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PAPER

Poly(dimethylsiloxane) cross-linked carbon paste electrodes for microfluidic electrochemical sensing

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Recently, the development of electrochemical biosensors as part of microfluidic devices has garnered a great deal of attention because of the small instrument size and portability afforded by the integration of electrochemistry in microfluidic systems. Electrode fabrication, however, has proven to be a major obstacle in the field. Here, an alternative method to create integrated, low cost, robust, patternable carbon paste electrodes (CPEs) for microfluidic devices is presented. The new CPEs are composed of graphite powder and a binder consisting of a mixture of poly(dimethylsiloxane) (PDMS) and mineral oil. The electrodes are made by filling channels molded in previously cross-linked PDMS using a method analogous to screen printing. The optimal binder composition was investigated to obtain electrodes that were physically robust and performed well electrochemically. After studying the basic electrochemistry, the PDMS-oil CPEs were modified with multi-walled carbon nanotubes (MWCNT) and cobalt phthalocyanine (CoPC) for the detection of catecholamines and thiols, respectively, to demonstrate the ease of electrode chemical modification. Significant improvement of analyte signal detection was observed from both types of modified CPEs. A nearly 2-fold improvement in the electrochemical signal for 100 μM dithiothreitol (DTT) was observed when using a CoPC modified electrode (4.0 ± 0.2 nA ($n = 3$) *versus* 2.5 ± 0.2 nA ($n = 3$)). The improvement in signal was even more pronounced when looking at catecholamines, namely dopamine, using MWCNT modified CPEs. In this case, an order of magnitude improvement in limit of detection was observed for dopamine when using the MWCNT modified CPEs (50 nM *versus* 500 nM). CoPC modified CPEs were successfully used to detect thiols in red blood cell lysate while MWCNT modified CPEs were used to monitor temporal changes in catecholamine release from PC12 cells following stimulation with potassium.

Introduction

The integration of electrochemical biosensors as part of microfluidic devices is attractive because of the size and volume compatibility between electrochemistry and microfluidics. One of the major challenges in this field, however, has been electrode fabrication. Traditional thin-film fabrication methods are limited to the use of either noble metals or reactive metals such as Cu. Noble metal electrodes are easily modified through self-assembled monolayers and exhibit fast electron transfer kinetics but readily foul in biological media.[1,2] One solution is the use of carbon-based electrode materials.[3] Carbon-based electrodes are attractive for electrochemical sensing due to their ability to withstand fouling and they possess a larger potential range compared to metal electrodes.[4–6] Common forms of carbon electrodes used in the development of biosensors include glassy carbon,[7,8] pyrolyzed carbon,[9] carbon pastes,[10,11] micromolded carbon ink,[12] carbon doped photoresist,[13] and carbon nanotubes (CNTs).[5,14–16] Each of these forms of carbon has unique advantages and disadvantages with regards to fabrication and use. For example, pyrolyzed carbon electrodes can be made in a wide range of sizes and shapes but require a substrate that can withstand the high temperatures necessary for photoresist pyrolysis.[9] Carbon doped photoresist electrodes overcome the need for high temperatures by making the photoresist conductive. Unfortunately, the large graphite particles cause light scattering during photopatterning, making it difficult to create small electrodes.[13] Finally, all of these methods generate raised electrode structures that disrupt the sealing of device layers and cause leakage around

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the electrodes. Of these materials, carbon pastes are one of the most attractive because they are easily fabricated and can be readily modified to enhance selectivity.[4–6,17,18] Unfortunately, they are generally unstable in flow based analysis devices such as those used in microfluidics. Here, a new method is reported for fabrication of carbon paste electrodes in poly(dimethylsiloxane) (PDMS) microfluidic devices that addresses these limitations.

Carbon paste electrodes (CPE) are amenable to miniaturization and have shown utility in a broad range of applications.[19,20] The mixture of graphite carbon and a binder (mineral oil, *etc.*) allows for ease of fabrication of CPEs; furthermore, CPEs can be modified with dopants, are size adjustable and can be easily integrated with various systems.[21] Recently, CPEs have shown promise as electrochemical sensors in microfluidic devices.[17,18] Various methods have been used for the integration of CPEs as sensors within microfluidic devices including filling tube sleeves with carbon paste[18,22] and screen printing of carbon paste on PDMS substrates.[17] Tube sleeve electrodes are not readily amenable to on-chip production because of their large size (on the order of several mm in diameter) which can lead to sealing issues. In contrast, screen-printed electrodes can be directly fabricated on-chip with the electrode size and shape controlled by the screens used for patterning on the microfluidic devices.[17] Screen printing also allows for simple, rapid, inexpensive mass-production of disposable electrochemical sensors.[23,24] Carbon paste for screen-printed electrodes can be classified as soft or rigid composites. This distinction is based largely upon their mechanical properties.[25] Soft composites are obtained by mixing graphite powder with mineral oils resulting in a chemically inert, electroinactive, pliable electrode material with moderate viscosity, low volatility and minimal solubility in aqueous solution.[21] Soft composites can be physically unstable, which becomes an issue in a microfluidic device where the solution flow can cause the soft paste to flow between electrodes. In contrast, rigid composites utilize mixtures of graphite powder and non-conducting polymeric binders to create rigid features resulting in a more robust electrode.[25]

