Published in final edited form as:
Anal Chem. 2007 December 1; 79(23): 9054–9059.

Coaxial-Flow System for Chemical Cytometry

Paul J. Marc[1], **Christopher E. Sims**[2], and **Nancy L. Allbritton**[2,*]

1*Department of Biomedical Engineering University of California, Irvine, California 92697*

2*Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290*

Abstract

Over the past decade, chemical cytometry performed by capillary electrophoresis (CE) has become increasingly valuable as a bio-analytical tool to quantify analytes from single cells. However, extensive use of CE-based chemical cytometry has been hindered by the relatively low throughput for the analysis of single adherent cells. In order to overcome the low throughput of CE-based analysis of adherent cells and increase its utility in evaluating cellular attributes, new higher throughput methods are needed. Integration of a coaxial buffer exchange system with CE-based chemical cytometry increased the rate of serial analyses of cells. In the designed system, fluid flow through a tube coaxial to the separation capillary was used to supply electrophoretic buffer to the capillary. This sheath or coaxial fluid was turned off between analysis of cells and on during cell sampling and electrophoresis. Thus, living cells were not exposed to the nonphysiologic electrophoretic buffer prior to lysis. Key parameters of the system such as the relative capillary-sheath positions, buffer flow velocities, and the cell chamber design were optimized. To demonstrate the utility of the system, rat basophilic leukemic cells loaded with Oregon Green and fluorescein were serially lysed and loaded into a capillary. Separation of the contents of 20 cells at a rate of 0.5 cells/min was demonstrated.

Keywords

Chemical Cytometry; Single Cell Analysis; Sheath Flow; Coaxial Flow; High Throughput Chemical Cytometry; Capillary Electrophoresis

Introduction

Chemical cytometry, the use of high-sensitivity analytical tools, such as mass spectrometry, electrochemistry and capillary separation methods, to characterize single cells, was defined by Dovichi and collaborators in 1999, giving a name to a field which had started almost 20 years earlier.[1] From the late 1980's to 1990's, Jorgensen, Kennedy, Ewing and Yeung pioneered methods to separate the contents of single cells using CE.[2-7] Over the past decade, a wide range of analytes has been measured in single cells using CE, illustrating the power of this method. CE has been used to quantify 100 proteins from a single cell, monitor individual mitochondrial properties, measure the activity of intracellular enzymes, and develop protein maps during the cell cycle.[8-18] Unfortunately, one disadvantage of CE as a tool for chemical cytometry is its relatively low throughput, typically 5-35 cells per day.[10,11,17,19] In order to fully characterize many biological attributes, the analysis of hundreds to thousands of cells is often necessary to obtain statistically relevant data. For example, 100's of Xenopus oocytes were analyzed to reveal the on/off switch of MAP kinases and 1000's of rat basophilic leukemia cells were analyzed to deconvolute specific signaling mechanisms of protein kinase C.[20,21]

* Corresponding author: NLA:, Email: nlallbri@unc.edu, Fax: 919-962-2388.

In order to improve the low throughput of the CE analysis of adherent cells, and increase the feasibility of implementing this technique routinely in evaluating cellular attributes, new high-throughput methods are needed for chemical cytometry performed by CE.

In an effort to increase the throughput of CE, two strategies, parallel and serial analysis, have been explored.[22-28] Parallel analysis increases throughput by relying on arrays of capillaries to increase the sampling population. One of the most well known implementations of parallel analysis was seen in the creation of high-throughput DNA sequencers for the human genome project.[22] Parallel analysis typically relies on the simultaneous analysis of multiple capillaries by either of two strategies. The first method relies on a specialized sheath flow cell in which there is concurrent illumination of all capillaries.[29,30] The second strategy utilizes epifluorescence detection with the detector traversing each capillary.[31-33] Both methods can be complex and require proper alignment of all capillaries over the sample which may be difficult with living cells. Serial analysis increases throughput by sequential injections of analyte into the same capillary.[26,34] This method frequently relies on the injection of subsequent samples before the previous sample has been detected. One example of this method is a flow-based injection system used to assess the protein content of cells at a rate of 10 cells in 40 minutes.[34] This method has the benefit of simplicity with good throughput. However, it is limited to nonadherent cells, utilizes a mixed physiological and electrophoretic buffer for separation, and possesses variable spacing of analyte plugs in the capillary. The limitation of performing CE analysis on non-adherent cells is an important one. Only 5% of mammalian cells naturally grow in suspension, primarily those of the hematopoietic system. The vast majority of cells grow in an adherent manner attached to a surface such as a basement membrane *in vivo* or a polymer surface *in vitro*. Removal of adherent cells from their growth substrate by trypsinization or other means is known to initiate a number of cellular events which can lead to artifacts in measurements of cell physiology. No parallel or serial CE system has been demonstrated for the separation of the contents of adherent cells.

Sheath flow methods were first introduced in flow cytometry and subsequently adapted for capillary electrophoresis. Sheath flow systems surround either the inlet or outlet of a capillary with a secondary buffer stream. The coupling of a sheath flow system to the outlet of a capillary permits highly sensitive detection of analytes and was initially developed by Dovichi, Kanberra, and co-workers.[22,29,30,35] A sheath flow on the outlet of the capillary has also allowed for the coupling of CE to the mass spectrometer.[26,36,37] The sheath flow increases sample volume, thus facilitating reliable and robust injection of the CE sample into the mass spectrometer. Coupling the inlet of a capillary to a coaxial sheath flow permits the controlled, localized delivery of buffer to a sample. This buffer is then available for loading into the capillary followed by electrophoretic separation. In neuroscience applications, dialysis studies of coaxially-delivered solutions have been used to reduce the total volume of fluid delivered to the capillary during sampling of extracellular fluid.[38] The use of sheath flow systems at either the outlet or inlet of a capillary has increased the versatility of capillary-based analyses.

