Linfen Yu[1,2]
Huaiqing Huang[1,2]
Xiuling Dong[3]
Dapeng Wu[1,2]
Jinhua Qin[1]*
Bingcheng Lin[1]

[1]Department of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, P. R. China
[2]Graduate school, Chinese Academy of Sciences, Dalian, P. R. China
[3]Dalian Third Municipal Hospital, Dalian, P. R. China

Received March 11, 2008
Revised June 19, 2008
Accepted June 23, 2008

Research Article

Simple, fast and high-throughput single-cell analysis on PDMS microfluidic chips

This paper demonstrated the chemical analysis of single cell on a cross PDMS microfluidic chip in a simple, fast, and high-throughput mode. The pre-stained cells were sequentially loaded into the cross section by hydrodynamic force, lysed by 0.2% SDS and subsequently the lysates were detected by LIF. Each cell can be lysed within 500 ms due to its high concentration of SDS at cross section resulted from the absence of electroosmosis after surface coating in microchannel. The reliability and quality of the analysis was confirmed by analysis of glutathione and rhodamine 123 in single K562 cells. In each run, approximately 100 cells could be analyzed in about 10 min, which demonstrated the comparatively high throughput. The proposed microfluidic method is simple, fast, and high throughput, which might be of significance in identifying the biological molecules involved in fast biochemical processes and studying heterogenous cells.

Keywords:
K562 cells / Microfluidic chip / Single-cell analysis DOI 10.1002/elps.200800331

1 Introduction

Cell analysis attracts increasing interests in biological, medical, and chemical fields. Most cell-based biological assays yield data averaged across large groups of cells; yet it is well known that individual cells, even those identical in appearance, differ in numerous characteristics [1, 2] Therefore, highly efficient and sensitive detection of the components in single cell will explain some important physiological processes and assist in detecting rare, abnormal cells in large populations of cells, thus potentially providing useful information for early disease diagnosis.

Because of the importance of the information that can be gained from individual cells, several techniques are available for the chemical analysis of single cells including CE [3–7], mass spectrometry [8–10], and electrochemistry [11]. Among these, CE has proved to be a valuable and important tool for studies of single-cell chemical composition due to its high separation efficiency and small sample consumption [3–7]. But until now, CE-based cell analysis still suffers from sophisticated cell manipulation and low throughput due to the one-dimensional structure of capillary [12, 13].

Microfluidic chip, also known as ''lab-on-a-chip'', offers a number of advantages in small sample consumption, flexible manipulation, and large-scale integration [14]. Especially, the size of microfluidic chip fits very well with cells; hence, it has attracted significant attention in cell analysis. Nowadays, several works on single-cell analysis on such integrated microdevices have been reported. McClain *et al.* [15] developed a rapid method of cell lysis by applying alternating current with direct current offsets and detection of fluorogenic dyes preloaded in cells. Wu *et al.* [16] and Hong *et al.* [17] produced complicated microfluidic chips that provided pneumatic valves and pumps for automated cell isolation, cell lysis, separation of amino acids, or purification of DNA or messenger ribonucleic acid. These works demonstrated high integration of cell analysis on microfluidic chip but the manipulation and chip design were comparatively complicated. Gao *et al.* and Sun *et al.* [18, 19] demonstrated the single-cell loading, docking and lysis by applying a set of potentials, and detected the glutathione (GSH) and reactive oxygen in human erythrocyte on a simple microfluidic chip. However, the analysis throughput was relatively low. Thus, a simple, fast, and high-throughput cell analysis is desirable.

In this paper, we demonstrated a simple way to rapidly lyse cell by SDS and analysis of single cell on a cross PDMS microfluidic chip in a simple, fast, and high-throughput mode. The pre-stained cells were sequentially loaded into the cross section by hydrodynamic force and then

Correspondence: Professor Bingcheng Lin, Department of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian, Liaoning 116023, P. R. China
E-mail: bclin@dicp.ac.cn
Fax: +86-411-84379065

Abbreviations: GSH, glutathione**; HPMC,** hydroxyprophylmethylcellulose**; NDA,** 2, 3-naphthalenedicarboxaldehyde**; Rh-123,** rhodamine 123

*Additional corresponding author: Professor Jinhua Qin, E-mail: jhquin@dicp.ac.cn

electrophoretically dispensed into the separation channel, in which the cells were lysed by 0.2% SDS and the lysate was detected by LIF. Each cell can be lysed within 500 ms due to its sufficiently high concentration of SDS at cross section when the microchannel was coated with epoxy-modified polymers and resulted in nearly zero EOF. The reliability and quality of the analysis was confirmed by analysis of rhodamine 123 (Rh-123) and GSH in single K562 cells.