Poly(dimethylsiloxane) (PDMS) is widely used as a microchip material due to its ease in fabrication and low cost. In this report, the use of PDMS as a binder to facilitate sealing of CPEs to microfluidic devices was investigated. The PDMS composition of both the CPE and the microfluidic substrate allows for better adhesion between the two materials. Another advantage to the use of carbon-PDMS electrodes is the reduction in capacitive current compared to bulk glassy carbon and other patternable electrodes.[26] The low capacitive current of these electrodes, however, also comes at the cost of reduced analyte signal. To overcome this, a large amount of graphite must be incorporated into the paste, which in turn results in mixing issues and electrode surface heterogeneity. Recently, PDMS was successfully used as a binder to create low-cost, patternable carbon-PDMS electrodes for chip-based electrochemical detection[26] and demonstrated compatibility within a PDMS microfluidic environment; however, since PDMS was the only binder used, the resulting electrodes exhibited slow electron transfer kinetics.

Here, we report the use of a mixed binder to create on-chip CPEs in microfluidic devices. The binder mix is a combination of a rigid composite (PDMS) and a soft composite (mineral oil) providing an improvement in physical stability and electrochemistry of the CPE compared to using PDMS or mineral oil alone. Optimal compositions of the binder mixture were investigated to obtain robust electrodes with low capacitive currents. Moreover, a new on-chip method for CPE fabrication is reported that is based on screen printing. Our method uses molded PDMS containing electrode channels filled with carbon paste where the channels provide well-defined electrodes for integration into microfluidic systems. Electrode performance was investigated by cyclic voltammetry in static solutions and amperometry with flow injection analysis. CPEs made using our methods can also be readily modified to improve selectivity. Mixed PDMS-mineral oil CPEs were modified with cobalt phthalocyanine (CoPC) and multi-walled carbon nanotubes (MWCNT) for the detection of thiols and catecholamines, respectively. The utility of the modified electrodes is shown with the detection of thiols in erythrocyte lysate and catecholamine release from PC12 cells.

Experimental

All chemicals used were of analytical grade. The following materials and chemicals were used as received: poly(dimethylsiloxane) (PDMS) Sylgard 184 elastomer kit (Dow Corning, Midland, MI), hydrochloric acid (Thermo Fisher), potassium chloride (Thermo Fisher), mineral oil (Thermo Fisher), sodium chloride (Thermo Fisher), graphite powder (Sigma Aldrich), nitric acid (Thermo Fisher) and silver conductive paint (SPI supplies, West Chester, PA). Copper wire was obtained from NTE Electronics (Bloomfield, NJ). A stock solution of dopamine hydrochloride (100 mM, Sigma Aldrich) was prepared in 0.01 M HCl and 0.1M NaCl which was subsequently diluted to 1 mM using the background electrolyte prior to analysis.

Electrode fabrication was achieved using a method analogous to screen printing. Channels were created in PDMS using soft lithography techniques.[27] Carbon paste (a mixture of electrocatalyst reagent, graphite powder, mineral oil and PDMS) was spread on the PDMS containing the electrode channels using a plastic artist's pottery scraper or screen printing squeegee. Prior to applying the carbon paste, Scotch Magic Tape™ was applied around the electrode channels to act as a border for the carbon paste for easy cleaning. Excess paste was removed by wiping the scraper over the electrode channel and any remaining paste removed by application and removal of a piece of tape over the paste until all excess carbon paste around the electrode area was removed, leaving the patterned electrodes. For the flow injection analysis, a second piece of PDMS containing a flow channel was plasma sealed to the piece of PDMS containing the electrodes in an orthogonal orientation. A 1 mm diameter hole was punched in one end of the flow channel as the inlet hole for connection to the off-chip injection valve and syringe pump (PHD 4400, Harvard Apparatus, Holliston, MA) and an 8 mm waste reservoir was punched at the opposite end of the channel. Finally, copper electrode leads were attached to the ends of the carbon electrodes using silver epoxy. The schematic diagram for the electrode fabrication process and the resulting electrode design are shown in Fig. 1. For cyclic voltammetry (CV) data collection, 8 mm PDMS fluidic reservoirs were plasma sealed over the electrodes leaving an 8 mm × 0.25 mm working electrode area within the well. For the flow system, the surface area of the working