Here, we describe the design and optimization of a coaxial-flow system for the inlet of a capillary. The goal was to develop a method to rapidly switch the buffer surrounding the mouth of the capillary between a physiologic buffer and an electrophoretic buffer. The physiologic buffer protected intact cells prior to cell lysis from the nonphysiologic electrophoretic buffer. The sheath supplied the electrophoretic buffer locally to the inlet of the capillary. In this system, the inlet of the capillary was surrounded by a coaxial tube that provided the sheath flow. The capillary inlet and coaxial tubing outlet were placed in a channel with a laminar stream of physiologic buffer. When fluid was moving through the sheath tubing, the laminar stream acted to sweep the elecrophoretic buffer away from upstream living cells. The flow of sheath buffer into the capillary and the mixing of this buffer with the laminar flow buffer in the channel were characterized for different channel geometries. The coaxial tube and channel flow velocities

were optimized to provide a consistent flow of 100% sheath fluid to the capillary during electrophoresis. In addition, the amount of shear stress on the cells with the optimized parameters was simulated using the finite element method. To demonstrate the utility of the coaxial flow buffer system, 20 cells were lysed in a serial manner and their contents separated at a rate 1 cell per 2 min. The coaxial-flow system also enabled the use of short capillaries (15 cm) for electrophoretic separations. Thus, this coaxial CE based chemical cytometry system increases the versatility and the rate of serial analyses of single cells.

Experimental Section

Materials

Pre-cleaned glass slides (75 × 25 × 1 mm) were purchased from Corning Glass Works (Corning, NY). Oregon Green 488 carboxylic acid diacetate (6-isomer) and fluorescein diacetate (mixed isomers) were purchased from Molecular Probes, Eugene, OR. Tissue culture materials were obtained from Gibco BRL, Gaithersburg, MD. Silicone O-rings (size 115) were purchased from McMaster-Carr (Los Angeles, CA). The Sylgard 184 silicone elastomer kit was purchased from Dow Corning (Midland MI). All other reagents were purchased from Fisher Scientific, Pittsburgh, PA.

Sheath flow instrumentation

An inverted microscope (Diaphot 200, Nikon) was fitted with a customized aluminum frame to mount the coaxial flow system. The microscope stage moved independently of the frame. A three-axis manipulator (MC1000, SD instruments, Grants Pass, OR) was connected to the frame and used to position the sheath flow assembly over the microscope stage. The capillary was mounted within the sheath flow assembly so that the sheath tubing surrounded the capillary (Figure 1). The coaxial sheath was a modified 18 or 20 gauge needle. The capillary possessed a 360 μm outer diameter and a 50 μm inner diameter. The capillary outlet extended beyond the end of the sheath tubing as specified in the Results and Discussion section. The distance that the capillary extended beyond the sheath tubing was determined by measuring the height of the capillary and the height of the sheath tubing above the glass surface of the laminar flow chamber using a 1″ dial indicator (MB 216, Brown and Sharp, North Kingstown, RI or 1″ digital, Cen-Tech). For electrophoresis, the electrophoretic buffer in the coaxial sheath was sodium dodecyl sulfate (20 mM) and sodium borate (10 mM, pH 9.3). See the supporting information for descriptions of the fluorescence detection and CE conditions.

Laminar flow and cell chambers

Three types of cell chambers were fabricated on a glass slide. Round cell chambers were constructed by attaching a silicon O-ring (size 115, 17.5 mm inner diameter, 22.2 mm outer diameter) to the glass surface using PDMS (Sylgard 184). The two other chambers were rectangular in shape with the side walls completely fabricated from PDMS. The dimensions of the rectangular chambers were 20 × 23 mm and 5 mm × 50 mm. For simplicity, the 20 × 23 mm chamber was denoted as the square chamber. The physiologic buffer flowing through the cell chamber was ECB cell buffer (NaCl, 135mM; KCl, 5mM; $MgCl_2$, 1mM; $CaCl_2$, 1mM; Hepes, 10 mM).

Control of buffer flow

The buffer flows through the sheath tube and through the cell chamber or laminar flow chamber were initiated by pressurizing their respective buffer reservoirs. The flow rate of the buffers was regulated using metering valves (MLS-MV, Beswick Engineering, Greenland, NH). Flow through the sheath tubing was switched on and off using a solenoid valve (AL4112, ASCO, Florham Park, NJ) with a switching time of 5 ms. Fluid removal from the laminar flow or cell

chamber was accomplished by applying suction to an 18 gauge needle positioned at the outlet end of the chamber. The fluid height in this chamber was adjusted by varying the vacuum and the height of the outlet.

Cell Sampling Protocol

To accomplish serial sampling of cells, the inlet end of the capillary was positioned 10-40 μm above a cell just prior to cell lysis while physiologic buffer was continually flowed through the cell chamber. Then the sheath flow and voltage were engaged simultaneously, thus exposing the cell to the electrophoretic buffer and the electric field which served to lyse the targeted cell and to inject its contents into the capillary. Electrophoresis was continued for 2 minutes during which time the fluorescent analytes (Oregon Green and fluorescein) from the lysed cell passed through the detection window. After this time the sheath flow and applied voltage were terminated. The next upstream cell to be sampled was positioned beneath the capillary inlet by translating the cell chamber mounted on the microscope stage. Upon positioning the cell, the process was repeated. The time between disengaging and reengaging CE power supply and electrophoretic buffer flow was 3-5 seconds.

Measurement of the buffer composition entering the capillary inlet

The amount of sheath fluid entering into the capillary was quantitated by adding trypan blue (0.1%) to the sheath fluid. Fluid at the inlet end of the capillary was sampled using hydrodynamic fluid flow (0.5-2 μL/min) through the capillary. While this flow rate is higher than that of the electro osmotic fluid flow rate (nL/min), it is still negligible compared to the sheath flow rate (>100 μL/min). The amount of trypan blue entering the capillary from the sheath tubing was quantitated with an absorbance detector (Linear 200 variable UV/Vis Detector, Grace, Deerfield, IL). The detector was set at a wave length of 569 nm with an integration time of 0.3 seconds. The detector was placed 42 cm from the capillary outlet, with a total capillary length of 63 cm.

