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Fabrication and Characterization of All-Polystyrene Microfluidic Devices with Integrated Electrodes and Tubing

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Abstract

A new method of fabricating all-polystyrene devices with integrated electrodes and fluidic tubing is described. As opposed to expensive polystyrene (PS) fabrication techniques that use hot embossing and bonding with a heated lab press, this approach involves solvent-based etching of channels and lamination-based bonding of a PS cover, all of which do not need to occur in a clean room. PS has been studied as an alternative microchip substrate to PDMS, as it is more hydrophilic, biologically compatible in terms of cell adhesion, and less prone to absorption of hydrophobic molecules. The etching/lamination-based method described here results in a variety of all-PS devices, with or without electrodes and tubing. To characterize the devices, micrographs of etched channels (straight and intersected channels) were taken using confocal and scanning electron microscopy. Microchip-based electrophoresis with repetitive injections of fluorescein was conducted using a three-sided PS (etched pinched, twin-tee channel) and one-sided PDMS device. Microchip-based flow injection analysis, with dopamine and NO as analytes, was used to characterize the performance of all-PS devices with embedded tubing and electrodes. Limits of detection for dopamine and NO were 130 nM and 1.8 μ M, respectively. Cell immobilization studies were also conducted to assess all-PS devices for cellular analysis. This paper demonstrates that these easy to fabricate devices can be attractive alternative to other PS fabrication methods for a wide variety of analytical and cell culture applications.

1 Introduction

Microfluidic devices have gained much interest as an analytical platform for studying a variety of biological systems. These devices offer fast and high throughput analysis, minimal dilution effects, and in many cases increased performance. Various detection processes have been integrated into microfluidic devices giving the advantage of close to real-time analysis proving crucial for cellular studies.^{1,2} A commonly used analytical detection method for a lot of these studies has been laser-induced fluorescence (LIF). Although this technique yields low limits of detection, the samples being analyzed often require derivatization in order to fluoresce. An analytical approach to overcome this limitation and offer the advantages of selectivity and sensitivity is electrochemical detection.^{3–5} To incorporate electrochemical detection onto microchips, previous work has described the fabrication of devices using sputter-coated metal electrodes on glass substrates and polydimethylsiloxane (PDMS) micro-channels.^{4,6,7} Despite offering the flexibility to modify electrodes for desired

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analyte selectivity, the production of these electrodes on glass substrates is expensive, time consuming, and requires specialized facilities.^{8,9} Previous work has also moved away from these glass devices by embedding fluidic tubing and electrodes in materials such as epoxy and polystyrene.^{9–11} These devices are more robust, can be fabricated in-house, are considerably cheaper, and offer polishing of the electrode surface when desired. However, these devices also use PDMS micro-channels making the device three-sides PDMS.

The transparency, inexpensive fabrication, and ability to reversibly seal to a variety of substrates make PDMS a widely used and advantageous material for microchip devices yet it has been shown that this may not be the best substrate for cell culture.^{12,13} PDMS is made up of hydrophobic dimethyl-siloxane oligomers that can have uncrosslinked monomers, which in turn leach into cell culture media leading to problems in cellular environments.^{13,14} Polystyrene (PS) has been studied as an alternative microchip substrate to PDMS.^{13,15,16} With cells being cultured in flasks made of PS, which is hydrophilic, and being more biologically compatible than PDMS, an all-PS device is desired. Other groups have studied ways to incorporate channels into PS and how to bond PS substrates together. Such studies include the method of hot embossing which uses a heated hydraulic press and metal molds¹⁴ and, a simpler approach uses commercially available Shrinky-Dinks® films.^{17,18} Khine has utilized the polystyrene nature of Shrinky-Dinks® films to create cheap, and simple microfluidic devices. Channels are created by using a syringe needle to scratch channel designs into the Shrinky-Dinks® before shrinking two films together, making the final device.^{17,18} Granting all this, the devices described above lack integration of detection and tubing. Separate approaches for bonding thermoplastics (other than PS) include thermal bonding,¹⁹ solvent and chemical bonding of poly (methyl methacrylate) (PMMA),²⁰ and the lamination of polyester films.^{21,22} While not with PS, work from Carrilho and Lucio do Lago demonstrated the ability to print ink channels onto polyester films, as well as forming channels through laser ablation and use a lamination method to bond and seal the ink channel material onto another polyester film.^{21,22} Landers group determined that printed ink channels gave greater adhesion in poly (ethylene-terephthalate) transparency film devices as compared to unprinted materials when using a lamination-based bonding technique.²³

