Published in final edited form as:

Electrophoresis. 2010 August ; 31(15): 2534–2540. doi:10.1002/elps.201000029.

Integration of On-Chip Peristaltic Pumps and Injection Valves with Microchip Electrophoresis and Electrochemical Detection

Amanda L Bowen and R. Scott Martin *

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

Abstract

A microfluidic approach that integrates peristaltic pumping from an on-chip reservoir with injection valves, microchip electrophoresis and electrochemical detection is described. Fabrication and operation of both the peristaltic pumps and injection valves were optimized to ensure efficient pumping and discrete injections. The final device uses the peristaltic pumps to continuously direct sample from a reservoir containing a mixture of analytes to injection valves that are coupled with microchip electrophoresis and amperometric detection. The separation and direct detection of dopamine and norepinephrine were possible with this approach and the utility of the device was demonstrated by monitoring the stimulated release of these neurotransmitters from a layer of cells introduced into the microchip. It is also shown that this pumping/reservoir approach can be expanded to multiple reservoirs and pumps, where one reservoir can be addressed individually or multiple reservoirs sampled simultaneously.

Keywords

Microchip Electrophoresis; Electrochemical Detection; Peristaltic pumps; Valving

1 Introduction

While the use of microfluidic devices to carry out electrophoretic separations is well-established [1,2], more recent work has demonstrated that these systems can be used to perform conventional assays on biological systems in an automated and high-throughput fashion [3–7]. The ability to integrate multiple processes in a manner where chip operation is reproducible and automatable is attractive in terms of both enabling the study of biological systems and ensuring that biologists and clinicians utilize the technology. An area of interest in this field is on-chip patterning of cells *via* cell culture or cell immobilization techniques, as microchip approaches can provide small and complex structures that mimic the *in vivo* environment that cells encounter [8,9]. By integrating a method to analyze the molecules that are released from cells, it will be possible to investigate processes such as how cells act in concert and how populations of cells communicate. While there have been reports of such integrated mimics that can monitor one specific analyte released from cells [3,10–12], it is also desirable to integrate cell culture/immobilization with analysis techniques such as electrophoresis and electrochemical detection. This will enable the separation and direct detection of a wide variety of molecules that are released from cells in near real-time. Ideally, the integrated device would be amenable to automation while also providing the temporal resolution needed for monitoring cellular release.

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

In this paper, we present work towards this type of microfluidic system, with a device that integrates peristaltic pumping from an on-chip reservoir with injection valves, microchip electrophoresis and electrochemical detection. The ability to couple injection valves with microchip electrophoresis and amperometric detection allows the separation and direct detection of dopamine and norepinephrine that is sampled from the on-chip reservoir. Fabrication and operation of both the peristaltic pumps and injection valves were optimized to ensure efficient pumping and discrete injections. The utility of the device was demonstrated by monitoring the release of neurotransmitters from a layer of immobilized cells introduced into the on-chip reservoir *via* PDMS micropallets. Finally, the ability to expand this pumping/reservoir approach to multiple reservoirs where one reservoir can be addressed individually or multiple reservoirs sampled simultaneously was investigated.

2 Materials and Methods

2.1 Microchip fabrication and operation

Fabrication and operation of PDMS-based bilayer valving microchips (Figure 1) followed previously published methods [13,14]. The electrophoresis channel width was 40 μm and the channel depths varied between 20 and 25 μm . The valving and pump channels were filled with water [4,14]. MAC valves (MAC Fluid Power Engineering, St. Louis, MO) were used to trigger injection valves #1 and #2 by means of a timer-based control unit. The peristaltic pumps were operated at 5 psi using an 8-channel valve controller from Fluidigm Corp. (San Francisco, CA). In these studies, PDMS injection valves were used as the interface between the hydrodynamic and electrophoresis flow regimes, with injection valve #1 being normally open and injection valve #2 being normally closed (Figure 2). An injection is made by shutting off the high voltage and actuating the valves for a few seconds (closing valve #1 and opening valve #2), with the duration of the actuation being used to control the injection plug size [4,14]. The buffer used consisted of 10 mM boric acid with 25 mM SDS (pH=8.0). Two voltage sources are required to operate the bi-layer valving microchip. In most studies a high voltage (+700 V) was applied to the buffer reservoir and a pushback voltage (+200 V) was used to eliminate stagnant sample at the valve interface.