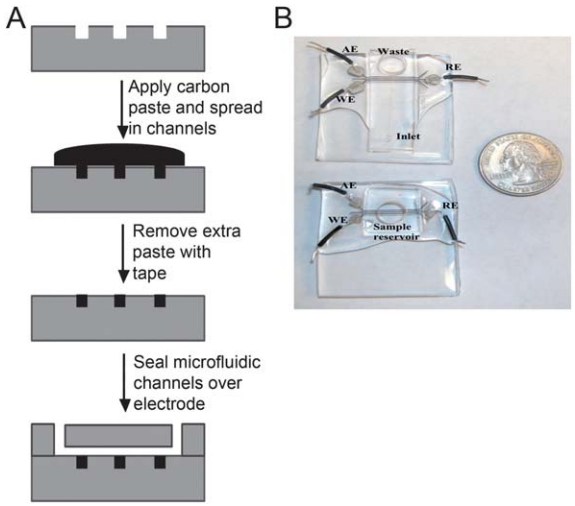

Fig. 1 Flow-based microfluidic system using CPEs. (A) Schematic for electrode fabrication process (side view) (B) Actual picture of electrode alignment in a microfluidic device for the flow injection system with amperometric detection (top) and for cyclic voltammetry study (bottom). Size of electrodes: 250 μm wide and 50 μm deep. Electrode spacing (center-to-center), 300 μm. Microfluidic channel dimension: 250 μm wide, 50 μm deep and 2.5 cm long.

electrode was approximately 0.0625 mm^2. Electrodes were cleaned using air plasma (20 min, 150 W, 0.8 torr) prior to chip assembly.

Commercially available potentiostats (CHI660b, CH Instruments and EA164 Quadstat, eDAQ) were used for amperometry and cyclic voltammetry experiments. Cyclic voltammograms were carried out at scan rates of 0.05, 0.1, 0.2, 0.25, 0.4 and 0.5 V s^{-1} from −0.2 V to 1.2 V with the initial potential set at 0.0 V. An average of three repetitions was done for each potential sweep rate. Amperometry was run at +0.6 V, +0.2 V, and +0.8V for dopamine, dithiothreitol (DTT), and glutathione (GSH), respectively. All experiments were conducted using a three-electrode system consisting of a screen printed carbon electrode as a working electrode and either a Ag/AgCl (3 M KCl) reference electrode and a platinum counter electrode in the waste reservoir or a set of integrated unmodified carbon paste electrodes. Noise was determined by measuring peak-to-peak current at 35 random points along the baseline.

For thiol detection, graphite electrodes were modified with cobalt phthalocyanine (CoPC). CoPC was added to graphite powder (12% w/w) and the mixture added to diethyl ether in a ratio of 1 : 10 (w/v). The solution was mixed for 2 h to ensure a homogeneous mixture of CoPC and graphite powder. Diethyl ether was evaporated prior to electrode fabrication. CoPC modified graphite powder was then mixed with oil and PDMS in a ratio of 5 : 3 : 2 (w:w:w) of graphite powder: mineral oil: PDMS. This mixture was used to fabricate electrodes as described above. For DTT detection, a Ag/AgCl (3 M KCl) reference electrode and a platinum counter electrode were placed in the waste reservoir. For GSH analysis, on-chip unmodified carbon paste electrodes were used as the reference and auxiliary electrodes.

Electrodes were also modified with multiwall carbon nanotubes (MWCNT) to improve electrode performance in the detection of dopamine. In this case, MWCNT were mixed with the graphite powder at 10% (w/w) prior to electrode fabrication. A 1 : 1 mixture of binder and graphite/MWCNT were combined to produce the carbon paste. Electrodes were fabricated as described above.

Red blood cell (RBC) lysate for GSH analysis was prepared according to the literature.[28,29] In brief, freshly collected human whole blood (5 mL) was centrifuged 1000 × g for 15 min at 4 °C to separate plasma and erythrocytes. The plasma was then discarded and the erythrocytes rinsed three times with phosphate buffer saline (PBS, pH 7.4). Portions of the erythrocytes were hemolysed in 1 mM Na_2H_2EDTA solution (1 : 1 v/v) with 10% (w/v) of 5-sulfosalicylic acid. The mixed solution was centrifuged at 1000 × g for 15 min at 4 °C. The supernatant containing RBC lysate was collected for GSH analysis. Dilutions of RBC lysate were carried out using PBS.

PC12 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and maintained in F-12 K medium supplemented with 10% fetal bovine serum. The cells were grown on poly-D-lysine-coated T-25 culture flasks (VWR International, Radnor, PA). Cell medium was replaced every 3 d and subcultured as needed. Cells were trypsinized at 37 °C for 10 min to release adherent cells from culture flask surface. Trypsin was neutralized by the addition of an equal amount of F-12 K medium. The cells were centrifuged and the supernatant aspirated from the pellet. The pellet was resuspended in 4 mL PBS. Cells (20 μL) were diluted 1 : 1 with 0.4% trypan blue for counting. Cells were washed 3x with PBS (pH 7.4) to remove any residual media prior to analysis.

Stimulation of the PC12 cells was carried out by exposing the cells to 80 mM K+ (KCl) in 1X PBS (pH 7.4) at room temperature. For the time course study, approximately 6.15 × 10^4 cells (300 μL) were placed in 1.5 mL microcentrifuge tubes and the high potassium buffer added (300 μL) to each tube at 1 min increments for 10 min total. The cells were centrifuged at 1000 × g for 5 min and 500 μL of supernatant removed and placed in a fresh tube for later analysis. Samples were kept on ice until analysis to prevent dopamine degradation.