To address the need for PS with integrated components such as fluidic tubing and electrodes, in this work we present an etching/lamination-based fabrication method for the production of all-polystyrene microfluidic devices that are robust, reusable, and contain multiple components. Both all-polystyrene devices with and without electrodes and tubing are characterized and demonstrate uses for various applications. First, the fabrication of an all-polystyrene device (which were termed PS-mini) is detailed using a PS base with integrated tubing/electrodes, etched channels, and a lamination-based bonding using a Shrinky-Dinks® (SD) printed layer. These devices are then characterized to show the strength of the bonding and the ability to produce devices with multiple channel designs, widths, and depths. The motivation for the production of an all-polystyrene device was first in relation to cell culture. The methodology described here eliminates the use of PDMS micro-channels and allows endothelial cells to fully immobilize in an all-polystyrene environment without any adhesion factor. Microchip electrophoresis studies were also conducted demonstrating the ability to etch a range of channel designs and further functionality of the device. Incorporating electrodes and tubing allows for electrode modification and the ability for all-PS devices to

act as a stand-alone detection device. This provides a 3-D electrode protruding into the etched polystyrene channel. Through microchip-based flow injection analysis and electrochemical detection, the PS-mini devices led to an LOD of 130 nM for dopamine. When used for the detection of a biologically important and an analytically challenging molecule, nitric oxide (NO), these integrated electrodes (modified with Pt-black) resulted in a LOD of 1.8 μ M.

2 Materials and Methods

2.1 Materials

The following chemicals and materials were used as received: Nano SU-8 developer, SU-8 50 photoresist (Microchem, Newton, MA, USA); silicon wafers (University Wafers, Boston, MA, USA); fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA); catechol, dopamine, boric acid, TES sodium salt, sodium dodecyl sulfate, potassium nitrate, Hanks balanced saline solution (HBSS), chloroplatinic acid hydrate, and lead (II) acetate trihydrate (Sigma Aldrich, St. Louis, MO, USA); Sylgard 184 (Ellsworth Adhesives, Germantown, WI, USA); 100 μ m gold wire (Alfa Aesar, Ward Hill, MA, USA); heat shrink tubes (Radioshack); isopropanol and acetone (Fisher Scientific, Springfield, NJ, USA); colloidal silver (Ted Pella, Redding, CA, USA); polishing pads (Buehler, Lake Bluff, IL, USA); disposable aluminum dishes (Fisher Scientific); NO tank (99.5%; Airgas, Radnor, PA, USA); PS powder (Goodfellow Cambridge, Huntingdon, England); Shrinky-Dinks[®] Crystal Clear (K & B Innovations, Inc. North Lake, WI, USA); isophorone (Ercon, Wareham, MA, USA); Apache laminator model AL13P (Apache Laminators).

2.2 Fabrication of PS-mini

The fabrication of polystyrene bases have been previously reported¹⁵ and using a modified approach, the PS-mini base fabrication is depicted in Figure 1. The bottom of an aluminum weighing dish (4.4 cm in diameter, 1.2 cm deep) was pierced twice, approximately 15 mm apart, using a syringe needle. On the vertical sides of the dish, two more holes were punched into the dish to insert the extending wire and extension of the capillary; the holes were 2 mm in diameter. A 100 μ m gold electrode (connected to a copper extending wire) and a 150 μ m ID capillary (360 μ m OD) were threaded through holes of the weighing dish, each occupying a different hole. To ensure the capillary ID was flat at the polystyrene surface, the capillary was looped through the bottom and held against the remaining capillary using shrink tubing. The dish, electrode, and tubing were positioned onto the hot plate surface. Polystyrene powder (250 μ m diameter) was poured around the electrodes/tubing, into the dish, and heated for approximately 8 hours at 250°C, eventually covering the top of the dish with aluminum foil to melt the topside of the device. After melting, the device was left to cool to room temperature before removing from the hot plate. The device was detached from the dish and wet polished using techniques previously reported.¹⁵