Sputtering of 200 Å titanium and 2000 Å palladium on high quality borosilicate glass was performed at Stanford University's Nanofabrication Facility. The decoupler design was patterned using positive resist and wet etching, as previously reported [15]. Electrodeposition of palladium onto the patterned decoupler was used to increase the decoupler surface area and more effectively dissipate hydrogen formation at the decoupler [13]. The length of the decoupler in these studies was 2000 μm , with the width being fixed by the separation channel. PDMS micromolding channels (10 $\mu\text{m} \times 1 \mu\text{m}$) were used to define structures for micromolding carbon ink electrodes [15–17]. A two electrode format was used with a CH Instrument potentiostat (812B, Austin, TX, USA) to apply +0.9 V to the carbon ink working electrode relative to a platinum quasi-reference electrode.

Injection plug sizes were imaged using fluorescein and a fluorescence microscope (IX71, Olympus America) equipped with a 100 W Hg arc lamp, fluorescein filters, and a cooled 12-bit monochrome Qicam Fast digital CCD camera (QImaging, Montreal, Canada). Streampix Digital Video Recording software (Media Cybernetics, Silver Spring, MD, USA) was used to capture images and Q Capture Pro software (QImaging, Montreal, Canada) was utilized for determining sample plug length

2.2 Cell studies

PC 12 cells (ATCC, Manassas, VA) were cultured (at 37°C and 7% CO₂) in 35 mm Petri dishes (Fisher Scientific, Springfield, NJ) that were pre-treated with polygen, a solution of

60.0 μL of collagen solution (3.0 mg/mL, Sigma-Aldrich, St. Louis, MO), 4.5 mL of a 0.01% poly-L-lysine solution (Sigma-Aldrich), and 9.0 mL of 30% ethanol, as previously described [4,18]. The cells were allowed to grow until ~90% confluency with the media (F-12K supplemented with 140 $\mu\text{g/mL}$ penicillin-streptomycin solution, 2.5% fetal bovine serum, and 15% horse serum, ATCC) being replaced every 1–2 days. Once the cells were confluent, on-chip cell experiments were performed. Circular PDMS micropallets (3.5 mm in diameter) were created from a thin layer of PDMS using a hole punch. For coating and cell immobilization, the micropallets were moved with tweezers into PDMS wells (4.5 mm in diameter) that were sealed onto a glass plate. Solutions were pipetted directly to the wells. Prior to immobilization, a 3 mg/mL collagen solution was allowed to sit over the micropallets in an incubator for 1 hr, the solution was removed *via* pipette, and the pallet was placed in a UV hood for at least 6 hours to complete evaporation of any residual solution. A cell suspension from a confluent Petri dish of PC 12 cells was made by scraping the cells (immersed in F-12K media) from the dish, placing the suspension in a 15 mL centrifuge tube (Benton-Dickinson), centrifuging at 110 g for 2 min, and adding fresh media to the tube so that the total volume was 2 mL. Forty μL of the resulting suspension was then added over each micropallet and allowed to sit in the incubator overnight. Prior to analysis, the cells were pre-loaded with dopamine and norepinephrine by placing 1 mM of each in media over the cells for 20 min [19]. After this period of time, the solution was removed from the well and the micropallet was rinsed four times with cell-compatible buffer (pH=7.4, containing 1.4 mM KCl, 72.5 mM NaCl, 0.5 mM MgCl_2 , 0.6 mM CaCl_2 , and 0.25 mM NaH_2PO_4) [4]. The PDMS micropallet was then transferred *via* tweezers to the microchip sample reservoir (SR, 4.5 mm in diameter, see Figure 1). As described in the results/discussion, the pallet was exposed to various buffer solutions as well as a nicotine stimulant solution [20], which was comprised of a 1 mM nicotine tartrate solution (prepared from salt, Sigma-Aldrich) in the cell-compatible buffer described above except with elevated CaCl_2 (2 mM).