Results and discussion

Optimization of binder composition

The long-term goal of this project is to create low-cost biosensors that can be used repeatedly for weeks to months. To meet this objective, carbon paste electrodes containing mineral oil as the binder were integrated into microfluidic devices. Initial attempts at creating stable biosensors in flow-based analytical devices were unsuccessful, however, due to leakage of carbon between electrodes after just 2 h in a flow injection device as shown in Fig. 2A. To increase the electrode's physical stability, PDMS was tested as an additive to the mineral oil binder expecting that the PDMS would cross-link with the existing PDMS of the channel resulting in physically stable electrodes. This approach proved successful with electrodes remaining intact for extended periods in flowing systems as shown in the photomicrographs of Fig. 2B.

Once a method for physically stabilizing the electrodes was demonstrated, the effect of binder composition on peak current (i_p) was studied for varying PDMS:mineral oil ratios.

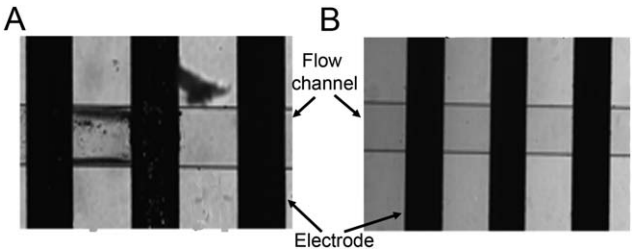

Fig. 2 Electrode photomicrographs within flow-based analytical devices after flow injection experiment for 2 h at a flow rate of 60 μL min^{-1}. (A) Electrodes made with mineral oil binder. (B) Electrodes made with 2 : 3 PDMS:mineral oil binder. Size of electrodes and dimension of microfluidic channels are the same as described in Fig. 1.

Traditionally, CPEs are used as prepared, however, our group has recently shown that use of an oxygen plasma increases peak current density, shifts the ratio of cathodic to anodic current closer to unity, and decreases peak-to-peak separation for carbon electrodes.[13] The benefits are the result of exposure of additional graphite surfaces during the plasma treatment process. As a result of these findings, all CPE electrodes were treated with air plasma prior to use to expose additional graphite from the solid binder. A series of studies were conducted to determine the optimal ratio of PDMS:mineral oil in the electrode binder material. The ratios studied were 1 : 0, 3 : 2, 1 : 1, and 2 : 3 (PDMS:mineral oil). In all cases, the ratio of graphite to binder was held constant at 1 : 1 (w:w). The difference between cathodic and anodic peaks (ΔE), capacitive current, and the effect of scan rate on peak current density were used to determine the best ratio of PDMS to mineral oil. Three separate electrodes on different chips were tested for each ratio. Table 1 shows the values for anodic peak current (I_{pa}), ΔE, and capacitive current for all binder ratios tested at 100 $mV*s^{-1}$ scan rate. Cyclic voltammograms at 100 $mV*s^{-1}$ for background electrolyte (0.01M HCl, 0.1M NaCl) and 1 mM dopamine using 1 : 0, 3 : 2, 1 : 1, and 2 : 3 PDMS:mineral oil ratios are shown in Fig. 3A, while Fig. 3B shows a scan rate study for the 1 : 1 ratio electrode. ΔE values at 100 $mV*s^{-1}$ scan rate for electrodes at the different ratios are 564.0 ± 55.7 mV, 221.6 ± 45.9 mV, 298.7 ± 71.8 mV, and 186.7 ± 43.0 mV (n = 3) for 1 : 0, 3 : 2, 1 : 1 and 2 : 3 (PDMS:mineral oil), respectively. All of the electrodes exhibit a linear relationship between the current density and the square root of the scan rate as predicted by the Randles-Sevcik equation indicating a diffusion limited reversible reaction[30] as shown in Fig. 4. It is interesting to note that as the amount of PDMS in the binder is increased, the electrode sensitivity decreases as indicated by a decrease in slope of current density *versus* square root

Table 1 Electrode characterization. Values are reported for 100 mV s^{-1} scan rate. BGE: 10 mM HCl, 100 mM NaCl

	ΔE (mV)	Capacitive Current (μA cm^{-2})	I_{pa} (μA)
All PDMS	564.0 ± 55.7	3.0 ± 1.3	1.7 ± 0.2
3 : 2	221.6 ± 45.9	29.2 ± 10.7	2.1 ± 0.3
1 : 1	298.7 ± 71.8	19.6 ± 7.5	2.4 ± 1.0
2 : 3	186.7 ± 43.0	22.6 ± 12.0	2.8 ± 0.8