To create channels in the polystyrene base, an organic solvent, isophorone, was used. A PDMS channel, made using soft photolithography techniques described previously^{24,25} was reversibly sealed against the capillary opening and electrode. Using a luer stub (20 gauge luer stub adapter, Becton Dickinson and Co., Sparks, MD, USA), a reservoir was punched at

the end of the PDMS channel. The isophorone was pumped through the embedded capillary and through the channel over the electrode. The PDMS channel acts as a pattern for the eventual etched channel. Multiple straight channel designs (channel widths ranging from 100 μm to 1000 μm and lengths 2 cm to 4 cm) as well as pinched, twin-tee channels, used for electrophoresis, were etched. Etched channel widths versus the PDMS defining channel used are approximately ~2–3 times wider due to the isotropic etching pattern. For the PS-mini devices used in these studies, a 4-hour etch time at 2.0 $\mu\text{L}/\text{min}$ yielded a channel depth of approximately 70 μm . For electrophoresis channels, a PDMS, pinched, twin-tee channel (80 μm wide and 110 μm depth) was used to etch into a PS base (made from an aluminum dish 6.7 cm in diameter, 1.6 cm in depth). A pinhole was punched at each end of the channels; one used to insert the capillary pumping isophorone and the others as outlet reservoirs. The etched channel dimensions were approximately 150 μm wide and 20 μm deep after a 45 minute etch time at 2.0 $\mu\text{L}/\text{min}$. After the etching time was complete, the remaining isophorone was vacuumed out of the PDMS channel before removing the channel from the PS base. The newly etched device was placed in an oven to evaporate the residual isophorone at 75°C for 2 hours. Channel depths were measured using a profilometer (Dektak³ ST, Veeco Instruments, Woodbury, NY, USA). Ink printed channel patterns were printed onto commercially available Shrinky-Dinks® (SD) using a laser jet printer (HP Laser Jet P1102w, Hewlett-Packard Development Company, LP, Palo Alto, CA). Sheets were printed with black ink and where a channel pattern was present, ink was absent. Using a standard hand-held hole-puncher (3 mm in diameter) holes were punched into the SD piece to serve as a reservoir to the underlying etched channel. The SD channel was positioned, ink side down, over the PS etched channel to match both channels and the reservoirs at the ends. Regular, one-sided tape was used to hold the SD in place before placing the device in a laminating pouch and sent through a laminator at 195°C. Finally, the laminating pouch was removed and revealed the completed, all-PS device (PS-mini). The tape can be removed if desired without any effect on the bonding. Fabrication of the all-PS device is depicted in Figure 2.

2.3 Immobilizing Cells-on-Chip

For immobilization studies of bovine pulmonary artery endothelial cells (bPAECs), culturing procedural steps were followed as described previously.²⁶ The preparation of the PS-mini device for cell immobilization was conducted by first taking an etched PS channel (1 mm width x 80 μm depth) and plasma treating for 1 minute in a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY). Following plasma treatment, SD was placed over the PS channel and the substrates were laminated together. After lamination, the PS-mini device was plasma treated a second time, along with two PDMS reservoirs, for 1 minute. This second treatment step was to treat the top of the SD and the PDMS reservoirs to create an irreversible seal between the PDMS and SD. Immobilization of bPAECs in the PS-mini device was conducted using a similar procedure, which is briefly described here. In T-25 cell culture flasks, ready to passage bPAECs were treated with 5 mL of HEPES buffer, 2 mL of 0.25% trypsin/EDTA, neutralized with 5 mL of trypsin neutralizing solution, before being scraped and centrifuged for 5 minutes. Cells were packed into a pellet in the bottom of the centrifuge tube and the supernatant solution was removed before adding 100 μL of fresh media to the pellet. The cells were suspended by titrating the media-pellet mixture before

