Published in final edited form as:

J Chromatogr A. 2016 June 17; 1451: 156–163. doi:10.1016/j.chroma.2016.05.015.

A microchip electrophoresis-mass spectrometric platform with double cell lysis nano-electrodes for automated single cell analysis

Xiangtang Li^a, Shulin Zhao^b, Hankun Hu^{c,d}, and Yi-Ming Liu^{a,d,*}

^aDepartment of Chemistry and Biochemistry, Jackson State University, 1400 Lynch St., Jackson, MS, 39217

^bCollege of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin, 51004, China

^cWuhan University Zhongnan Hospital, Wuhan 430071, China

^dWuhan Yaogu Bio-tech, Wuhan 430075, China

Abstract

Capillary electrophoresis-based single cell analysis has become an essential approach in researches at the cellular level. However, automation of single cell analysis has been a challenge due to the difficulty to control the number of cells injected and the irreproducibility associated with cell aggregation. Herein we report the development of a new microfluidic platform deploying the double nano-electrode cell lysis technique for automated analysis of single cells with mass spectrometric detection. The proposed microfluidic chip features integration of a cell-sized high voltage zone for quick single cell lysis, a microfluidic channel for electrophoretic separation, and a nanoelectrospray emitter for ionization in MS detection. Built upon this platform, a microchip electrophoresis - mass spectrometric method (MCE-MS) has been developed for automated single cell analysis. In the method, cell introduction, cell lysis, and MCE-MS separation are computer controlled and integrated as a cycle into consecutive assays. Analysis of large numbers of individual PC-12 neuronal cells (both intact and exposed to 25 mM KCl) was carried out to determine intracellular levels of dopamine (DA) and glutamic acid (Glu). It was found that DA content in PC-12 cells was higher than Glu content, and both varied from cell to cell. The ratio of intracellular DA to Glu was 4.20 ± 0.8 ($n=150$). Interestingly, the ratio drastically decreased to 0.38 ± 0.20 ($n=150$) after the cells are exposed to 25 mM KCl for 8 min, suggesting the cells released DA promptly and heavily while they released Glu at a much slower pace in response to KCl-induced depolarization. These results indicate that the proposed MCE-MS analytical platform may have a great potential in researches at the cellular level.

Corresponding Author: Professor Yi-Ming Liu, ; Email: yiming.liu@jsums.edu, Tel. +1-601-979-3491

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Microchip electrophoresis-mass spectrometry; Single cell analysis; Automation; PC-12 cells; Dopamine; Glutamic acid

1. Introduction

Single cell analysis is carried out in various biomedical studies [1–5]. Capillary electrophoresis (CE) is the analytical technique of choice for single cell analysis due to its compatibility to small volume /mass samples and short run times among other advantages [6–12]. However, automation of CE-based single cell analysis has been a challenge. Several approaches were proposed. One of them was based on continuous cell introduction /cell lysis and CE separation [13]. A continuous cell flow was generated in a capillary and all cells in the flow were lysed at the outlet that was adjacent to the inlet of a CE separation capillary. The CE analysis with laser induced fluorescence detection was fully automated. However, since cells were randomly positioned in the flow, overlapping of CE peaks was sometimes unavoidable. Another approach was investigated in a recent study [14]. A micro-fabricated cell trap array was deployed in the automated CE-LIF method. Individual cells were trapped in cell-sized wells on a microfluidic chip, and then each well was sequentially moved to beneath the inlet of a CE separation capillary. After lysing the cell with laser pulses, the content was injected into the capillary for analysis. By using this method, two fluorescent dyes loaded up into PC-12 cells were determined.

Microfluidic chips are the best platforms for manipulating cells and analyzing single cells [15–18]. Microchip electrophoresis (MCE), which can be regarded as CE performed on microfluidic chips, has been proven to be a speedy and highly efficient separation technique with many attractive microfluidic features such as precise control of flows, automation, and volumetric reduction of samples, reagents and waste. It has been utilized in various biochemical and chemical applications since introduced by Manz et al [19–21]. Automated single cell analysis by MCE with laser induced fluorescence detection was reported [22]. In the method, the electrokinetic gated injection technique [23] was deployed for single cell injection and the cells were lysed in the separation channel. Two factors certainly affect the number of cells injected: cell density of the cellular sample and aggregation of cells. Since all cells injected are lysed the analytical signal obtained may be from multiple cells or cell clusters instead of single cells. MCE coupled with mass spectrometry (MCE-MS) integrates cell manipulation, cellular content electrophoretic separation, and structure-specific MS detection into one platform. Therefore, it is the most promising technique for automated single cell analysis. Although various analytical methods based on MCE-MS have been reported [24–31], little study has been carried out on automated single cell analysis by this technique so far [32]. The main challenges include injecting/lysing single cells in a controlled and automated manner, and achieving effective MCE-MS separation/detection as well.