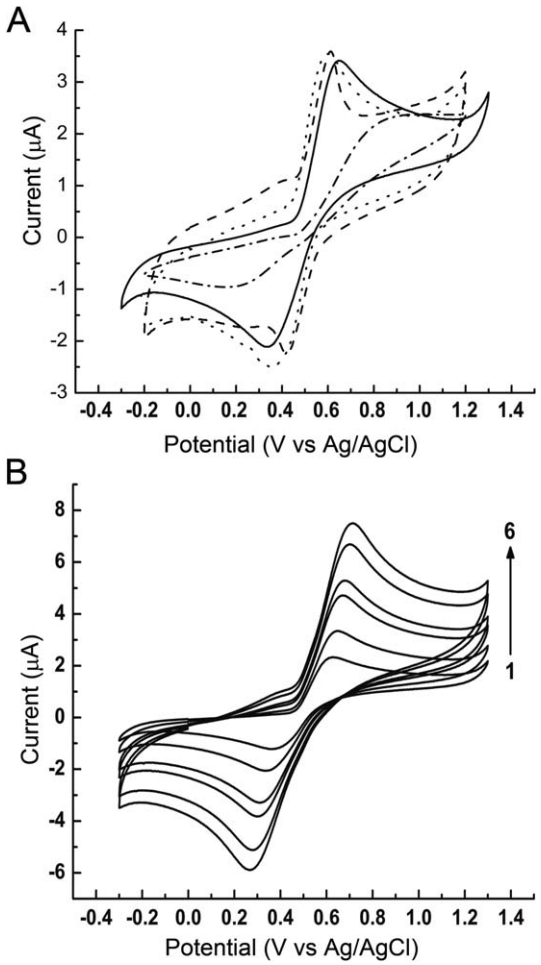

Fig. 3 Comparison of electrode performance for the detection of dopamine at unmodified carbon paste electrodes. (A) Cyclic voltammograms of 1 mM dopamine at four different binder composition electrodes: 1 : 0 (-·-), 3 : 2 (---), 1 : 1 (—), and 2 : 3 (···) (PDMS:mineral oil). Conditions: scan rate of 0.1 V s^{-1} between −0.2 and 1.2 V for 1 : 0, 3 : 2, and 2 : 3 binder composition and between −0.3 and 1.3 V for 1 : 1 binder composition in 10 mM HCl, 100 mM NaCl. (B) Cyclic voltammograms of 1 mM dopamine in 10 mM HCl, 100 mM NaCl at varying scan rates (1–6) of 100, 200, 250, 400, 500, and 1000 mV/s.

of the scan rate plot. The experimentally obtained peak oxidation current densities for dopamine (1 mM) are slightly less than expected for all ratios of chips tested based on the Randles-Sevcik equation given a diffusion coefficient of 4.2 × 10^{-6} cm^2 s^{-1}, scan rate of 100 mV s^{-1} and a measured geometric electrode area of 0.02 cm^2. Based on these conditions, the expected peak oxidation current density for dopamine (1 mM) should be 3.47 μA cm^{-2}, while the measured current density was 2.41 μA cm^{-2}. The deviation is likely due to the fact that the Randles-Sevcik equation assumes a uniform electrode surface. The carbon paste electrodes are a porous surface and contain a mixture of conducting graphite particles and non-conducting binder. From this study, it can also be noted that the CPEs deviate slightly from ideal electrode behavior. In a completely reversible reaction, one would expect the slope of current density *versus* scan rate$^{1/2}$ for oxidation and reduction to be equal and opposite; however, only the slopes for the oxidation and reduction

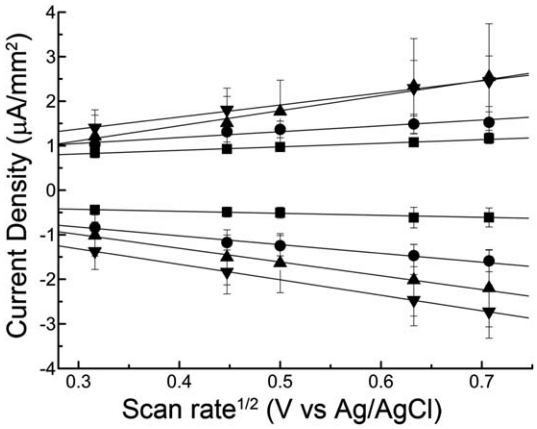

Fig. 4 Current density-scan rate study for 1 mM dopamine in 10 mM HCl, 100 mM NaCl at four different binder composition electrodes: 1 : 0 (■), 3 : 2 (●), 1 : 1 (▲), 2 : 3 (▼) (PDMS:mineral oil). All show a linear dependence of the current density as a function of the square root of the scan rate. All electrodes were run in triplicate at each scan rate.

reactions for the 1 : 1 binder composition were near equal and opposite with a ratio of 0.9. In contrast, the CPE made with all PDMS exhibited a ratio of just 0.6.

The capacitive current was also determined for each binder composition. Capacitive current is an important measure of surface area and also contributes to background noise for CV-based measurements. For the electrodes with all PDMS binder, the capacitive current was measured to be 3.0 ± 1.3 (n = 3) μA cm^{-2} which was statistically lower than the other three binder compositions containing mineral oil measured at 29.2 ± 10.7 μA cm^{-2}, 19.6 ± 7.5 μA cm^{-2}, and 22.6 ± 12.0 μA cm^{-2} for the 3 : 2, 1 : 1, and 2 : 3 ratios, respectively (unpaired t-test, $p < 0.05$). None of the three mixed binder systems, however, were statistically different.