distributing into one reservoir of the PS-mini device. Diffusion of the cell solution occurred down the channel before adding fresh media to the outlet reservoir. The filled device was placed into an incubator at 37°C and 5% CO₂ and left for 2 hours. Cell adhesion in the channel was observed after 2 hours in both the plasma treated and untreated PS channels; images. To show live cells versus dead cells, a fluorescent dyeing procedure was adopted and applied (100 µg/mL of acridine orange solution).²⁷

2.4 Modified Electrodes

For studies involving nitric oxide analysis, platinum-black (Pt-black) depositions have been used to modify electrodes.²⁶ A 100 µm gold electrode was modified with Pt-black which can be deposited either before laminating the SD channel or after. If modification before bonding was desired, a PDMS reservoir was placed over the electrode, filled with 3.5% chloroplatinic acid w/v and 0.005% lead (II) acetate trihydrate, and a deposition step was performed using cyclic voltammetry (potentials were scanned from +0.6 V to −0.35 V at a rate of 0.02 Vs^{−1} vs Ag/AgCl). Each deposition was conducted in 3 sweep segments.²⁶ For in-channel deposition, the Pt-black solution was pushed through the embedded capillary to fill the channel and reservoir. The same potentials and time were used to deposit the Pt-black and the capillary and channel were flushed thoroughly with water after the deposition was complete.

2.5 Imaging

Multiple images depicting etched channels and integrated electrodes and tubing are shown in Figure 3. Using a confocal microscope (Keyence VK-9170 Violet Laser Scanning Confocal Microscope, Keyence Corp.), Figure 3A displays a 3-D image of a straight etched channel with 100 µm wide and 25 µm depth dimensions. Fluorescence imaging was used to capture Figure 3C showing a 350 µm etched channel, sealed with printed SD, ink channels of 500 µm width, and filled with fluorescein using an upright microscope (Olympus EX 60 equipped with 100 W Hg Arc lamp and cooled 12 bit monochrome Qicam Fast Digital CCD camera, QImaging, Montreal, Canada). Excluding Figures 3A and 3C, scanning electron microscopy (SEM) (FEI Inspect F50 SEM with Schottky Field Emission as electron source) was used to image etched straight channels with embedded, modified electrodes and tubing. SEM samples were sputter coated with gold particles using (Denton Vacuum, LLC Desk V) with a timed sputter setting of 30 seconds at 20 mA. Figures 6A and 6C were captured using an Olympus BX51 microscope equipped with an Infinity 3 camera.

2.6 Electrophoresis and Microchip-based Flow Analysis

Electrophoresis studies were conducted using a pinched-gated injection scheme. The length of the separation channel was approximately 30 mm and 7.5 mm push-back channel lengths with a 200 µm intersection between pinched channels. Using a blank piece of PDMS, reservoirs were punched to match the ends of the etched channel and placed over the PS. Electrophoresis buffer (10 mM boric acid with 25 mM SDS at pH = 9.2) was used to fill the channel and 500 µM fluorescein as the sample. Continuous fluorescein injections were conducted by filling the channel by applying +700 V potential to both buffer and sample reservoirs, leaving the remaining waste reservoirs at 0 V. The fill time was set at 2 seconds. Injections/separations were carried out by applying a HV (+1000 V) to the buffer reservoir,

+600 V to both sample and sample waste reservoirs, with the remaining buffer waste reservoir set to ground (0 V). The separation was carried out for 5 seconds before the potentials were switched back to the fill step. A detection window ($335\ \mu\text{m} \times 30\ \mu\text{m}$ in size) was positioned 2 cm from the intersection and measured the fluorescence of the sample injection as the plug passed through the detection window.