Results and Discussion

Design of a Coaxial-Flow System for Chemical Cytometry

Chemical cytometry using CE is typically performed by exchanging the physiologic buffer around the cell either immediately before or after lysis. The separation is then performed in a nonphysiologic, but optimized electrophoretic buffer.[9,10,19,39] A goal of this work was to design a system capable of serially analyzing cellular analytes that are rapidly modified within the cell. Ideally, for such measurements the cell should not be exposed to the electrophoretic buffer prior to cell lysis in order to prevent any undesired modifications to the analytes resulting from stresses imposed by nonphysiologic conditions.[11,12,14] Transfer of the capillary inlet to an electrophoretic buffer must then occur after lysis. Manual movement of the capillary between a physiologic and electrophoretic buffer is possible, but is generally slow and not amenable to automation. For this reason, a CE system in which the electrophoretic buffer was supplied through a tube coaxial to the capillary was designed (Fig. 1). The flow of the electrophoretic buffer was regulated by a valve which could be rapidly opened at the initiation of electrophoresis and closed at other times. When flowing from the coaxial tube, the electrophoretic buffer surrounded the capillary inlet. The capillary inlet within the coaxial tube was placed in a cell chamber through which a physiologic buffer flowed. The physiologic buffer provided all cells upstream of the capillary with an appropriate environment to maintain cell health. Cells downstream of the capillary were exposed to a mix of the physiologic and electrophoretic buffers. The goal of the designed system was to provide the inlet of the capillary with the electrophoretic buffer during the separation of the cell contents without exposing unsampled cells upstream to this buffer. To serially analyze cells, the capillary must then be translated in the upstream direction of the chamber (towards the inlet of the physiologic buffer).

To determine whether the system could function as designed, five variables required optimization. These were: the diameter of the coaxial tube, the shape and size of the cell chamber, the velocity of the physiologic buffer stream, the length of the capillary protruding beyond the coaxial tube (Δcapillary), and the velocity of the electrophoretic buffer exiting the coaxial tube (Fig. 1A). The velocity of the physiologic buffer was a function of the cross sectional area of the cell chamber as well as the volumetric flow rate of the buffer through the chamber. The velocity of the electrophoretic buffer depended on the cross sectional area of the coaxial tube and the volumetric flow rate of the electrophoretic buffer. These variables were sequentially optimized.

Optimization of the Diameter of the Coaxial Tube

At a fixed volumetric flow rate for the electrophoretic buffer, the diameter of the coaxial tube controls the velocity of the electrophoretic buffer as well as the distance upstream of the capillary that the electrophoretic buffer travels. The distance that the electrophoretic buffer travels upstream of the coaxial tube also determines whether this buffer stream envelopes the capillary inlet and the amount of electrophoretic buffer entering the capillary during electrophoresis. In order to optimize the diameter of the coaxial tube, the chamber geometry (23 X 20 mm square), and electrophoretic buffer flow rate (0.17 mL/min) were fixed, while the diameter of the coaxial tube, the volumetric flow rate of the physiologic buffer, and Δcapillary were varied. The diameters tested for the coaxial tube were 0.9 and 1.2 mm. The volumetric flow rate for the physiologic buffer was varied between 1.1 and 10 ml/min. Δcapillary values of 0.1 to 2.5 mm were examined. Trypan blue (0.1%) was added to the electrophoretic buffer flowing through the coaxial tube and the amount of trypan blue exiting the capillary was measured for the different coaxial tube diameters as Δcapillary and the volumetric flow rate of the physiologic buffer were varied. The percentage of electrophoretic buffer entering the capillary was defined as 100 times the optical density of the fluid in the capillary divided by the optical density of the electrophoretic buffer with trypan blue. The percentage electrophoretic buffer entering the capillary for the two coaxial tube diameters was plotted for a fixed volumetric flow rate of the physiologic buffer (Fig.2A). The smaller coaxial tube diameter (0.9 mm) resulted in a broader range of Δcapillary for which the capillary filled with 100% electrophoretic buffer. This was most likely due to the higher linear velocities produced by the smaller coaxial tube, causing the electrophoretic buffer to travel a greater distance in the z direction out from the coaxial tube. The greater z distances attained by the electrophoretic buffer permitted a more complete coverage of the capillary inlet. At all volumetric flow rates, larger Δcapillary were attainable with complete filling of capillary with the electrophoretic buffer for the smaller coaxial tube. A larger Δcapillary is desirable when positioning the capillary over a cell just prior to cell lysis since the coaxial tube with nonphysiologic electrophoretic buffer will be as far as possible from the cell. The smaller coaxial tube (0.9 mm) was used in all subsequent experiments.

Selection of an Optimal Cell Chamber Shape

When cells are serially analyzed, the electrophoretic buffer emerging from the coaxial tube should not interact with the unsampled cells. If cells near the outlet of the cell chamber are analyzed first, those upstream from the chamber outlet should be shielded by the physiologic buffer flowing across the chamber. To determine whether upstream cells were shielded from the electrophoretic buffer, trypan blue was loaded into the buffer flowing through the coaxial tube and the pattern of flow in the cell chamber was imaged as the coaxial flow was initiated. Three different cell chambers were tested: a round chamber (17.5 mm ID), a square chamber (20 × 23 mm), and a rectangular chamber (5 × 50 mm). Δcapillary was fixed at values spanning from 0.1 to 1.5 mm, while the volumetric flow rate of the physiologic buffer was varied from 1 to 10 ml/min, and the volumetric flow rate of the electrophoretic buffer was varied from 0.04 to 0.5 ml/min. These flow rates were chosen on the basis of estimation of the minimal fluid

rates that are reliably metered, yield good upstream separation of the physiologic and electrophoretic buffers, and result in low shear stress on the base of the chamber.