In this work, a MCE-MS method deploying the double electrode cell lysis technique was developed for automated single cell analysis. In order to improve the single cell counts in the

analysis, the cell lysis zone was minimized by deploying two nano-electrodes for lysing cells. In the method, cell introduction, cell lysis, and MCE-MS separation were integrated as three individual steps into one cycle of single cell analysis and controlled by a computer. Experimental conditions, including voltage and time for cell lysis, MCE separation and MS detection conditions were investigated. Automated consecutive assays of large numbers of individual cells to determine intracellular levels of dopamine (DA) and glutamate (Glu) in PC-12 neuronal cells were demonstrated. Further, the method was applied to measuring changes in intracellular DA and Glu levels in PC-12 cells under KCl stimulation.

2. Experimental

2.1. Materials

Polydimethylsiloxane (PDMS) prepolymer and the curing agent were purchased from Dow Corning (Midland, MI). Fused silica capillary (254 μm ID, 360 μm OD) was obtained from Polymicro Technologies (Tucson, AZ). Glass slides were obtained from Silicon Valley Microelectronics (Santa Clara, CA). Hexamethyldisilazane (HMDS) was from Ultra Pure Solutions (Castroville, CA). Dopamine and glutamic acid were purchased from Sigma–Aldrich Chemical (St. Louis, MO). All other reagents used were of analytical grade or better. Milli-Q water was used throughout the work. All solutions were filtered through a nylon 0.22 μm syringe filter before use. Two PBS solutions were prepared and used in this work. One of them (PBS1) contained 0.14 M NaCl, 2.08 mM KCl, 9.25 mM Na_2HPO_4 , and 1.76 mM KH_2PO_4 . The other (PBS2) contained 0.14 M NaCl, 25 mM KCl, 9.25 mM Na_2HPO_4 , and 1.76 mM KH_2PO_4 . PBS1 was used to dilute and wash cells. PBS2 was used for the stimulation tests. The pH value of both solutions was adjusted to 7.4 with a 1 M NaOH solution.

2.2 Cell culture and treatment

Rat PC-12 cells (obtained from ATCC, Manassas, VA, USA) were cultured in complete RPMI-1640 media supplemented with 10% heat inactivated horse serum, 5% fetal bovine serum (FBS), and 1% penicillin streptomycin solution in a 5% CO_2 and 100% humidity atmosphere at 37°C. The medium was replaced every 2–3 days. Cells were sub-cultured every 4–5 days. Before tests the cell media was replaced with PBS1.

2.3. Preparation of cellular samples for MCE-MS analysis

PC-12 cells turned to aggregate, forming clusters during culture or even tests. The clusters were disassociated into single cells before MCE-MS analysis. The procedure was to first pipette in and out the cells for several min and then transfer 250 μL cell suspension into a centrifuge tube containing 1.25 mL PBS1 solution. The diluted cell suspension was centrifuged at 1000 rpm for 3 minutes. The cell pellet was re-suspended in 1.50 mL PBS1 solution. This centrifugal and re-suspension step was repeated three times. The cell suspension was diluted step-wise to obtain a cell density of 10^4 cells/mL. Cell viability was determined for the diluted cell suspension by using a standard staining method with trypan blue.