CoPC modified carbon paste electrodes

Thiol compounds play an important role in numerous biological functions.[31,32] Cysteine is an important structural element in many proteins while glutathione (GSH) plays a major role in intracellular oxidative stress defense as it reacts readily with free radicals and reactive oxygen species.[33] Fluorescence, UV and electrochemical detection have been employed previously for total thiol analysis.[34,35] Both fluorescence and UV detection generally require derivatization prior to detection to obtain selectivity.[35] Electrochemical detection, on the other hand, can be used to directly detect thiols without derivatization owing to the moderate oxidation potential of thiols.[34,36] Unmodified electrode materials such as gold and platinum can be used to detect thiols but require high applied potential (>+1.0 V) and often lead to electrode fouling after just a few runs.[37,38] Carbon electrodes can also be used to detect thiols but suffer from low peak currents due to less than ideal interactions between the carbon and the thiols. To overcome these problems, an electrocatalytic reagent is often added to decrease the detection potential and increase peak current. Cobalt phthalocyanine (CoPC) has been widely used as a redox mediator that lowers the overpotential for thiols.[38–40] Carbon paste has been used as an electrode material with CoPC.[29,38,39] Here, CoPC was doped into the PDMS:mineral oil CPEs to demonstrate the compatibility of the new fabrication method with chemical modification.

DTT was used as a model analyte to demonstrate the utility of CoPC modified carbon paste electrodes for thiol detection. The performance of bare CPEs and CoPC modified CPEs was compared using cyclic voltammetry and amperometry. Fig. 5A shows cyclic voltammograms of CoPC modified CPE (solid line) and unmodified CPE (dashed line) of 1 mM DTT in MES buffer (30 mM, pH 7). With the conditions used for these experiments, DTT was not detected using unmodified CPEs whereas the oxidation peak of DTT was observed using CoPC modified CPE as expected.[41] The two-step electrocatalytic process begins with the electrochemical oxidation of Co(II)phthalocyanine followed by the chemical oxidation of DTT as it regenerates the Co(II) phthalocyanine.[39] The optimal amount of CoPC added to the CPE was also investigated, and it was found that a CPE consisting of a 12% mixture of CoPC in graphite (w/w) provided the

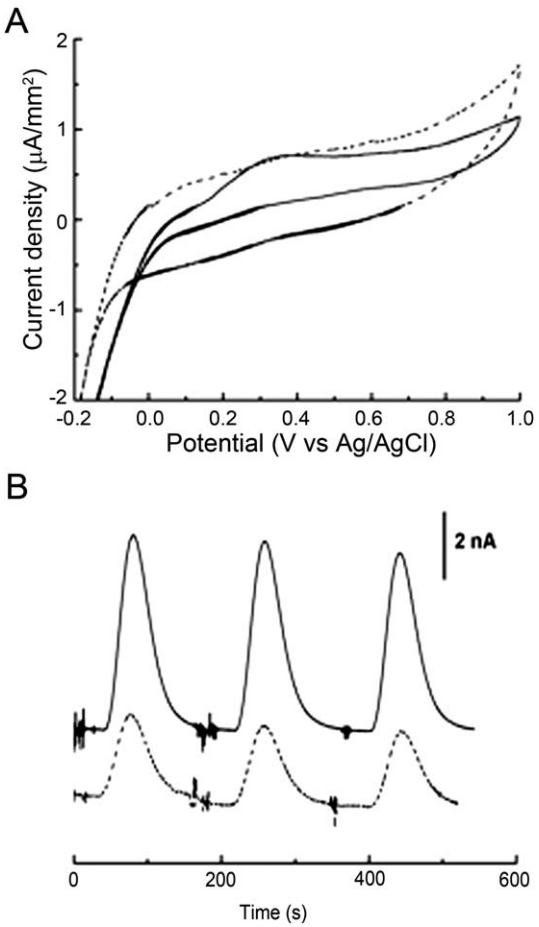

Fig. 5 Comparison of working electrode performance for DTT detection between CoPC modified CPE (solid line) and unmodified CPE (dashed line). (A) Cyclic voltammograms of 1 mM DTT in 30 mM MES buffer, pH 7. (B) Flow injection analysis with amperometric detection of 100 μM DTT using 30 mM MES, pH 7, as a running buffer. Paste composition ratio is 5 : 3 : 2 (graphite:oil:PDMS). Off-chip reference and auxiliary electrodes were used. Applied potential: 0.2 V (*vs.* Ag/AgCl (3 M KCl)) for both types of working electrodes with Pt wire as an auxiliary electrode. Flow rate: 60 μL min^{-1}.

best performance for DTT detection (data not shown). The composition of the binder to create the paste was also investigated and found that the ratio of 2 : 3 (PDMS:mineral oil) provided the best electrode composition in terms of robustness and electrochemical performance. Finally, flow injection analysis using amperometric detection was employed to study electrode performance and provide continuous analysis of DTT. At +0.2 V (*vs.* Ag/AgCl (3 M KCl)), a higher signal was obtained for 100 μM DTT using CoPC modified CPEs (4.0 ± 0.2 nA, n = 3) as compared to bare CPEs (2.5 ± 0.2 nA, n = 3) (Fig. 5B). Limit of detection (LOD, defined as the concentration that gives a signal 3x larger than the baseline noise) for DTT using CoPC modified CPE and unmodified CPE systems were 2.5 ± 0.2 μM (n = 5) and 16.8 ± 1.3 μM (n = 5), respectively.