Laminar flow of the electrophoretic buffer toward the cell chamber outlet did not occur in the square or round chambers. In both cases the electrophoretic buffer mixed with the physiologic buffer throughout the chamber. Thus the square and round chambers were unsuitable for serial analyses of cells in the chamber. However, the rectangular chamber displayed a stable laminar flow with the electrophoretic buffer flowing towards the chamber outlet for physiologic buffer flow rates of 1-4 mL/min and electrophoretic buffer flow rates of 0.04-0.2 mL/min. In addition, the electrophoretic buffer traveled a distance of less than 2 mm upstream in the cell chamber (Fig. 1C). The smaller the upstream incursion of electrophoretic buffer, the closer the cells can be spaced for analysis. Since the rectangular chamber yielded a stable laminar flow for the coaxial buffer, this chamber was used for all ensuing experiments.

Identification of the Optimal Physiologic Buffer Velocity

The velocity of the physiologic buffer flowing across the capillary tip influences the amount of electrophoretic buffer entering the capillary. The physiologic buffer velocity was varied from 1 to 9 mm/s (Table S1), while the volumetric flow rate of the electrophoretic buffer was maintained constant at 0.12 mL/min (0.9 mm/s). For each of the tested physiologic buffer velocities, Δcapillary was also varied (0.8-1.5 mm). The percentage of electrophoretic buffer entering the capillary was measured for the different values of Δcapillary and physiologic buffer velocities (Fig.2B). A greater percentage of the buffer in the capillary originated from the coaxial tube when Δcapillary was small and the physiologic buffer velocities were low. In particular, a physiologic buffer velocity of 1.7 mm/s yielded 100% entry of electrophoretic buffer into the capillary for all tested Δcapillary. At this velocity, the Δcapillary could be as large as 1.5 mm, a value which places unsampled cells a substantial distance from the coaxial tube even when the capillary is positioned just above a cell prior to lysis. As seen in Figure 1C, with a sheath flow velocity of 0.9 mm/s, a physiologic velocity of 1.7 mm/s, and a Δcapillary of 0.75 mm, the CE buffer flow upstream is a little over 1 mm.

To estimate the shear stress placed on a cell located beneath the flowing physiologic buffer, finite element methods were used to model the system with and without a capillary. The incompressible Navier-Stokes equation was used to generate the stationary models. Refer to supporting information for further discussion of the model parameters. At a flow rate of 1.7 mm/s for the physiologic buffer, the maximum shear stress induced on a cell located on the floor of the chamber was between 0.02-0.03 dynes/cm^2. These shear stresses are in the lower range of those exerted on cells in microfluidic experiments (2-0.0005 dynes/cm^2), an order of magnitude less than the chemotaxis studies, and orders of magnitude lower than those experienced by vascular endothelial cells.[40-45] For these reasons, in subsequent experiments, we utilized a physiologic buffer flow velocity of 1.7 mm/s.

Optimization of the Electrophoretic Buffer Velocity in the Coaxial Tube

To determine the range of flow velocities through the coaxial tube that yielded complete filling of the capillary with electrophoretic buffer, the percentage of electrophoretic buffer in the capillary was measured as the electrophoretic buffer velocity was varied from 0.3-0.9 mm/s (0.04-0.12 mL/min). Δcapillary was also altered (0.8-1.5 mm), but all other parameters were fixed at the optimal values described in the prior sections. For an electrophoretic buffer flow velocity equal to or greater than 0.5 mm/s (0.07 ml/min), 100% electrophoretic buffer filled the capillary for all Δcapillary. Flow velocities of less than 0.5 mm/s did not completely fill the capillary with the electrophoretic buffer from the coaxial tube. Since electrophoretic buffer flow velocities of 0.5-0.9 mm/s resulted in 100% filling of the capillary at all Δcapillary, these flow velocities were used in all following experiments.

Electrophoretic Buffer Leakage Upon Flow Termination

When the capillary is aligned over a cell just prior to cell lysis, the electrophoretic buffer flow rate must be zero since this buffer is toxic to living cells. Thus it is important to insure that there is no leakage of the electrophoretic buffer from the coaxial tube as well as to determine the time constant for turning off the electrophoretic buffer. Trypan blue was mixed with the electrophoretic buffer and the velocity of this buffer through the coaxial tube was set at a constant value. The percentage of electrophoretic buffer entering the capillary was then monitored for 200 s. After this time the valve regulating the electrophoretic buffer flow was closed and the percentage of electrophoretic buffer in the capillary measured for an additional 250 s. This experiment was repeated at different electrophoretic buffer velocities (0.3-0.9 mm/s), physiologic buffer velocities (1.3-2.2 mm/s) and Δcapillary (0.1-0.8 mm). For all tested physiologic and electrophoretic velocities, fluid leakage was apparent for a Δcapillary of less than 0.5 mm (Fig. 3). At a Δcapillary of 0.75 mm, the percentage of electrophoretic buffer entering the capillary monotonically decreased when the electrophoretic-buffer valve was closed. For this reason a Δcapillary of 0.75 mm was used in all subsequent experiments.