3 Results and Discussion

3.1 Integration of peristaltic pumps

Our group recently developed a 3-dimensional microchip device that integrates the immobilization of PC 12 cells in microchannels with on-chip valving, microchip electrophoresis, and fluorescence detection [18]. In this device, the stimulated release of dopamine from a microchannel containing PC 12 cells was monitored *via* an electrophoretic separation and an on-chip derivatization step. While this work demonstrated the ability to integrate multiple processes, there were limitations to the approach. The first was the need for derivatization prior to fluorescence detection. This could be circumvented by integrating a direct detection mode for catecholamines, such as electrochemical detection. The second issue involved the need to immobilize the cells directly in the fluidic network. This non-modular cell culture approach becomes a concern in high throughput studies, as only one layer of cells can be analyzed per device and if any portion of the device fails the cell layer cannot be reused. This could be addressed by introducing the cells into the device in a modular fashion *via* reservoirs. The use of reservoirs for placing cells in microfluidic devices has been used for islets of Langerhans [21,22] and the immobilization of endothelial cells [3,12].

The device shown in Figure 1 was developed to address these challenges. One key aspect of the device is a means to continually sample from a reservoir where cells can be placed while also enabling the discrete introduction of the sampled solution into a separate part of the device where an electrophoretic separation and electrochemical detection takes place. In this work, on-chip PDMS-based peristaltic pumps were utilized to sample from the reservoir while separately controlled PDMS valves were used for the discrete injection events. The

on-chip peristaltic pumps were used in order to minimize dead volumes and Taylor dispersion that can occur when using off-chip sampling while working towards a fully integrated and automated device. These types of self-priming pumps have been previously described [23–26] and use 3 evenly spaced valves that are actuated in a sequential order. Another important part of the device shown in Figure 1 is the coupling of microchip electrophoresis with electrochemical detection; this enables the direct detection of catecholamine neurotransmitters that are being studied here. This coupling was achieved by use of a sputtered palladium decoupler and carbon ink microelectrodes [15,17,27]. As we recently described, the ability to use electrodepositions of palladium onto a sputtered palladium electrode results in a decoupler that has an increased surface area that can more effectively dissipate hydrogen produced at the decoupler when high ionic strength samples are electrophoretically separated [13].

Characterization of the pump and injection valve location/dimensions was essential to achieve reproducible pump actuation, maximum pump flow rate, and proper injections. To continually sample from the reservoir towards the injection interface, the pump valve that is first actuated (valve #1 in Figure 1) was placed closest to the sample reservoir. The first design that was used placed the pump valves close to the injection valves, as shown in Figure 1B. With this initial design, the flow channel was 100 μm in width while both the pump and injection valves were 200 μm in width so that actuation area of each was $100 \times 200 \mu\text{m}$ (the spacing between each pump valve was 200 μm). Within using this design for a few minutes, flow was seen leaking into the separation channel (*via* fluorescein and fluorescence detection) because injection valve #2 was not closing completely. This was remedied by increasing the flow channel width to 200 μm so that actuation area of both the injection and pump valves was $200 \mu\text{m} \times 200 \mu\text{m}$. In this case, no apparent leakage was seen into the separation channel, as injection valve #2 was able to fully close; however, the larger valve actuation area often resulted in incomplete pump valve actuation with the pump valves having a tendency to stick to the flow channel. In addition, the close proximity of the injection and pump valves around the injection intersection created a high amount of pressure in that area. This in turn routinely caused the reversibly sealed PDMS chip to detach from the glass surface. These problems were circumvented by moving the peristaltic pumps away from the intersection, with pump valve #3 being 1.5 mm from the intersection (shown in Figure 1C) and valve #1 being 5.0 mm from the sample reservoir. The flow channel in this design remained 200 μm and, to ensure proper closing of the injection valves, the injection valve actuation area was fixed at $200 \mu\text{m} \times 200 \mu\text{m}$. Changing the peristaltic pumps actuation area to $200 \mu\text{m} \times 100 \mu\text{m}$ helped to ensure proper pump valve function and less valve sticking. The flow rate and linear velocity created by the pumps in the flow channels were calculated by measuring the channel dimensions and the time required for a fluorescein plug to travel a known distance. With the optimized design shown in Figure 1C and an optimal pump frequency (discussed below), an average linear velocity of $167 \pm 14 \mu\text{m/s}$ and an average flow rate of $49 \pm 4 \text{ nL/min}$ were achievable ($n = 3$ different chips).