Amperometric sensing for glutathione in RBC lysate

To further evaluate the performance of CoPC modified CPEs, the detection of a second thiol compound, GSH, in a biological matrix, RBC lysate, was performed. One of the most important functions of glutathione in human body is its antioxidant property to protect cells from free radicals and reactive oxygen species while maintaining an appropriate intracellular redox status.[33] RBC lysate solution was prepared in 1X PBS for the GSH study. The solution containing GSH was injected to the microfluidic device *via* an off-chip injection valve and PBS as the running buffer. As shown in Fig. 6A, duplicate injections of 0.5% RBC lysate with various amounts of added H_2O_2 are monitored amperometrically. H_2O_2 oxidizes GSH to glutathione resulting in a decrease of GSH in the sample, observed as a decrease in the GSH signal. Fig. 6B shows the dose response curve for this experiment and demonstrates a good linear relationship between the amount of added H_2O_2 and the thiol signal in the RBC lysate ($y = -0.0023x + 0.6157$, $R^2 = 0.9874$). This microfluidic device with an on-chip three electrode system can be used over several injections of RBC lysate with no electrode fouling and a very reproducible signal (%RSD = 1.61%, n = 10) (data not shown).

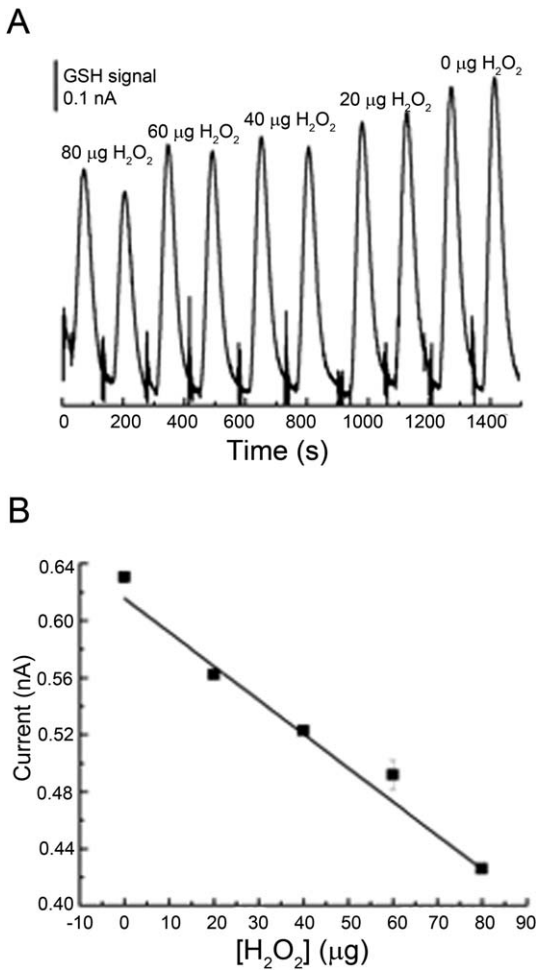

Fig. 6 (A) Flow injection signal of GSH in 0.5% RBC lysate in PBS with different added amount of H_2O_2. (B) Dose response curve of GSH as a function of added H_2O_2. Experimental condition: Working electrode: 12% CoPC modified CPE, Applied potential: 0.8 V *vs.* CPE, Auxiliary electrode: CPE, Running buffer: Phosphate buffered saline (pH 7.4), flow rate: 60 μL min^{-1}.

Multi-walled carbon nanotube modified carbon paste electrodes for detection of dopamine

The ability to quantitatively detect catecholamines such as dopamine is important because of their known role in the progression of Parkinson's and Alzheimer's diseases.[42,43] To demonstrate the further utility of the electrode fabrication method, multi-walled carbon nanotube (MWCNT) modified electrodes were fabricated for the measurement of catecholamine release from cultured PC12 cells. MWCNTs act to improve mass transfer, and thus, sensitivity of the CPEs.[44–47] Fig. 7 shows flow injection analysis data for the detection of 10 μM dopamine at both an unmodified and a MWCNT modified CPEs. The lowest detectable concentration of dopamine for the MWCNT modified CPEs was 50 nM with a signal to noise ratio (S/N) of 27 while the lowest concentration detectable for unmodified CPE was 500 nM (S/N = 4.6). Injections of dopamine below 50 nM did not result in measurable peaks, and the mechanism for this rapid drop-off in signal was not investigated further here. This order of magnitude improvement in the lowest detectable dopamine concentration when adding the MWCNT is likely caused by an increase in surface area of the electrode and thus the peak currents obtained for the electrode. The collection efficiencies for 500 nM dopamine at the two electrode types are nearly equal at 0.54% for unmodified CPEs and 0.53% for MWCNT modified CPEs. The experimentally determined peak area for 500 nM dopamine was compared to the theoretical charge to determine the collection efficiency of the CPEs. The theoretical charge was determined using the relation of N = Q/nF, where N is the number of moles, F is Faraday's constant, n is the number of electrons transferred (for catecholamines such as dopamine, n = 2), and Q is the charge of the peak in nC. Both of these collection efficiencies are quite low. This is likely due to the fact that the surface area of the electrode is very small (0.0625 mm^2) compared to the diffusional distances in the fluidic channel. By increasing the surface area of the electrode in the channel and decreasing the channel height, we would expect to see marked improvements in the collection efficiency of these electrodes.