Serial Analysis of Cells with the Coaxial Flow System

The ability of the coaxial CE system to increase the throughput of analysis for adherent cells was demonstrated using the optimized parameters from the prior experiments: a physiologic buffer velocity of 1.7 mm/s, an electrophoretic buffer velocity of 0.9 mm/s (0.11 ml/min), and a Δcapillary of 0.75 mm. RBL cells were cultured in a rectangular flow chamber and then loaded with Oregon Green and fluorescein. See supporting information for cell culture and dye loading parameters. A future goal will be to lyse cells on sub-second timescales prior to exposure to the electrophoretic buffer; however, for these feasibility experiments each cell was lysed by exposing it to the electrophoretic buffer and an electric field by simultaneously initiating sheath flow and electrophoresis over the selected cell. The sampling procedure consisted of positioning the capillary over a cell, initiating the sheath flow, and loading the cell contents into the capillary by initiation of electrophoresis. The contents of the cell were separated in the capillary as the capillary was translated to the next cell. Prior to positioning the capillary over the next cell, the flow of the electrophoretic buffer and the voltage were turned off. The sampling process was then repeated and electrophoresis reinitiated. The contents of twenty adherent cells were sequentially analyzed within 40 min using this strategy (Fig. 4). Two peaks with migration times corresponding to that of Oregon Green and fluorescein were observed for each cell. The amount of dye as well as the ratio of the two dyes varied significantly between the cells. These results are consistent with prior analyses of Oregon Green and fluorescein in single cells.[46,47]

Conclusion

The feasibility of a coaxial-CE system to rapidly analyze the contents of adherent cells in a serial manner has been demonstrated. Oregon Green and fluorescein were separated and detected from adherent cells at a rate of 0.5 cells/min. Further improvements are contemplated in the system in order to optimize injections of cellular contents into the capillary and to increase throughput. In this demonstration, the cells were lysed by combined exposure to the electrophoretic buffer and the applied electric field. This lysis procedure presents a limitation as cell lysis is likely on the order of several seconds. Differences in the positioning of the capillary and the sheath flow in combination with slow release of cell contents can be expected to introduce variability in the efficiency of loading cell contents for analysis. Future versions of the system will incorporate fast cell lysis either by electrical or laser methods in order to completely disrupt cells on millisecond or faster time scales. Fast cell lysis will serve to rapidly terminate cellular reactions, thus enabling the serial analysis of cellular analytes which change on timescales of a few seconds.[11,12,14,47] Improvements in the speed and reproducibility of

analysis will be attained by further automating the capillary's movement in combination with the flow valves and applied voltage. While the cells to be analyzed must be approximately 1-2 mm apart to prevent exposure of upstream cells to the electrophoretic buffer, large numbers of cells could be analyzed sequentially through the use of a serpentine flow chamber. The sequential nature of the method also facilitates the analysis of the response of cells over time to agonists or inhibitors.”

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Special thanks to Lavanya Rau for assistance with flourescence detection, to Simon Mwongela for his input on electrophoretic buffers, and to G.P. Li, Mark Bachman, and Yuli Wang for their advice and guidance.

References

1. Krylov SN, Zhang Z, Chan NW, Arriaga E, Palcic MM, Dovichi NJ. Cytometry 1999;37:14–20. [PubMed: 10451502]
2. Olefirowicz TM, Ewing AG. J Neurosci Methods 1990;34:11–15. [PubMed: 2259233]
3. Kennedy RT, Oates MD, Cooper BR, Nickerson B, Jorgenson JW. Science 1989;246:57–63. [PubMed: 2675314]
4. Kennedy RT, Jorgenson JW. Anal Chem 1989;61:436–441. [PubMed: 2719258]
5. Gilman SD, Ewing AG. Anal Chem 1995;67:58–64. [PubMed: 7864392]
6. Hogan BL, Yeung ES. Analytical Chemistry 1992;64:2841–2845. [PubMed: 1294009]
7. Wallingford RA, Ewing AG. Anal Chem 1988;60:1972–1975. [PubMed: 3228199]
8. Hu S, Lee R, Zhang Z, Krylov SN, Dovichi NJ. J Chromatogr B Biomed Sci Appl 2001;752:307–310. [PubMed: 11270869]
9. Zhang Z, Krylov S, Arriaga EA, Polakowski R, Dovichi NJ. Anal Chem 2000;72:318–322. [PubMed: 10658325]
10. Whitmore CD, Hindsgaul O, Palcic MM, Schnaar RL, Dovichi NJ. Anal Chem. 2007
11. Meredith GD, Sims CE, Soughayer JS, Allbritton NL. Nat Biotechnol 2000;18:309–312. [PubMed: 10700147]
12. Lee CL, Linton J, Soughayer JS, Sims CE, Allbritton NL. Nat Biotechnol 1999;17:759–762. [PubMed: 10429239]
13. Li H, Sims CE, Wu HY, Allbritton NL. Anal Chem 2001;73:4625–4631. [PubMed: 11605840]
14. Li H, Sims CE, Kaluzova M, Stanbridge EJ, Allbritton NL. Biochemistry 2004;43:1599–1608. [PubMed: 14769036]
15. Shoemaker GK, Lorieau J, Lau LH, Gillmor CS, Palcic MM. Anal Chem 2005;77:3132–3137. [PubMed: 15889901]
16. Shoemaker GK, Palcic MM. Anal Bioanal Chem 2007;387:13–15. [PubMed: 16955262]
17. Hu S, Le Z, Krylov S, Dovichi NJ. Anal Chem 2003;75:3495–3501. [PubMed: 14570202]
18. Whitmore CD, Olsson U, Larsson EA, Hindsgaul O, Palcic MM, Dovichi NJ. Electrophoresis 2007;28:3100–3104. [PubMed: 17668449]
19. Hu S, Zhang L, Cook LM, Dovichi NJ. Electrophoresis 2001;22:3677–3682. [PubMed: 11699905]
20. Teruel MN, Meyer T. Science 2002;295:1910–1912. [PubMed: 11884760]
21. Ferrell JE, Machleder EM. Science 1998;280:895–896. [PubMed: 9572732]
22. Dovichi NJ, Zhang J. Angew Chem Int Ed Engl 2000;39:4463–4468. [PubMed: 11169637]
23. He Y, Zhong W, Yeung ES. J Chromatogr B Analyt Technol Biomed Life Sci 2002;782:331–341.
24. Hofman-Bang J, Behr ER, Hedley P, Tfelt-Hansen J, Kanters JK, Haunsoe S, McKenna WJ, Christiansen M. Clin Genet 2006;69:504–511. [PubMed: 16712702]
25. Snyder TM, McGown LB. Appl Spectrosc 2005;59:335–339. [PubMed: 15901315]