Microchip-based flow analysis was carried out on the all-PS, PS-mini devices containing embedded capillary ($150\ \mu\text{m}$ ID and $360\ \mu\text{m}$ OD) and an embedded electrode ($100\ \mu\text{m}$ gold wire) that was positioned approximately 15 cm from the capillary. For the detection of dopamine 10 mM TES buffer (pH = 7.4) was used, while NO studies utilized HBSS buffer (pH 7.4). In both studies, the respective buffers were pumped continuously at $4.0\ \mu\text{L}/\text{min}$ using a $500\ \mu\text{L}$ syringe (SGE Analytical Science) and syringe pump (Harvard 11 Plus, Harvard Apparatus, Holliston, MA, USA). Using a four-port injector (Vici Rotor, Valco Instruments, Houston, TX, USA), $200\ \text{nL}$ injections were made of the respective samples. A platinum wire and silver/silver chloride (Ag/AgCl) electrode functioned as the auxiliary and reference electrodes, respectively.

Nitric oxide gas samples were prepared following a previously described procedure.^{28,29} Using argon gas, approximately $50\ \text{mL}$ HBSS buffer was degassed for 30 minutes in a glass vessel which was later sealed with a suba seal septa. In a second tube, HBSS buffer was degassed for 30 minutes and the gas flow was then switched to nitric oxide gas and allowed to concentrate the buffer with NO for another 30 minutes. The stock of NO was $1.9\ \text{mM}$.²⁹ A $5\ \text{mL}$ volumetric flask was suba sealed and degassed with argon for 5 minutes; a needle-tip probe was used to insert through the airtight suba seal. Depending on the desired concentration, an amount of NO sample (varying from $250\ \mu\text{L}$ to $20\ \mu\text{L}$) was added to the degassed volumetric flask and topped off with degassed HBSS buffer to the indicated line. Immediately following preparation, the sample was used in microchip-based flow analysis using the previously described four-port inject-ion method and HBSS buffer.

3 Results and Discussion

3.1 Fabrication and Assembly

The formation of channels in PS occurs by exposing isophorone to PS, which dissolves the material. However, because isophorone was continuously pumped over the PS forming a defined structure, the term “etch” is used here to best describe the process. Other all-PS devices, briefly mentioned in the introduction, require expensive equipment as well as elaborate fabrication/assembly, all of which lead to devices lacking integrated detection and fluidic interconnects. Previous work has elucidated the incorporation of embedded electrodes and tubing in a polystyrene layer;^{10,15} nonetheless, these devices still required the use of PDMS flow channels. For reasons briefly discussed in the introduction, eliminating PDMS and fabricating flow channels out of PS provided a hydrophilic, more biologically compatible substrate than PDMS. As shown in Figure 2, we have etched channels into a polystyrene base containing electrodes and tubing, and used a lamination method to seal commercially available Shrinky-Dinks® (SD) that have a printed channel network, resulting in a complete all-PS device. The heat from the laminator melts the printed ink, which acts as an adhesive between the SD and PS creating a very tight seal between the two substrates. It

is important to note that the SD does not shrink during this process and that the fabrication, etching or bonding process did not occur in a cleanroom, making this approach attractive to those with limited fabrication equipment.