2.4. Fabrication of microfluidic chip

The microchip design is shown in Figure 1A. The chip was composed of a glass substrate (Telic Company, Valencia, CA) and a PDMS cover bearing the channels. The procedure used to create channels in the PDMS cover was described previously [28,29]. Briefly, to create a master for fabricating the PDMS covers, a silicon wafer was coated with HMDS and then with SU-8 2010 negative photoresist using a spin coater. After preexposure bake at 65°C for 30 min, the coated wafer was exposed to a near-UV light source through a negative chrome mask that contained the desired channel features. Following a post exposure bake at 95°C for 10 min, the wafer was developed with SU-8 developer to obtain the master. To fabricate covers, a PDMS monomer solution was prepared by mixing PDMS prepolymer and curing agent at a 10:1 ratio. After being degassed under vacuum, the monomer solution was poured onto the master spinning at 1800 rpm, obtaining a ~100 µm thick membrane. After 2 hours curing at 50°C, a cofferdam (~2 mm deep) was placed on top of the first layer of the PDMS cover, and filled with the pre-polymer mixture, but leaving a ~5mm long section on the cover's edge uncovered. After curing for 3 hours the PDMS cover was removed from the silicon plate, bearing 60 µm wide × 20 µm deep channels. Access holes of 3-mm-diameter were drilled in the PDMS cover at channel terminals, forming four reservoirs. The microchip was made by bonding the glass substrate and the PDMS cover together through heating for 5 min via an air plasma cleaner (10.5 W and 500mTorr, Harrick Plasma, Ithaca, NY). The nanoESI emitter was formed at a corner of the microchip by ~100 µm PDMS cover and beveled glass substrate. Three gold microelectrodes were fabricated on the glass substrate using a 108 auto sputter coater (Ted Pella, Inc.) under a vacuum pressure of 50 mTorr. The resultant gold film was about 50 nm in thickness. The electrode for electrospray had a width of 100 µm and the two for cell lysis were 25 µm wide. The center-to-center distance of the two lysis electrodes was ~80 µm.

2.5. MCE-MS system

The system consisted of an ion trap mass spectrometer equipped with a nano-ESI source (LCQ Deca, ThermoFinnigan, San Jose, CA) and the MCE platform, including the microfluidic chip, a multi-channel high voltage power supply (Shandong Normal University, Jinan, China), and a syringe pump. The microchip was fixed on a XYZ-translational stage and so positioned that the nanoESI emitter tip was about ~1.0 mm away from the MS orifice. Xcalibur software (ThermoFinnigan) was used to control the mass spectrometer and process MS data. House-written software was used for controlling the potentials applied to the microchip for cell injection, MCE separation and nanoESI operation.

MCE conditions were: MCE separate channel, 35 mm long × 60.0 µm wide × 20.0 µm deep; cell injection, 100V at sample reservoir and 0V at waste reservoir for 1 s; cell lysis, 250V at Au1 and 0V at Au2 for 1 sec; MCE separation, 3050V at buffer reservoir and 1500V at Au3 for 58 s; MCE background electrolyte, 25mM ammonium acetate/acetic acid buffer (pH 4.2)/methanol (1:1); make-up flow (MUF), MCE background electrolyte at a flow rate of 100 nL/min. MS operating conditions were optimized in positive ion mode as follows: sheath gas flow rate, 0 units; auxiliary gas flow rate, 0 units; capillary temperature, 250 °C; capillary voltage, 15V; ion source voltage, 0V; relative collision energy, 28%; isolation

width, 1.0 u; activation time, 30ms. Selected-reaction monitoring mode (SRM) was used for quantification. A schematic of the MCE-MS set up is shown in Fig 1B.

2.6. Single cell assay

Prior to each run, the channels were flashed with 0.5% hydrogen peroxide for 5 minutes, 1M NaOH for 5 minutes, and then background electrolyte for 3 minutes. Cell suspension (~20 μ L) was transferred to the sample reservoir, and background electrolyte to the other three reservoirs. A cell flow was generated by applying a voltage of 100V between sample and waste reservoirs for 1 s. A voltage of 250V was then applied between the two cell lysis electrodes for 1 s. MCE-MS started by applying potentials of 3050V at background electrolyte reservoir and 1500V at ESI electrode (Au3) for 58 s. After that, another cycle of analysis started till the desired counts of analysis were reached. All these voltage applications were controlled by software written in house.

2.7. Study on KCl-induced release of dopamine and glutamic acid from PC-12 cell

Cells were washed with PBS1 twice prior to exposing to 25 mM KCl-containing PBS2 stimulant solution for 3, 5 and 8 min, respectively. At the end of exposure time, the cells were spun down, washed twice with SPB1, and re-suspended in PBS1 for single cell analysis to determine intracellular dopamine and glutamic acid. Fifty individual cells were assayed in each test. Three replicate tests were performed for each exposure time with cells collected from three different culture dishes.