26. Williams WA, Hendrickson A, Gillaspy AF, Dyer DW, Lewis LA. Anal Biochem 2003;313:183–185. [PubMed: 12576078]
27. Kulka S, Quintas G, Lendl B. Analyst 2006;131:739–744. [PubMed: 16732362]
28. Zacharis CK, Tempels FW, Theodoridis GA, Voulgaropoulos AN, Underberg WJ, Somsen GW, de Jong GJ. J Chromatogr A 2006;1132:297–303. [PubMed: 16919652]
29. Takahashi S, Murakami K, Anazawa T, Kambara H. Analytical Chemistry 1994;66:1021–1026.
30. Kamahori M, Kambara H. Methods Mol Biol 2001;163:271–287. [PubMed: 11242952]
31. Pang H, Pavski V, Yeung ES. J Biochem Biophys Methods 1999;41:121–132. [PubMed: 10626770]
32. Kheterpal I, Scherer JR, Clark SM, Radhakrishnan A, Ju J, Ginther CL, Sensabaugh GF, Mathies RA. Electrophoresis 1996;17:1852–1859. [PubMed: 9034766]
33. Huang XC, Quesada MA, Mathies RA. Anal Chem 1992;64:2149–2154. [PubMed: 1416049]
34. Chen S, Lillard SJ. Anal Chem 2001;73:111–118. [PubMed: 11195493]
35. Zhang JZ, Chen DY, Wu S, Harke HR, Dovichi NJ. Clin Chem 1991;37:1492–1496. [PubMed: 1893573]
36. Michalke B. Electrophoresis 2005;26:1584–1597. [PubMed: 15822064]
37. Liu CC, Zhang J, Dovichi NJ. Rapid Commun Mass Spectrom 2005;19:187–192. [PubMed: 15593250]
38. Kottegoda S, Shaik I, Shippy SA. J Neurosci Methods 2002;121:93–101. [PubMed: 12393165]
39. Arkhipov SN, Berezovski M, Jitkova J, Krylov SN. Cytometry A 2005;63:41–47. [PubMed: 15584019]
40. Walker GM, Sai J, Richmond A, Stremler M, Chung CY, Wikswo JP. Lab Chip 2005;5:611–618. [PubMed: 15915253]
41. Nerem RM, Alexander RW, Chappell DC, Medford RM, Varner SE, Taylor WR. Am J Med Sci 1998;316:169–175. [PubMed: 9749558]
42. Li YS, Haga JH, Chien S. J Biomech 2005;38:1949–1971. [PubMed: 16084198]
43. Yin H, Zhang X, Pattrick N, Klauke N, Cordingley HC, Haswell SJ, Cooper JM. Anal Chem. 2007
44. Schaff UY, Xing MM, Lin KK, Pan N, Jeon NL, Simon SI. Lab Chip 2007;7:448–456. [PubMed: 17389960]
45. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL. Lab Chip 2005;5:401–406. [PubMed: 15791337]
46. McClain MA, Culbertson CT, Jacobson SC, Allbritton NL, Sims CE, Ramsey JM. Anal Chem 2003;75:5646–5655. [PubMed: 14588001]
47. Sims CE, Meredith GD, Krasieva TB, Berns MW, Tromberg BJ, Allbritton NL. Anal Chem 1998;70:4570–4577. [PubMed: 9823716]

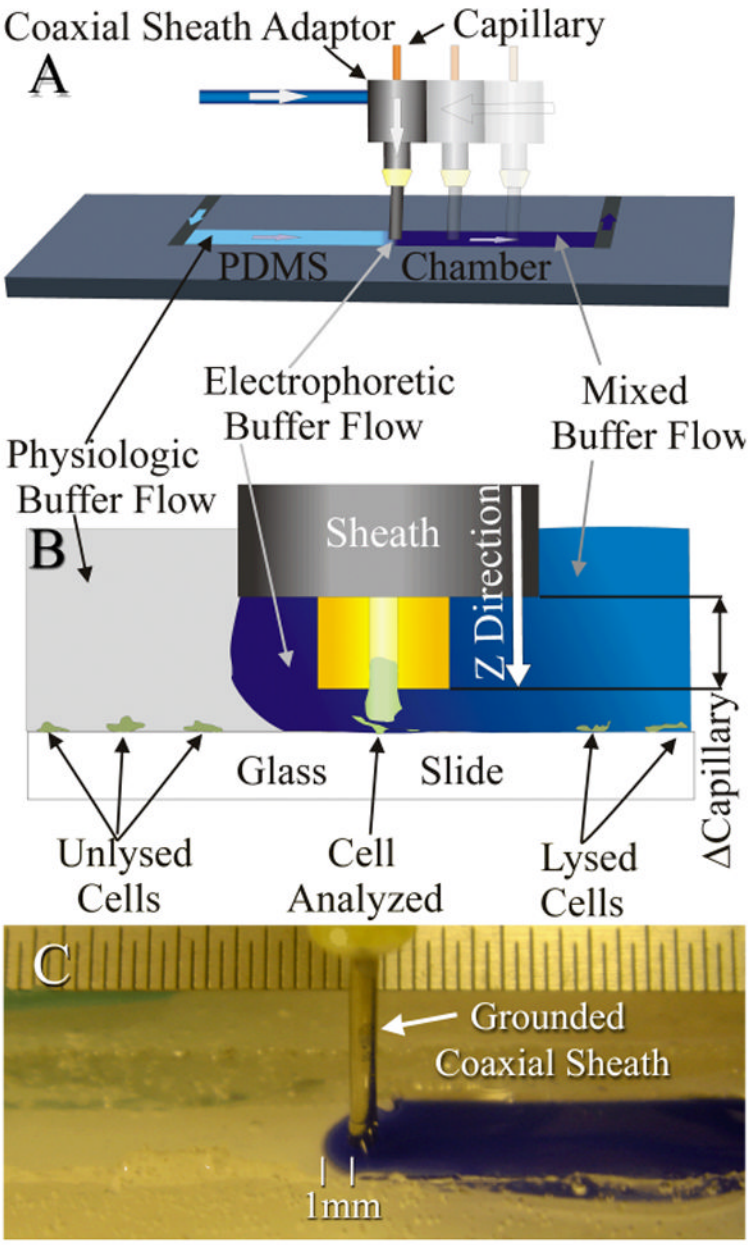

Figure 1.

