement was seen there as well, with a great increase in the sensitivity for the pillar (72.1 pA/μM) relative to the flat electrode (4.2 pA/μM). The LOD was also significantly improved to 20 ± 3 nM (calculated for three different pillars and on three different days), which was much lower than the 600 nM LOD found for a flat microelectrode.

4 Concluding remarks

In this study, we have presented a novel way of fabricating and integrating epoxy-embedded electrodes with microchip-based analysis systems. This approach can be used to embed electrodes of various sizes and compositions, and the resulting electrode base has the important advantage of being polishable with commercially available electrode polishing kits. In this study, we have characterized bare electrodes with a simple electrodeposition process. The resulting electrode base has the important advantage of being polishable with commercially available electrode polishing kits. In this study, we have characterized bare and modified electrodes through flow injection analysis and used multiple electrodes to integrate microchip electrophoresis with electrochemical detection. A special exciting feature of this approach is the ability to create pillar electrodes with a simple electrodeposition process. The resulting pillar can easily be integrated within a fluidic network and results in significant improvements of both the sensitivity and LOD. The use of a pillar electrode to increase the electrode area does not lead to a large increase in the overall detector length, which will be important for the future work in integrating these electrodes in a separation system where contributions to band broadening from the detector must be minimized. These initial studies lay the foundation for the future work in exploring other configurations that are possible with this approach. This includes the use of different electrode materials such as glassy carbon and platinum, the integration of pillar electrodes with a palladium decoupler for microchip electrophoresis, and fabrication/utilization of multiple pillars for signal enhancement, similar to what has been done recently with planar electrodes [16, 44, 45].

This described project was supported by Award Number R15GM084470 from the National Institute of General Medical Sciences.

The authors have declared no conflict of interest.

5 References