A variety of organic solvents were previously tested (acetone, γ -Butyrolactone, chloroform, dichloromethane, and isophorone) before isophorone was selected for etching purposes. When acetone, γ -Butyrolactone, chloroform, and dichloromethane were used, the PS was left with bubbles and discoloration. The etching rates were too rapid and there was often leaking between the PDMS sacrificial layer and the underlying PS. However, with isophorone, it was observed that the channels formed were uniform, smooth, and discrete. As shown in Figure 3, distinct channel dimensions are achieved with this etching process. By continuously pumping isophorone through the PDMS channel, the PS slowly dissolves away and leaves a well-defined channel profile behind. The bottoms of the etched channels are very smooth along with the channel walls. Because of the organic composition of isophorone, the PDMS channel swells slightly and causes the PS to etch isotropically (the resulting etched channel was ~2–3 times wider than the PDMS channel). This phenomenon renders a rounded channel with a wider top edge and slightly narrower bottom. Channel depths are dependent on etch time, giving deeper channels the longer isophorone is pumped over PS. Optimized channel widths and depths for flow analysis studies were $680 \pm 20 \mu\text{m}$ wide, with depths of $63 \pm 8 \mu\text{m}$ ($n = 3$ different devices). These dimensions were achieved using fresh isophorone, a $200 \mu\text{m}$ wide by $120 \mu\text{m}$ tall PDMS channel, and a 4 hour etch time at $2.0 \mu\text{L}/\text{min}$ flow rate. The average height of the electrode protruding into the etched channels was approximately $55 \pm 13.2 \mu\text{m}$ ($n = 3$), occupying 68.8% of the channel. Cell culture channels used a $400 \mu\text{m}$ wide PDMS channel and allowed to etch for the same time and flow rate and achieved 1.0 mm wide and $60 \mu\text{m}$ deep channels. The ink-printed channels on SD were made to have widths slightly bigger than the corresponding etched channel. It was found that when the ink-printed channels (on the SD layer) were the same width as the etched channel (in PS), some residual ink particles were “in the channel” and caused bubbles to form during flow. Therefore, a wider, printed channel SD layer was sealed against the PS, with the printed channel being approximately $100 \mu\text{m}$ wider than the largest portion of the etched channel. This resulted in a strong seal, without bubbles or leaking between the two substrates. More importantly, the solution was only contained within the etched channel and did not conform to the wider, ink-printed channel. This can be seen clearly in Figure 3C; fluorescein did not diffuse to the SD ink-channel width. Strength of the bond between the PS and SD was tested by gradually increasing the flow of 1 mM fluorescein, using a syringe pump. Starting at a flow rate of $5 \mu\text{L}/\text{min}$, the system was allowed to run for 60 seconds before increasing the flow rate in $5 \mu\text{L}/\text{min}$ increments. After reaching $100 \mu\text{L}/\text{min}$, the flow rate was then increased by increments of $10 \mu\text{L}/\text{min}$. A flow rate of $2 \text{ mL}/\text{min}$ was reached, still without any leaking or delaminating of the seal, before concluding that the seal was sufficiently strong enough for flow analysis studies typically conducted. The maximum linear velocity reported for the PS-mini device was $111.11 \text{ cm}/\text{sec}$ (higher flow rates were not tested). It should also be noted the SD cover is easily removed by simply peeling off the material using a razor. This allows the device to be cleaned and sealed against another SD cover by lamination if desired. In many of these studies, devices were used multiple times.

3.2 Cells in PS-mini Device

Ultimately, the inspiration for an all-PS device was to eliminate the need for PDMS flow channels and create a device that was biologically compatible for cellular analysis studies. In previous work, PDMS channels were plasma treated to increase the hydrophilicity of the polymer and provide a more suitable environment for cells. However, over time, PDMS reverts back to its hydrophobic nature, and with cell culture studies, it has been shown that toxins (PDMS oligomers) can leach into the channel after a day of culture.^{5,13} With the PS-mini device, having all sides of the channel be PS, this allowed cells to be in an environment similar to that of a cell culture flask. In Figure 4, a study was conducted comparing bPAECs in an untreated PS-mini device versus a plasma treated PS-mini device. From Figure 4A, some confluence can be seen however, the cells are not fully immobilized after a 2 hour incubation period. Compared to a plasma treated PS-mini device, after 2 hours, the bPAECs are fully immobilized, elongated, and much more confluent, as shown in Figure 4B. The fluorescent image (Figure 4C) allows the visibility of live cell nuclei shown as bright fluorescent dots versus the dead cells. An *in situ* approach for channel treatment was also studied. From previous studies, we also found that corona discharge treatment can be used to modify the surface energy of PS and increase the hydrophilicity.³⁰ It should be noted that all cell immobilization studies were conducted without the application of an adhesion factor, such as collagen.