Coaxial capillary electrophoresis system. A) Shown is a side view of the capillary with coaxial tube and cell chamber with physiologic buffer flow. B) Shown is a close-up, side view of the capillary tip with the coaxial or sheath tube. The capillary is positioned above the targeted cell. A voltage is applied across the capillary at the same time that the electrophoretic buffer is delivered to the tip of the capillary through the coaxial tube. Electrophoretic buffer is swept away by a physiological buffer flow preventing upstream cells from being exposed to the electrophoretic buffer. After electrophoresis is complete coaxial flow is temporarily turned off as the capillary is positioned above another cell upstream. C) Close up photo of the 0.9 mm coaxial sheath in the rectangular chamber with a physiologic buffer velocity of 1.7 mm/s and a coaxial sheath flow 0.9 mm/s with a Δcapillary of 0.75 mm. Note that the coaxial sheath fluid (in blue) travels slightly over 1 mm up steam.

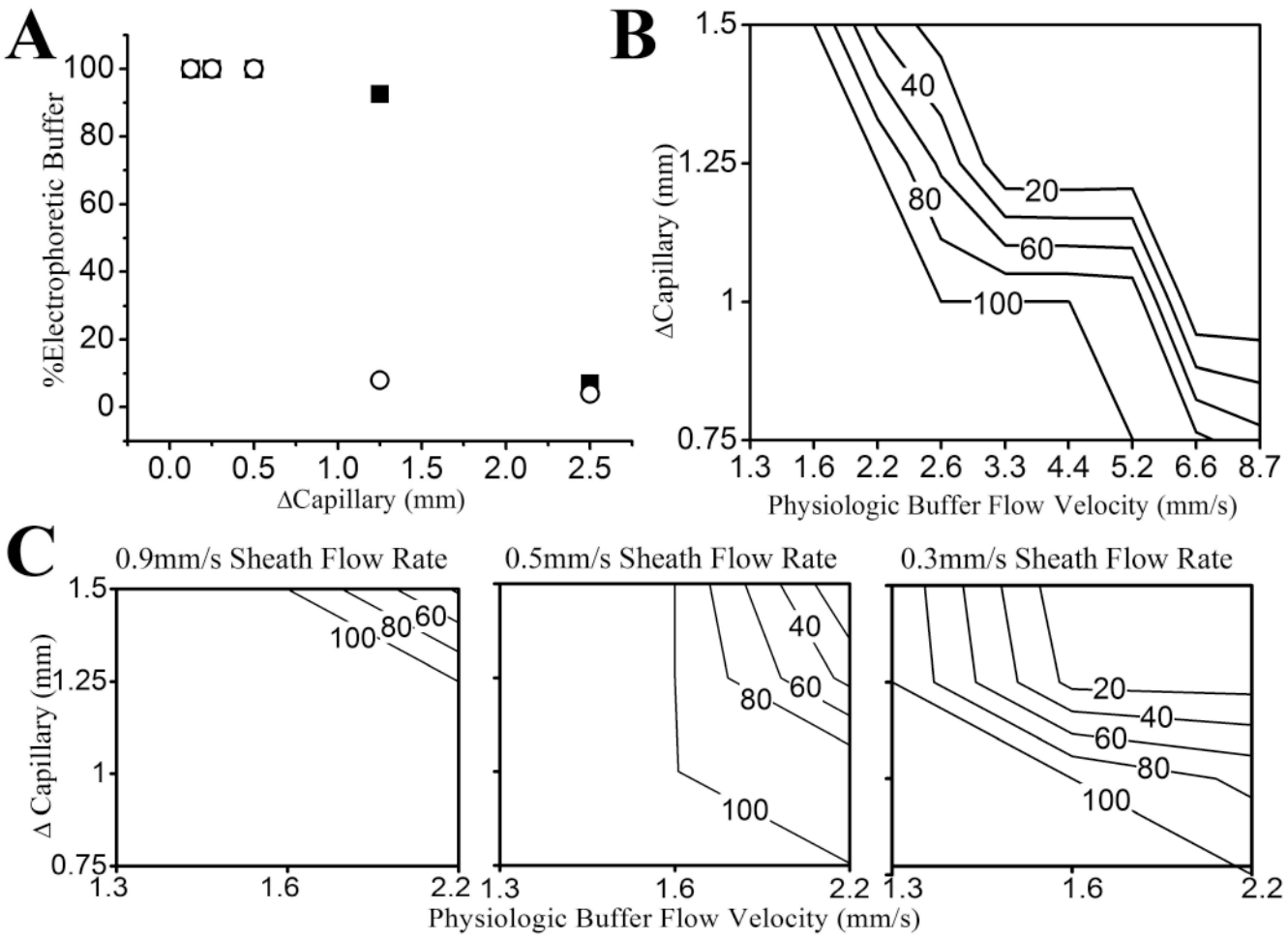

Figure 2.

Optimization of the flow rates and dimensions for the coaxial CE system A) Shown is the percentage electrophoretic buffer entering the capillary as a function of Δcapillary for two different-diameter coaxial tubes, 1.2 mm (solid squares) 0.9 mm (open circles). Other system parameters were a sheath flow rate (0.17 ml/min), physiologic buffer rate (5.5 ml/min), and a square chamber geometry. B) The percentage electrophoretic buffer entering the capillary was plotted as a function of the velocity of the physiologic buffer and Δcapillary for a constant coaxial sheath flow velocity of 0.9 mm/s in the rectangular chamber. The topographical lines represent the percentage electrophoretic buffer entering the capillary. C) The percentage electrophoretic buffer entering the capillary was plotted as a function of physiologic buffer velocity and Δcapillary for various coaxial sheath flow velocities (0.3-0.9 mm/s) in the rectangular chamber. The topographical lines are the percentage electrophoretic buffer in the capillary.