3. Results and discussion

3.1. Microchip design

Based on the recent development of several MCE-MS microchips in our lab [28, 29, 33], the present design was intended to achieve automated single cell analysis. A major change in the design is integrating two gold nano-electrodes into the microchip for single cell lysis by using the double electrode technique [34–37]. The nano-electrodes (Au1 and Au2 in Fig. 1A) are made cross the channel intersection measuring approximately $80\text{ }\mu\text{m} \times 60\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$ (i.e. 96 pico-liter). However, since the electrodes are ~80 nm in thickness the effective cell lysis zone (i.e. the volume between the two lysis electrodes) is estimated to be $80\text{ }\mu\text{m}$ (distance between the two electrodes) $\times 100\text{ }\mu\text{m}$ (width of electrode) $\times 0.08\text{ }\mu\text{m}$ (thickness of electrode), i.e. 0.64 pico-liter, which is comparable with the volume of individual mammalian cells such as PC-12 cells (~2 picoliter). Minimizing the lysis zone is expected to improve the probability of single cell lysis. Another advantage of this chip design is to allow lysing cells at any specific time from a continuous cell flow without interfering with MCE separation nor MS detection. In an analysis, a continuous cell flow is generated electrokinetically by applying a voltage between sample and waste reservoirs. When the voltage for electrokinetic pumping is removed, the cell flow stops. The cell (or cells) in the effective lysis zone are lysed by applying a voltage between the nano-electrodes, Au1 and Au2. After the cell is lysed, MCE-MS is started by applying voltages at background electrolyte reservoir and at ESI electrode Au3.

3.2. Single cell lysis

In automated single cell analysis, it's critical that every sample plug injected contains only one cell. This is normally achieved by appropriately diluting the cellular sample (i.e. a cell suspension) [34, 38]. However, sometimes it can be hard to get satisfactory results by this approach, particularly when the cells aggregate, forming clusters. In this work, we made efforts to address this issue by minimizing the effective cell lysis zone. In cell lysis by the double electrode technique, a cell must be exposed to a strong-enough electrical field for a certain period of time in order to be completely lysed. If the effective cell lysis zone, i.e. the volume between the two electrodes, becomes small enough to compare with that of a single cell, single cell lysis can be achieved even when multiple cells are injected into the cell lysis zone (i.e. the channel intersection). Cell lysis in the present MCE-MS platform with double nano-electrodes was visually studied by microscopic imaging. When a voltage of 100v was applied at sample reservoir with ground at the waste, cells were seen moving slowly and passing through the cell lysis zone. Cell integrity remained intact under such a low electrical field as viewed under a microscope. When the voltage was removed cells stopped, floating in the channel intersection. Cell lysis was achieved by applying a voltage between the two nano-electrodes Au1 and Au2. It was noticed that at a voltage below 150V cells were lysed slowly (normally >5 s for a complete lysis). In this work a voltage of >200V was deployed to lyse a cell within in 1 sec so that a reasonable sample through-put for the analysis could be obtained. Fig. 2A shows the microscopic images of cell lysis at 250v for 1 s. In this case a cellular sample of very high cell density was chosen for the test to ensure multiple cells would be retained in the channel intersection. It's worth noting that only one cell is lysed although multiple cells are present in the lysis zone. Because the effective cell lysis zone formed by the two nano-electrodes is extremely small (i.e. ~0.64 pico-liter), only the cell (or cells) locating in the same layer with the two nano-electrodes are lysed. This is highly significant for improving the probability of single cell count (i.e. only one cell is lysed and analyzed in an assay). The present lysis technique will be particularly useful for single cell analysis in the cases when cells aggregate. However, it should be pointed out that if the lysis voltage is set too high, all cells present in the intersection can be lysed as shown in Fig 2B. This is because the volume of effective cell lysis zone is dependent both upon the size of cell lysis electrodes and upon the voltage applied to them for cell lysis. When a higher voltage is applied, the volume becomes larger. This study demonstrates for the first time that with an appropriate lysis voltage single cell lysis can be achieved even in presence of multiple cells by using the double nano-electrode technique proposed herein. The probability of single cell assay can be maximized by a combination of appropriately diluting the cellular sample and selecting an appropriate lysis voltage (250V in this case), which largely depends upon the physicochemical properties of cells to be analyzed. It should be pointed out that considering the heterogeneity from cell to cell within the same cell line, the lyse voltage chosen should be high enough to ensure every single cell in the effective lysing zone is lysed quickly and completely. The most effective way to determine an appropriate cell lyse voltage is to perform a series of cell lysing tests on the platform monitored under a microscope.