The injection sequence using the optimized design is depicted in Figure 2. An injection is made into the electrophoresis channel by opening injection valve #2, closing injection valve #1, and turning off the separation/pushback voltages (Figure 2A). After a sufficient plug is generated, valve #2 is closed and valve #1 is opened, and the separation/pushback voltages are re-applied. The injection volume is dependent upon the valve actuation time and the optimal pump frequency. In previous work from our lab where a syringe pump was used to pump fluids into the device at 0.3 $\mu\text{L/min}$, typical injection valve actuation times varied from 0.2 to 1.0 sec [4,13,14]. In this work, the reduced pump flow rate necessitated the use of longer injection valve actuation times, typically 5.0 to 7.0 sec. Using the optimized pump design (Figure 1C), the size of plug that is injected into the electrophoresis channel was determined at various pump frequencies using a constant injection valve actuation time

(Figure 2C). As can be seen, the maximum injection size was seen at a pump frequency of 2.5 Hz, which resulted in a plug size of $1065 \pm 4 \mu\text{m}$ ($n=4$, $916 \pm 3 \text{ nL}$ plug volume).

3.2 Device characterization

One of the advantages of integrating multiple processes on-chip is a decrease in dead volume which, as compared to off-chip processes, leads to improvements in temporal resolution when monitoring dynamically changing systems. In order to evaluate how well the device shown in Figure 1 responds to external changes in concentration, a study monitoring the increase of dopamine concentration in the sample reservoir was performed. Figure 3A shows the results of this study, with 7 sec. injections repeated every 25 sec. Six injection events, each followed by electrophoretic separation and amperometric detection, were made from a 250 μM dopamine solution before the concentration of dopamine in the reservoir was changed to 500 μM . A change in signal is evident one separation after the concentration change and within two separations the change in signal is complete. The rise time (time during which signal increases from 10% to 90% of the maximum intensity) for the dopamine concentration change study was calculated to be 70 sec. This is somewhat limited by the separation time, which could be shortened to reduce the rise time. These values are much improved compared to previous studies from our lab when an off-chip sampling process (microdialysis sampling) is coupled with microchip electrophoresis and electrochemical detection (330 sec. to see a change in signal and a rise time of 150 sec.) [4]. This can be explained by the much reduced dead volume that is possible when processes are integrated on-chip.

In order to evaluate the separation capability of the device, dopamine and norepinephrine were chosen, as these analytes are somewhat difficult to differentiate since they only differ by one hydroxyl group. A mixture was introduced into the sample reservoir and the peristaltic pumps were used to continuously flow this solution past the injection interface. The results of using the injection valves to introduce a sample plug (620 pL in volume) followed by an electrophoretic separation and amperometric detection with carbon ink microelectrodes [15–17] is shown in Figure 3B. Use of a 3-cm separation channel led to a baseline separation between the analytes (resolution = 1.6). As we [13] and others [28,29] have previously shown, this separation resolution can be increased by increasing the channel length. It was found here that use of an 8-cm electrophoresis channel with a serpentine design to separate the same mixture increased the resolution to 4.4. A 3-cm electrophoresis channel and a mixture of dopamine and norepinephrine were used to determine the limit of detection (LOD) for the device (in all cases at a S/N of 3). Using a single carbon ink microelectrode led to a LOD of 9.3 μM for dopamine and 7.5 μM for norepinephrine. As was recently shown by Amatore *et al.*, the use of serially configured dual electrodes operated at the same potential (generator-generator mode) with optimized electrode widths and spacings can lead to an increased signal-to-noise ratio, as compared to a single electrode of the same overall surface area [30]. In the case of oxidative processes, this is due to the ability of fresh, un-oxidized analyte to diffuse to the surface of the second electrode, as opposed to a larger single electrode where analyte may become depleted at the electrode surface. This trend was also seen in this device. Use of two carbon ink electrodes (10 μm in width, each separated by 100 μm) operating at the same potential (+0.9 V) led to an improved LOD (3.6 μM for dopamine and 2.2 μM for norepinephrine). The LOD could be improved into the nanomolar range for both analytes (400 nM for dopamine and 600 nM for norepinephrine) by using 4 carbon ink electrodes (10 μm in width, each separated by 100 μm) operated at +0.9 V. The remaining studies used two electrodes operated at the same potential.