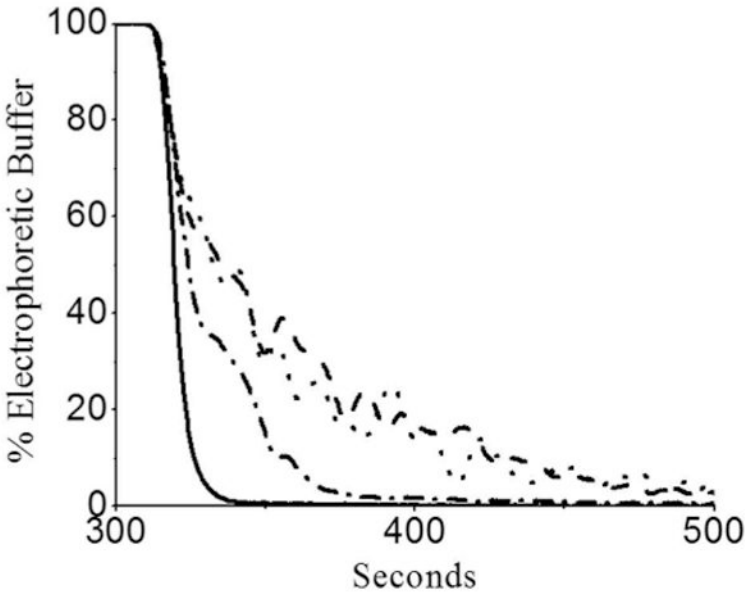

Figure 3.

Decay of electrophoretic buffer flow upon closure of the elecrophoretic-buffer valve. For varying Δcapillary, the percentage electrophoretic buffer entering the capillary was measured over time. The tested Δcapillary were 0.75 mm (solid), 0.5 mm (dotted-dashed), 0.3 mm (dotted), and 0.1 mm (dashed). The physiologic buffer velocity: was 1.7 mm/s and the electrophoretic buffer flow rate was 0.1 ml/min.

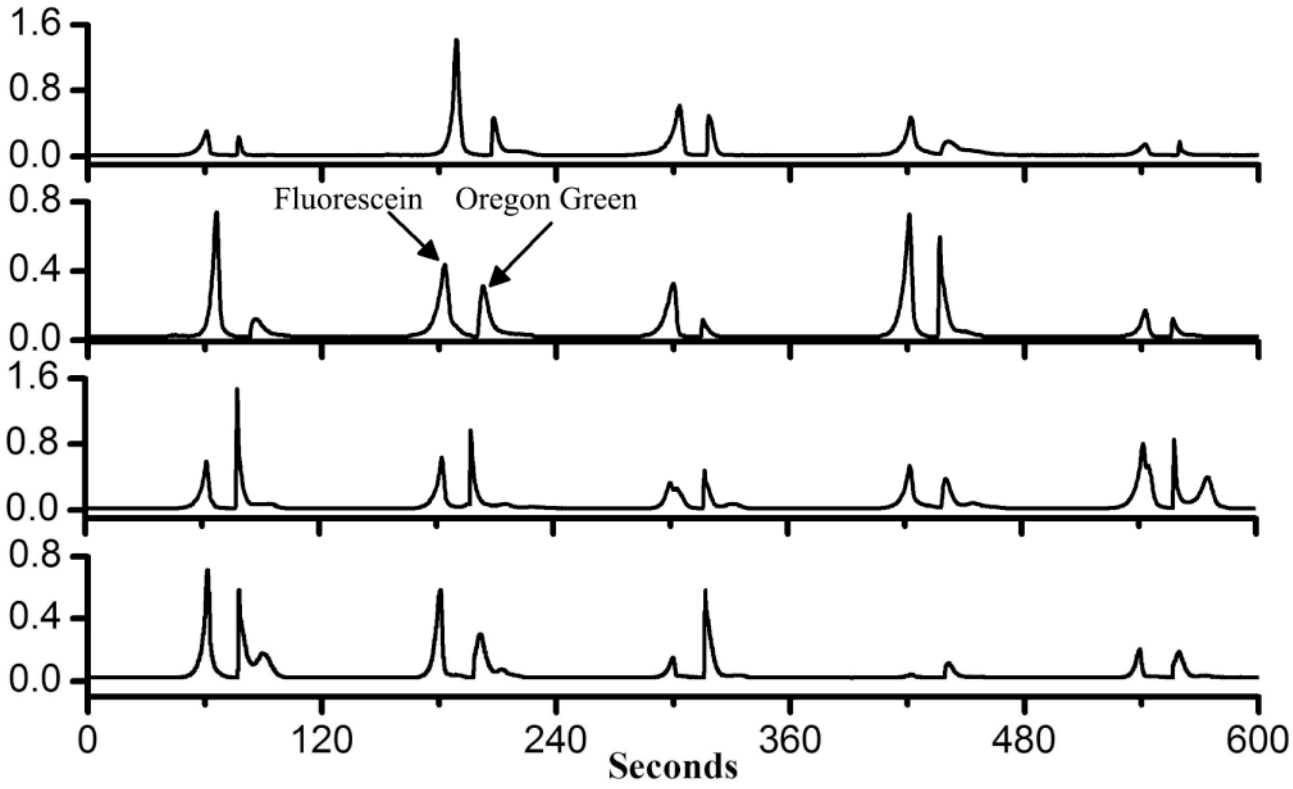

Figure 4.
Serial lysis and analysis of adherent cells using the coaxial CE system. Twenty cells were sequentially lysed and their contents (Oregon Green and fluorescein) separated as described in the text.