3.3. Analytical performance of the proposed MCE-MS method

Analytical figures of merit were assessed for the proposed MCE-MS method by analyzing a series of standard mixtures of dopamine (DA) and glutamic acid (Glu), two major

neurotransmitters in PC-12 neuronal cells. Fig. 3 shows an electropherogram obtained from analysis of a mixture containing 150 nM each of DA and Glu. Under the selected conditions, the two compounds were base-line separated within 1.5 min that was significantly faster than CE or HPLC separations [39]. Calibration curves were prepared by analyzing a series of standard mixtures at various concentrations ranging from 50 to 750 nM. Transitions m/z 154 $\rightarrow$ 137 for DA and m/z 148 $\rightarrow$ 130 for Glu were used for quantification. The following calibration curves based on peak height versus analyte concentration were obtained:

DA	$Y = 0.740 X + 0.052$	$r^2 = 0.998$
Glu	$Y = 0.229 X + 0.012$	$r^2 = 0.997$

where Y was peak height, X was analyte concentration in nM. Detection limits ($S/N=3$) were estimated to be 8.3 nM and 15.6 nM for DA and Glu, respectively. The assay sensitivity is far better than those of CE-MS methods due to two reasons: 1) nano-electrospray ionization is deployed and 2) eluent dilution in coupling of CE separation with mass spectrometric detection is minimized [10–12]. These results indicate that the present MCE-MS method is sufficiently sensitive for chemical analysis of DA and Glu present in single PC-12 cells. Assay repeatability was determined by repeatedly analyzing two standard mixtures of DA and Glu (75 nM each and 350 nM each, respectively) for six times. Relative standard deviations (RSD) of the concentrations measured were all <8.2% for both DA and Glu. It is worth noting that since the data acquisition sampling rate was limited by the MS instrument used in this work the separation efficiency may be compromised, and quantification based on peak heights might be affected. Therefore, replicate measurements are performed in order to obtain valid results of intracellular contents.”

3.4. Single cell analysis

To attest the applicability of the present MCE-MS platform in single cell analysis, a run was set to assay consecutively 50 PC-12 cells in a cell suspension of 1×10^4 cells/mL in 50 min. In each assay, cell injection (for 1 s), cell lysis (for 1 s), and MCE-MS separation (for 58 s) were computer controlled by switching voltages applied at reservoirs and electrodes Au1~Au3. Fig.4 shows the results of DA and Glu contents from all 50 assays. Both DA and Glu were detected in all cells assayed. However, contents vary from cell to cell. Based on the peak height results, single cell assay counts 72%, multiple cell assay (i.e. two or three cells are analyzed in an assay) counts 18%, and vein assay counts 10%. It's worth noting that although the double nano-electrode technique is deployed to promote single cell lysis the single cell count is not 100%. One reason for this is that PC-12 cells aggregate, forming clusters. If a cell cluster happens to be such positioned that multiple cells are exposed to the high electrical field between the two nano-electrodes (i.e. the effective cell lysis zone), multiple cells are lysed. Thanks to the specificity of MS detection, it's easy to distinguish a single cell assay from a multiple cell assay based on the peak heights. When intracellular DA and Glu contents are calculated, those results from multiple cell assays are excluded. The assay results shown in Fig. 4 prove that the proposed MCE-MS platform is effective and robust for long-time runs of single cell analysis. The capability of analyzing a large number of individual cells allows obtaining intracellular chemical information with statistical

significance, which makes the proposed MCE-MS method well suited for studying cellular release of neurotransmitters under chemical stimuli. Finally, although only DA and Glu are involved in this study, the present MCE-MS platform is expected to be useful for studying other intracellular compounds because most organic compounds are detectable by MS detection.