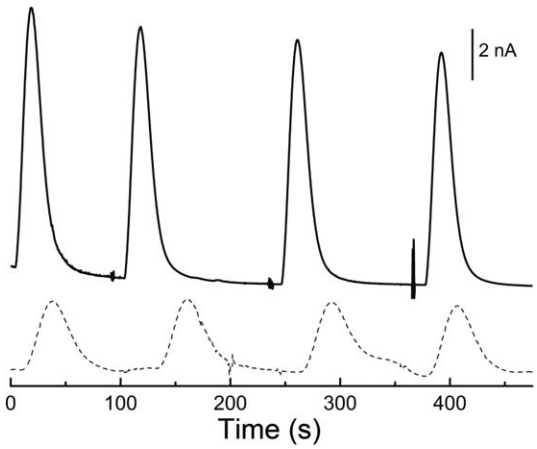

Fig. 7 Flow injection analysis of dopamine. Comparison of unmodified CPE (dashed line) and CNT modified CPE (solid line). Sequential injections of 10 μM dopamine. Off-chip reference and auxiliary electrodes were used. Applied potential: 0.6 V (*vs.* Ag/AgCl (3 M KCl)) with Pt wire as an auxiliary electrode. Flow rate: 60 μL min^{-1}.

Amperometric sensing for release of catecholamines from PC12 cells

A plot of peak area *versus* time following cellular exposure to high concentrations of potassium is shown in Fig. 8. In this study, 6×10^4 cells were used at each time point. The data shows that the majority of catecholamines appear to be released 3 min after cellular stimulation. The calculated concentration of catecholamines released at 3 min using the flow injection analysis system with MWCNT modified CPEs was estimated to be 3.6 μM (58.3 pM/cell). Previous publications indicate that PC12 cell vesicles contain an average catecholamine concentration of 110 μM and release just 0.06% of this concentration, or 67 μM, during exocytotic events.[48,49] Another report by Martin's group showed that 1.3×10^4 cells released a catecholamine concentration of 20–160 μM dopamine following calcium stimulation.[50] The amount of catecholamines detected in our system is lower than numbers published previously, an effect that can be attributed to the very low collection efficiency of this non-optimized CPE design. Also of note is the sharp decrease in catecholamine signal following the high level of catecholamine secretion at 3 min. These results support the common theory that catecholamine re-uptake occurs in neuronal cells such as PC12 cells.

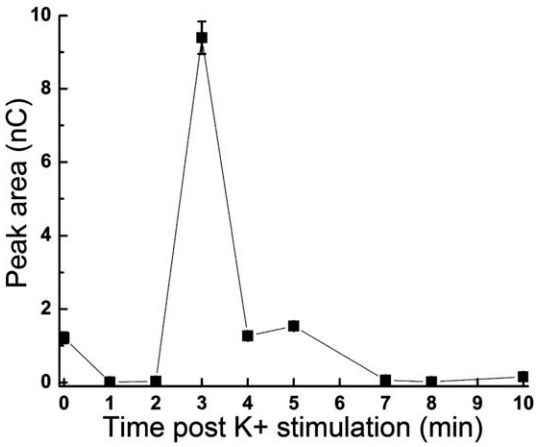

Fig. 8 Effect of potassium on catecholamine release from PC12 cells. 6.15×10^4 cells were stimulated at each time point. Supernatant was removed and stored on ice until analysis. All experimental conditions same as in Fig. 7.

Conclusion

We have demonstrated the use of a mixed PDMS-mineral oil binder to create a low cost, patternable, highly robust, carbon paste electrodes. Microfluidic electrochemical sensors were created using an on-chip fabrication method based largely on screen printing techniques. The robustness of the electrode was obtained from the cross-linking of PDMS components between the electrode and microfluidic substrate. A decrease in sensitivity (decrease in slope of current density *versus* square root of the scan rate) was observed as the amount of PDMS was increased in the electrode binder material. A binder composition of equal amounts of PDMS and mineral oil showed nearly ideal behavior based on the Randles-Sevcik equation. Although an increase in capacitive current was observed when mineral oil was added to the binder, ΔE was lower and the anodic peak current was higher in the CPEs made with hybrid binder compositions when compared to the CPEs made with all PDMS binder indicating improved electrode performance. The same CPEs were modified with dopants to demonstrate the ease with which CPEs can be tailored for detection of specific analytes. In this case, these electrodes were designed for the detection of thiols and catecholamines by modification with CoPC or MWCNTs, respectively. These modified electrodes exhibited a higher response to these classes of analytes at low detection potentials compared to unmodified CPE. Furthermore, the mixed binder CPEs did not exhibit electrode fouling when performing analysis in complex biological matrices including RBC lysate (thiol detection) and PC12 cell supernatant (detection of catecholamine release). Our proposed method is readily adaptable, can be applied to various electrode materials and is amenable to chemical modification.

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