3.3 Electrophoresis

To display the versatility of etching channels in PS, a pinched injection, twin-tee electrophoresis channel design was etched into a PS base, as shown in Figure 5A and 5B. The etching process gives smooth, flat, channel walls not only with straight channel designs but with electrophoretic twin-tee channels as well. The confocal images displayed in Figure 5A and 5B show defined, etched channels of the intersection of the pinched injection (twin-tee). Fluorescence injections were conducted using etched electrophoresis channels with 150 μm wide by 20 μm deep dimensions and 500 μM fluorescein. Due to the channel dimensions, more importantly the width of the etched channels, an all-PS device was unable to be used. With the printed SD layer, bubbles were continuously forming and present in the channel. This could be due to Joule heating and the SD layer not allowing the gasses to dissipate efficiently to counteract the effect. Instead, a channel-less piece of PDMS was used as the top layer to conduct electrophoresis. Fluorescein was used as the test analyte and, as shown in Figure 5C, reproducible injections were achieved. The average peak height was 44.73 AFU ($n = 20$) with a relative standard deviation of 2.8%. Individual injections were conducted to calculate the efficiency of the device and were calculated at 1,180 plates ($n = 6$) and 3.8% relative standard deviation.

3.4 Microchip-based Flow Injection Analysis

Dopamine and nitric oxide detection were conducted using microchip-based flow injection analysis with straight channel, PS-mini devices. Dopamine is an important neurotransmitter involved in the functions of motor control as well as behavior. The molecule has been studied both *in vitro* and *in vivo* studies. The embedded tubing from the PS device was directly inserted into an off-chip, four-port injector to carry out 100 μM dopamine injections,

displaying the reproducibility, detection limits, and linearity of the PS-mini device). As shown in Figure 6A, the 100 μm gold electrode was protruding vertically within the channel and had a very smooth and flat surface at the top. In Figure 6B, the reproducible injection data displays an average peak height of 0.12 ± 0.01 nA with a RSD of 7.2% ($n = 10$). The detection of the electrode was studied by conducting a calibration curve using dopamine concentrations ranging from 200-1 μM . Linearity was calculated to be $r^2 = 0.999$ showing direct correlation with the peak height and concentration injected. The limit of detection for the PS-mini device was 130 nM.

For the detection of nitric oxide (NO), which is a small, gaseous molecule that plays a vital role in the vasodilation of blood vessels^{31–33}, the gold electrode was modified with Pt-black deposition. The Pt-black catalyzes nitric oxide and enhances the signal of detection.^{26,34–36} Others have previously detailed that a platinized electrode offers the advantages of providing faster electron transfer kinetics for NO oxidation while also increasing the surface area of the microelectrode.³⁵ The electrode was coated with a layer of Pt-black on the sides of the electrode as well as the top surface. By assembling the PS-mini device and filling the channel with the Pt-black solution, the deposition could be conducted in-channel allowing for the sides of the electrode to be fully coated. In Figure 3D and E, a side view of a Pt-black deposited electrode is shown. A top view of the modified electrode was imaged and displayed in Figure 6C. As shown in Figure 3D, a valley around the electrode is present. This phenomenon varies from device to device, with some devices having this to a lesser extent. Various concentrations were used to construct a calibration curve to determine the linearity and limit of detection of the modified electrode within the PS-mini device. Nitric oxide concentrations ranged from 190-7.6 μM were injected. The limit of detection was 1.8 μM with a linearity coefficient calculated to be $r^2 = 0.988$ (see Figure 6D). We have previously utilized a platinized electrode array in microchip devices for NO detection, with a LOD of 9 nM.²⁶ The exploration of etched arrays to improve detection limits for NO in these all-PS devices will be explored in the future.