3.5. Study on DA and Glu release from PC-12 cells

DA and Glu are two major neurotransmitters in PC-12 neuronal cells [40]. DA is involved in initiating many behavioral responses towards various stimuli, and Glu is the major excitatory neurotransmitter in the mammalian central nervous system [41, 42]. It's well documented that KCl induces neuronal depolarization, causing neurotransmitter release from cells. In this work, the MCE-MS method was applied to quantify simultaneously DA and Glu present in individual PC-12 cells treated with KCl elevated PBS for different time periods. For each cell group 50 cells were assayed to gain statistical significance. Fig. 5 shows several representative TIC and extracted ion electropherograms obtained from these assays. As can be seen, in each single cell assay DA and Glu peaks are well identified with no interference from other endogenous compounds. From the peak heights both DA and Glu can be quantified precisely. All results from single cell assays indicate that intracellular DA content is significantly higher than that of Glu in intact PC-12 cells. Data from analysis of 600 individual PC-12 cells (50 cells from each of three replicate tests at 4 different exposure times) show that KCl depolarization causes decreases in extracellular levels of both DA and Glu as shown in Fig. 6A. Interestingly, PC-12 cells release DA very promptly and heavily in response to KCl-induced depolarization. Within 3 min more than 85% intracellular DA disappears. Under KCl stimulation, Glu is also released by PC-12 cells, but at a much slower pace. This can also be seen by studying the ratio trend of intracellular DA to Glu shown in Fig. 6B. The ratio changed from 4.20 in intact PC-12 cells to 0.38 in cells exposure to 25 mM KCl for 8 min.

4. Conclusions

A microchip electrophoresis-mass spectrometric (MCE-MS) method has been developed for automated single cell analysis. The present MCE-MS platform features double nano-electrodes for cell lysis to enhance the single cell assay count. Results obtained from consecutive assays of 600 individual PC-12 cells indicated that the intracellular level of dopamine was significantly higher than that of glutamic acid, and both varied from cell to cell. We found it very interesting that in response to KCl-induced neuronal depolarization PC-12 cells released dopamine very quickly and heavily (85% of the content within 3 min after exposure), but released glutamic acid at a much slower pace. To our knowledge this is the first demonstration of deploying the double nano-electrode cell lysis technique to enhance automated MCE-MS analysis of single cells.

Acknowledgments

Financial support from US National Institutes of Health (GM089557) is gratefully acknowledged.

References

1. Zenobi R. Single-cell metabolomics: analytical and biological perspectives. *Science*. 2013; 342:1243259. [PubMed: 24311695]
2. Stender AS, Marchuk K, Liu C, Sander S, Meyer MW, Smith EA, Neupane B, Wang G, Li J, Cheng J-X. Single cell optical imaging and spectroscopy. *Chem. Rev.* 2013; 113:2469–2527. [PubMed: 23410134]
3. Passarelli MK, Ewing AG, Winograd N. Single-cell lipidomics: characterizing and imaging lipids on the surface of individual *Aplysia californica* neurons with cluster secondary ion mass spectrometry. *Anal. Chem.* 2013; 85:2231–2238. [PubMed: 23323749]
4. Ibáñez AJ, Fagerer SR, Schmidt AM, Urban PL, Jefimovs K, Geiger P, Dechant R, Heinemann M, Zenobi R. Mass spectrometry-based metabolomics of single yeast cells. *PNAS*. 2013; 110:8790–8794. [PubMed: 23671112]
5. Romanova EV, Aerts JT, Croushore CA, Sweedler JV. Small-volume analysis of cell–cell signaling molecules in the brain. *Neuropsychopharmacology*. 2014; 39:50–64. [PubMed: 23748227]
6. Borland LM, Kottogoda S, Phillips KS, Allbritton NL. Chemical analysis of single cells. *Annu. Rev. Anal. Chem.* 2008; 1:191–227.
7. Lapainis T, Rubakhin SS, Sweedler JV. Capillary electrophoresis with electrospray ionization mass spectrometric detection for single-cell metabolomics. *Anal. Chem.* 2009; 81:5858–5864. [PubMed: 19518091]
8. Trouillon R, Passarelli MK, Wang J, Kurczy ME, Ewing AG. Chemical analysis of single cells. *Anal. Chem.* 2012; 85:522–542. [PubMed: 23151043]
9. Galler K, Bräutigam K, Große C, Popp J, Neugebauer U. Making a big thing of a small cell—recent advances in single cell analysis. *Analyst*. 2014; 139:1237–1273. [PubMed: 24495980]
10. Klepárník K. Recent advances in the combination of capillary electrophoresis with mass spectrometry: From element to single-cell analysis. *Electrophoresis*. 2013; 34:70–85. [PubMed: 23161493]
11. Klampfl CW. CE with MS detection: a rapidly developing hyphenated technique. *Electrophoresis*. 2009; 30:S83–S91. [PubMed: 19517505]
12. Pantková P, Gebauer P, Boček P, Kivánková L. Recent advances in CE-MS: Synergy of wet chemistry and instrumentation innovations. *Electrophoresis*. 2011; 32:43–51. [PubMed: 21171112]
13. Chen S, Lillard SJ. Continuous cell introduction for the analysis of individual cells by capillary electrophoresis. *Anal. Chem.* 2001; 73:111–118. [PubMed: 11195493]
14. Dickinson AJ, Armistead PM, Allbritton NL. Automated capillary electrophoresis system for fast single-cell analysis. *Anal. Chem.* 2013; 85:4797–4804. [PubMed: 23527995]
15. El-Ali J, Sorger PK, Jensen KF. Cells on chips. *Nature*. 2006; 442:403–411. [PubMed: 16871208]
16. Wheeler AR, Thordset WR, Whelan RJ, Leach AM, Zare RN, Liao YH, Farrell K, Manger ID, Daridon A. Microfluidic device for single-cell analysis. *Anal. Chem.* 2003; 75:3581–3586. [PubMed: 14570213]
17. McClain MA, Culbertson CT, Jacobson SC, Allbritton NL, Sims CE, Ramsey JM. Microfluidic devices for the high-throughput chemical analysis of cells. *Anal. Chem.* 2003; 75:5646–5655. [PubMed: 14588001]
18. Zare RN, Kim S. Microfluidic Platforms for Single-Cell Analysis. *Annu. Rev. Biomed. Eng.* 2010; 12:187–201. [PubMed: 20433347]
19. Harrison D, Verpoorte EMJ, Fetting JC, Paulus A, Lüdi H, Widmer HM. Planar chips technology for miniaturization and integration of separation techniques into monitoring systems—capillary electrophoresis on a chip. *J. Chromatogr.* 1992; 593:253–258.
20. Hulvey MK, Frankenfeld CN, Lunte SM. Separation and detection of peroxynitrite using microchip electrophoresis with amperometric detection. *Anal. Chem.* 2010; 82:1608–1611. [PubMed: 20143890]
21. Breadmore MC. Capillary and microchip electrophoresis: challenging the common conceptions. *J. Chromatogr. A*. 2012; 1221:42–55. [PubMed: 22000781]