3.3 Cellular Analysis

The ability to use peristaltic pumps to continuously sample from a reservoir led to the investigation of reservoir-based cell immobilization where cells are introduced into the reservoir on micropallets. The micropallets were made from a cured layer of PDMS, as it has been previously shown that PC 12 cells can be immobilized onto this substrate [18,31]. While culturing of cells on SU-8 micropallets has been described by Allbritton's group [32], the approach that is used here involves immobilizing PC 12 cells onto PDMS micropallets and, when analysis of the cell layer is desired, the pallet is introduced into the sample reservoir. Micrographs of a collagen-coated micropallet with immobilized PC 12 cells are shown in Figures 4A and B. Figure 4A shows the micropallet inside the sample reservoir and Figure 4B is a micrograph of a pallet after buffer rinses (discussed below).

The ability of the integrated microchip device to monitor the release of dopamine and norepinephrine from PC 12 cells immobilized on the micropallet is demonstrated in Figure 4C. Before the cell-coated micropallet was placed in the sample reservoir, standards of dopamine and norepinephrine (100 μM each) were prepared in the stimulant solution and analyzed (top trace). A cell-coated micropallet was then added to the sample reservoir. Initially, the micropallet was rinsed 4 times with a cell-compatible buffer and the 4th addition of buffer was analyzed (bottom trace). Next, a nicotine stimulant solution containing elevated Ca^{2+} was added to the reservoir [20, 33]. Once the stimulant was added, the peristaltic pumps transferred solution from the reservoir towards the injection interface, where a discrete injection was made into the electrophoresis channel. The resulting electropherogram (2nd trace from top) clearly shows the separation and detection of dopamine and norepinephrine released from the cells. It is worthy to note that a separation between the 2 analytes is possible even though the sample is of relatively high ionic strength, which can cause destacking issues [34, 35]. Since the cells were preloaded with dopamine and norepinephrine prior to analysis [19], a pallet with no cells was placed in the sample reservoir, exposed to the same pre-loading and rinsing steps as the cell-coated pallet, and analyzed in a similar fashion. As shown in the third trace, no residual dopamine or norepinephrine was seen. By using the data shown in Figure 4 to compare the cellular release data to injection of standards, it was found that the released concentration of dopamine and norepinephrine from a pallet with a cell density of $\sim 2170 \text{ cells/mm}^2$ was $36.7 \pm 2.9 \mu\text{M}$ and $56.8 \pm 4.5 \mu\text{M}$, respectively.

3.4 Use of multiple reservoirs

While the above studies used a single sample reservoir and one set of peristaltic pumps, it is possible to use multiple reservoirs and several sets of peristaltic pumps to integrate more processes. The device shown in Figure 5A was used to demonstrate this principle. Two sets of peristaltic pumps were used to address each reservoir. Initially, only the pumps addressing the top catechol reservoir were actuated, with the pumps that sample from the dopamine reservoir being fully closed. The catechol solution was pumped to the injection interface and a discrete injection into the electrophoresis channel was performed. The separation and detection of this solution is shown in Figure 5B(i). Next, the dopamine pumps were actuated and the two flow streams mixed and also discretely injected into the separation channel. The separation and detection of this mixture is shown in Figure 5B(ii). It can be envisioned that this type of device could be expanded even further to include multiple reservoirs for rapid calibration curves followed by sampling from a cell-containing reservoir for full automated cellular analysis.