4 Conclusion

In this paper, a lamination-based method was utilized to fabricate an all-PS device. The PS-mini device fabrication was optimized by selecting the appropriate solvent and varying etching times for the needs of different experiments. Characterization of the PS-mini device was conducted by confocal and scanning electron microscopy, cell culture, electrophoresis, and electrochemical detection of dopamine and nitric oxide through microchip-based flow analysis. Imaging provided evidence that etching channels into polystyrene gives an isotropic etch and offers smooth channels, this also included proof that polystyrene was dissolved around embedded electrodes yielding a 3-D electrode protruding into the channel. The PS-mini device was also studied as an alternative cell-culture platform as compared to PDMS devices. With the findings described in this paper, these all-PS devices provided a more biologically compatible substrate for bPAECs showing cell immobilization and confluency, without the need of an adhesion factor. In addition to showing that the etched channels can be used for electrophoresis, it was shown that the ability to create an all-PS device with 3-D electrodes allows the integration of electrochemical detection making it its own stand-alone analysis device. Dopamine and nitric oxide studies demonstrated great

promise in the detection and operation ability of the PS-mini. Future work will involve creation of a PS-mini cell culture platform that can be incorporated with a separate analysis chip to help study cellular releasates in a chip-to-chip based format.

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Figure 1. Encapsulation of tubing and electrodes in polystyrene. (A) Placement of 150 μm ID (360 μm OD) fused silica capillary and 100 μm diameter gold electrode in aluminum weighing dish. (B) Commercially available polystyrene powder is poured into dish, around electrodes. (C) Polystyrene is heated at 250 $^{\circ}\text{C}$ and left to completely melt over an 8 hour period. After melting, polystyrene is allowed to cool to room temperature.

Figure 2.

Fabrication of all-polystyrene device (PS Mini). Tubing and electrodes are first encapsulated in polystyrene (PS) as depicted in Figure 1. A PDMS channel is then aligned with capillary and electrode over the PS base. Using the inserted capillary, isophorone is pumped through the PDMS channel at a desired flow rate and time. The remaining isophorone is vacuumed out of the channel, before the PDMS is removed, and the PS is left to evaporate off the residual isophorone for 2 hours in an oven at 75 °C. A channel pattern is printed onto the Shrinky-Dink (SD) film (note that ink is absent where an underlying channel is present). The SD layer is hole-punched (to make a reservoir) before positioning above the etched channel. Using tape, the SD channel is securely placed over the etched channel before being placed in a laminating pouch and sent through the laminator at 195 °C. After this bonding step, the completely assembled device is removed from the lamination pouch.

Figure 3.

Etched channel in polystyrene base with embedded electrodes and tubing: (A) Confocal image of 100 μm channel with approximately 25 μm depth. (B) SEM image of straight channel etched; channel depth 80 μm; (C) Shrinky-Dink sealed over channel with fluorescein fill. Fluorescein stays within etched channel dimensions and does not conform to ink defined channel of Shrinky-Dink; (D) and (E) SEM images of platinum-black deposited gold electrode, in etched channel. Electrode height in channel is approximately 80 μm; (F) SEM image of embedded capillary showing etched channel.

Figure 4. bPAECs in All-PS device. (A) Micrograph of bPAECs in untreated PS Mini channel after 2 hour incubation period. Dimension of channel 1000 μm wide and 60 μm deep. (B) Micrograph of bPAECs in plasma treated PS Mini channel after 2 hour incubation time. (C) Micrograph of dyed bPAECs in channel.

Figure 5.

Electrophoresis channels etched in polystyrene. (A) Confocal image at intersection of etched, pinched channel design; dimensions are 150 μm wide and 20 μm deep. (B) 3-D image of etched channel displays smooth etch and defined edges of channel. (C) Reproducible fluorescein (500 μM) injections using etched channels ($n = 20$). Injection field strength = 225 V/cm and push back field strength = 145 V/cm.

Figure 6. Microchip-based flow analysis of a PS Mini device. (A) Micrograph of gold electrode in etched channel. (B) Reproducible dopamine injections using PS Mini device ($n=10$). (C) Micrograph of platinum-black modified, 100 μm gold electrode in etched device. (D) Calibration curve of nitric oxide gas detection using PS Mini device. Concentrations of nitric oxide are 7.6 – 190 μM; yielding a LOD of 1.8 μM.