22. Zhang X, Li Q, Chen Z, Li H, Xu K, Zhang L, Tang B. Electrokinetic gated injection-based microfluidic system for quantitative analysis of hydrogen peroxide in individual HepG2 cells. *Lab Chip*. 2011; 11:1144–1150. [PubMed: 21298131]
23. Jacobson SC, Koutny LB, Hergenroeder R, Moore AW Jr, Ramsey JM. Microchip capillary electrophoresis with an integrated postcolumn reactor. *Anal. Chem*. 1994; 66:3472–3476.
24. Hoffmann P, Häusig U, Schulze P, Belder D. Microfluidic glass chips with an integrated nanospray emitter for coupling to a mass spectrometer. *Angew. Chem. Intl. Ed*. 2007; 46:4913–4916.
25. Mellors J, Gorbounov V, Ramsey R, Ramsey J. Fully integrated glass microfluidic device for performing high-efficiency capillary electrophoresis and electrospray ionization mass spectrometry. *Anal. Chem*. 2008; 80:6881–6887. [PubMed: 18698800]
26. Nordman N, Sikanen T, Aura S, Tuomikoski S, Vuorensola K, Kotiaho T, Franssila S, Kostianen R. Feasibility of SU-8-based capillary electrophoresis-electrospray ionization mass spectrometry microfluidic chips for the analysis of human cell lysates. *Electrophoresis*. 2010; 31:3745–3753. [PubMed: 21077242]
27. Lecault V, White AK, Singhal A, Hansen CL. Microfluidic single cell analysis: from promise to practice. *Curr. Opin. Chem. Biol*. 2012; 16:381–390. [PubMed: 22525493]
28. Li X, Zhao S, Liu Y-M. Evaluation of a microchip electrophoresis-mass spectrometry platform deploying a pressure-driven make-up flow. *J. Chromatogr. A*. 2013; 1285:159–164. [PubMed: 23473508]
29. Li X, Xiao D, Sanders T, Tchounwou PB, Liu Y-M. Fast quantification of amino acids by microchip electrophoresis–mass spectrometry. *Anal. Bioanal. Chem*. 2013; 405:8131–8136. [PubMed: 23929191]
30. Ohla S, Belder D. Chip-based separation devices coupled to mass spectrometry. *Curr. Opin. Chem. Biol*. 2012; 16:453–459. [PubMed: 22673066]
31. Kitagawa F, Otsuka K. Recent progress in microchip electrophoresis–mass spectrometry. *J. Pharm. Biomed. Anal*. 2011; 55:668–678. [PubMed: 21130595]
32. Mellors JS, Jorabchi K, Smith LM, Ramsey JM. Integrated microfluidic device for automated single cell analysis using electrophoretic separation and electrospray ionization mass spectrometry. *Anal. Chem*. 2010; 82:967–973. [PubMed: 20058879]
33. Li X, Xiao D, Ou X-M, McCullum C, Liu Y-M. A microchip electrophoresis-mass spectrometric platform for fast separation and identification of enantiomers employing the partial filling technique. *J. Chromatogr. A*. 2013; 1318:251–256. [PubMed: 24354006]
34. Han F, Wang Y, Sims CE, Bachman M, Chang R, Li G, Allbritton NL. Fast electrical lysis of cells for capillary electrophoresis. *Anal. Chem*. 2003; 75:3688–3696. [PubMed: 14572031]
35. Lu H, Schmidt MA, Jensen KF. A microfluidic electroporation device for cell lysis. *Lab Chip*. 2005; 5:23–29. [PubMed: 15616736]
36. Wang H-Y, Bhunia AK, Lu C. A microfluidic flow-through device for high throughput electrical lysis of bacterial cells based on continuous dc voltage. *Biosens. Bioelectron*. 2006; 22:582–588. [PubMed: 16530400]
37. Church C, Zhu J, Huang G, Tzeng T-R, Xuan X. Integrated electrical concentration and lysis of cells in a microfluidic chip. *Biomicrofluidics*. 2010; 4:044101.
38. Cecala C, Sweedler JV. Sampling techniques for single-cell electrophoresis. *Analyst*. 2012; 137:2922–2929. [PubMed: 22288071]
39. Perry M, Li Q, Kennedy RT. Review of recent advances in analytical techniques for the determination of neurotransmitters. *Anal. Chim Acta*. 2009; 653:1–22. [PubMed: 19800472]
40. Shi B, Huang W, Cheng J. Determination of neurotransmitters in PC 12 cells by microchip electrophoresis with fluorescence detection. *Electrophoresis*. 2007; 28:1595–1600. [PubMed: 17447240]
41. Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci*. 1990; 13:171–182. [PubMed: 1970230]
42. Liu WW, Wilson RI. Glutamate is an inhibitory neurotransmitter in the *Drosophila* olfactory system. *PNAS*. 2013; 110:10294–10299. [PubMed: 23729809]