4 Concluding Remarks

This work has shown that it is possible to integrate peristaltic pumping from an on-chip reservoir with injection valves, microchip electrophoresis and electrochemical detection. The fabrication and optimization of the peristaltic pumps was described as was the effect of using multiple carbon ink microelectrodes for amperometric detection. The ability to use the peristaltic pumps to continuously sample from the reservoir enables the introduction of cells into the device *via* PDMS-based micropallets. The ability to couple microchip electrophoresis with amperometric detection allows the separation and direct detection of dopamine and norepinephrine released from the cells. Future work that results from these studies could include the use of multiple reservoirs for fully automated calibration and cellular analysis as well as the use of different cell lines in each reservoir to investigate processes such as cell-to-cell communication.

Acknowledgments

This project described was supported by Award Number R15GM084470 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

References

1. Vilkner T, Janasek D, Manz A. *Anal Chem* 2004;76:3373–3386. [PubMed: 15193114]
2. West J, Becker M, Tombrink S, Manz A. *Anal Chem* 2008;80:4403–4419. [PubMed: 18498178]
3. Genes LI, Tolan NV, Hulvey MK, Martin RS, Spence DM. *Lab Chip* 2007;7:1256–1259. [PubMed: 17896007]
4. Mecker LC, Martin RS. *Anal Chem* 2008;80:9257–9264. [PubMed: 19551945]
5. Dishinger JF, Reid KR, Kennedy RT. *Anal Chem* 2009;81:3119–3127. [PubMed: 19364142]
6. Luk VN, Wheeler AR. *Anal Chem* 2009;81:4524–4530. [PubMed: 19476392]
7. Tolan NV, Genes LI, Subasinghe W, Raththagala M, Spence DM. *Anal Chem* 2009;81:3102–3108. [PubMed: 19301907]
8. El-Ali J, Sorger PK, Jensen KF. *Nature* 2006;442:403–411. [PubMed: 16871208]
9. Martin RS, Root PD, Spence DM. *Analyst* 2006;131:1197–1206. [PubMed: 17066186]
10. Oblak TD, Root PD, Spence DM. *Anal Chem* 2006;78:3193–3197. [PubMed: 16643013]
11. Spence DM, Torrence NJ, Kovarik ML, Martin RS. *Analyst* 2004;129:995–1000. [PubMed: 15508026]
12. Hulvey MK, Martin RS. *Anal Bioanal Chem* 2009;393:599–605. [PubMed: 18989663]
13. Bowen AL, Martin RS. *Electrophoresis* 2009;30:3347–3354. [PubMed: 19739137]
14. Li MW, Martin RS. *Electrophoresis* 2007;28:2478–2488. [PubMed: 17577199]
15. Mecker LC, Martin RS. *Electrophoresis* 2006;27:5032–5042. [PubMed: 17096314]
16. Kovarik ML, Torrence NJ, Spence DM, Martin RS. *Analyst* 2004;129:400–405. [PubMed: 15116230]
17. Kovarik ML, Li MW, Martin RS. *Electrophoresis* 2005;26:202–210. [PubMed: 15624172]
18. Li MW, Martin RS. *Analyst* 2008;133:1358–1366. [PubMed: 18810283]
19. Moore JM, Papke JB, Cahill AL, Harkins AB. *Am J Physiol Cell Physiol* 2006;291:C270–281. [PubMed: 16467400]
20. Chen TK, Luo G, Ewing AG. *Anal Chem* 1994;66:3031–3035. [PubMed: 7978300]
21. Dishinger JF, Kennedy RT. *Anal Chem* 2006;79:947–954. [PubMed: 17263320]
22. Roper MG, Shackman JG, Dahlgren GM, Kennedy RT. *Anal Chem* 2003;75:4711–4717. [PubMed: 14674445]
23. Cellar NA, Burns ST, Meiners JC, Chen H, Kennedy RT. *Anal Chem* 2005;77:7067–7073. [PubMed: 16255611]