Highlights

- A microchip electrophoresis-mass spectrometric method is developed for analysis of single cells.
- Double nano-electrode cell lysis technique is deployed for the first time in single cell analysis.
- Intracellular levels of dopamine and glutamic acid in PC-12 neuronal cells are determined.
- KCl-induced changes in intracellular dopamine and glutamic acid levels are measured.

Fig.1.

A) Microchip design proposed for automated single cell analysis by MCE-MS, and B) schematic of the MCE-MS set-up.

Fig. 2. Microscopic images of cell lysis by the proposed double nano-electrode cell lysis technique, showing: (A) only one cell is lysed at 250V for 1 s although multiple cells are present in the channel intersection, and (B) if the lysis voltage used is set too high (e.g. 600V for 1 s in this case) multiple cells (3 cells in this case) can also be lysed. Arrows are pointing to the cells lysed.

Fig. 3.

Separation of a standard mixture containing dopamine and glutamic acid (150 nM each) on the proposed MCE-MS platform: (A) TIC of m/z 154 for dopamine and 148 for glutamic acid; (B) and (C) MS² spectra of Glu and dopamine, respectively, verifying the peak identities: dopamine (t_R = 1.17 min) and glutamic acid (t_R = 1.31 min).

Fig. 4. Automated MCE-MS quantification of dopamine and glutamic acid in individual PC-12 neuronal cells.

Fig. 5.
TIC and extracted m/z 154 (for dopamine) and m/z 148 (for glutamic acid) electropherograms obtained from consecutive assays of: (A) individual intact PC-12 cells, and (B) PC-12 cells exposed to 25 mM KCl for 8 min.

Fig. 6. Changes in intracellular DA and Glu levels (A), and ratio of DA/Glu (B) in PC-12 cells exposed to 25 mM KCl for different time periods. Data shown are mean $\pm$ SD, n=150.