24. Leach AM, Wheeler AR, Zare RN. *Anal Chem* 2003;75:967–972. [PubMed: 12622393]
25. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. *Science* 2000;288:113–116. [PubMed: 10753110]
26. Wu H, Wheeler A, Zare RN. *Proc Natl Acad Sci* 2004;101:12809–12813. [PubMed: 15328405]
27. Lacher NA, Lunte SM, Martin RS. *Anal Chem* 2004;76:2482–2491. [PubMed: 15117187]
28. Paegel BM, Hut LD, Simpson PC, Mathies RA. *Anal Chem* 2000;72:3030–3037. [PubMed: 10939363]
29. Ramsey JD, Jacobson CT, Culbertson CT, Ramsey JM. *Anal Chem* 2003;75:3758–3764. [PubMed: 14572041]
30. Amatore C, Da Mota N, Lemmer C, Pebay C, Sella C, Thouin L. *Anal Chem* 2008;80:9483–9490. [PubMed: 19007242]
31. Li MW, Spence DM, Martin RS. *Electroanalysis* 2005;17:1171–1180.
32. Salazar GT, Wang Y, Young G, Bachman M, Sims CE, Li GP, Allbritton NL. *Anal Chem* 2007;79:682–687. [PubMed: 17222037]
33. Sombers, LA.; Ewing, AE. *Electroanalytical Methods for Biological Methods*. Brajter-Toth, A.; Chambers, JQ., editors. Marcel Dekker; New York: 2002. p. 279-327.
34. Gillogly JA, Lunte CE. *Electrophoresis* 2005;26:633–639. [PubMed: 15690436]
35. Weinberger, R. *Practical Capillary Electrophoresis*. Academic Press; San Diego, CA: 2000.

Figure 1.

A) Straight channel bilayer pneumatic valve microchip with the incorporation of peristaltic pumps. The pumps operate in sequential order moving the analytes from the sample reservoir towards the injection interface. The labels are as follows: B, buffer reservoir; BW, buffer waste; SR, sample reservoir (4.5 mm in diameter); SW, sample waste; and PB, pushback. Injection valves are also labeled. **B)** Micrograph of pump/injection interface with a $200\ \mu\text{m} \times 100\ \mu\text{m}$ actuation area for both the injection valves and peristaltic pumps ($100\ \mu\text{m}$ flow channel). **C)** Micrograph of interface with a $200\ \mu\text{m} \times 200\ \mu\text{m}$ actuation area for injection valves and $100\ \mu\text{m} \times 200\ \mu\text{m}$ actuation area for peristaltic pumps ($200\ \mu\text{m}$ flow channel), with pump valve #3 being 1.5 mm from intersection.

Figure 2. Operation of peristaltic pumps and injection sequence. **A)** Injection into electrophoresis channel with injection valve #2 being opened and valve #1 being closed, **B)** Injected plug with valve #2 fully closed and valve #1 being open. **C)** Size of plug injected into electrophoresis channel as a function of the peristaltic pump frequency ($n = 4$ for each frequency) using a constant injection valve actuation time (7 sec.).

Figure 3. Characterization of the microchip device. **A)** Concentration change experiment where the concentration of dopamine in the reservoir was changed from 250 to 500 μM . Injection volume = 670 pL. **B)** Typical separation and amperometric detection (+0.9 V vs. platinum quasi-reference) of 50 μM dopamine (DA) and norepinephrine (NE).

Figure 4.

A) Micrograph (2X) showing PDMS micropallet with immobilized PC 12 cells placed into the sample reservoir. **B)** Micrograph (10X) of micropallet that has been rinsed four times with buffer prior to analysis. **C)** Electropherograms demonstrating the ability of the microchip device to measure the stimulated release of dopamine (DA) and norepinephrine (NE) from a micropallet with immobilized PC 12 cells. A constant injection volume of 780 pL was used for each separation.

Figure 5.

A) Straight channel bilayer multi-reservoir mixing device with injection valves and two sets of peristaltic pumps. **B)** Electropherograms resulting from i) contents of catechol (Cat) reservoir being pumped to the interface and ii) contents of both Cat and dopamine (DA) reservoirs being pumped to the injection interface.