2 Materials and methods

2.1 Reagents and buffer preparation

All chemical reagents were of analytical or HPLC reagent grade. Rh-123 (Ex 470 nm, Em 520 nm) and SDS were obtained from Sigma Chemicals (St. Louis, MO, USA). 2, 3-Naphthalenedicarboxaldehyde (NDA, Ex 470 nm, Em 520 nm) was obtained from Molecular Probes (Eugene, OR, USA). Sylgard 184 PDMS prepolymer and curing agent were purchased from Dow Corning. All other chemicals, unless otherwise noted above, were from Shanghai Regents Company (China). An aliquot of 20 mM borate (pH 9.2) containing 0.2% SDS was selected as BGE, which was optimized in Qin's work [20].

2.2 Cells culture and cell preparation

The K562 cells were cultured in RPMI 1640 medium, supplemented with 10% v/v fetal bovine serum, L-glutamine (2 mM), penicillin (100 Ug/mL), and streptomycin (100 Ug/mL). All cell culture reagents were obtained from Gibco BRL (Gaithersburg, MD). The cells were grown in 25-mL glass culture flasks and maintained at 37°C with 5% CO^2. Before the experiment the cells were incubated and labeled with 5×10^{-6} M Rh-123 or incubated with 1×10^{-4} M NDA and 5×10^{-6} M Rh-123 for an additional period of 30 min in the presence of a culture medium. Then cells were centrifuged at 126g for 5 min and washed using PBS (containing 9 g/L sodium chloride, 1 mM sodium phosphate monobasic, and 3 mM sodium phosphate dibasic; pH was adjusted to 7.4 with 1 M sodium hydroxide) twice in order to remove possible leftover components from the medium; finally, the cells were suspended in PBS buffer again for use. The cell density was about $\sim 5 \times 10^6$ cells/mL.

2.3 Microchip fabrication and surface coating

The schematic illustration of the single cross PDMS microchip is shown in Fig. 1. The microchannels were 70 μm wide and 25 μm deep. The PDMS microfluidic chips were fabricated according to the established rapid prototyping protocols [21]. The master composed of a positive relief of SU-8 2025 photoresist (Microchem, Newton, MA) was made on a 5 cm glass wafer by soft lithography. A 10:1 v/v mixture of PDMS prepolymer and curing agent was used and cured over the SU-8 master at 80°C for at least 2 h. After cooling at room temperature, the PDMS was stripped off the master. Four holes (3 mm diameter) used as the sample and buffer reservoirs were punched. The channel between reservoirs S and SW was used for sampling cell and the channel between B and BW was used for the separation of intracellular component as shown in Fig. 1.

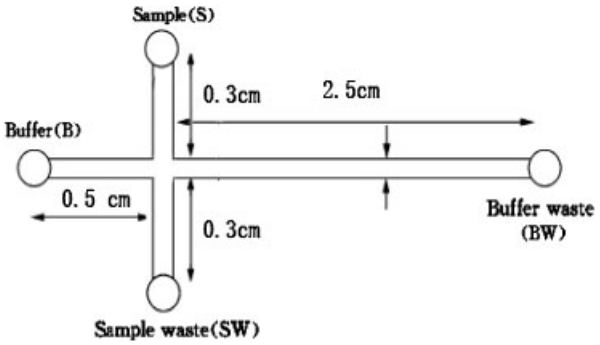

Figure 1. Geometric layout of PDMS microfluidic chip. All channels are 25 μm deep, 70 μm wide.

In order to reduce adsorption and electroosmosis, the surface of microchannels were treated according to the approach of epoxy-modified hydrophilic polymer coatings as described in Wu's paper [22]. Epoxy-modified polymer aqueous solution with poly(dimethylacrylamide-co-glycidyl methacrylate) was synthesized by Wu (Dalian Institute of Chemical Physics, China). Briefly, PDMS microfluidic chip and microscope slides (75 mm × 25 mm) were all pretreated by oxygen plasma for 60 s in a Harrick plasma cleaner/sterilizer (PDC-23G, 100 W). After that, they were brought together immediately for irreversible bonding. Within 1 min, polymer aqueous solution was filled into the microchannel and pumped for a few minutes. The microfluidic chip filled with polymer solution was placed for 15 min at room temperature. The solution was completely pumped out and the PDMS microchip was directly heated in 110°C for 10 min. Before use, the channel was washed with purified water for 5 min. After coating, the surface of the microchannel resulted in nearly zero EOF with little change at least within 2 wk.

2.4 Detection system

A homemade confocal microscope LIF system was used in this experiment. This system was mounted on a motor controlled *X-Y-Z* translation stage. A power adjustable diode laser (473 nm, 10 mW; Viasho, Beijing, China) was used as the excitation source. The laser beam was passed through a band-pass filter (XF1072, 475AF20; Omega Optical, USA); then it was reflected by a dichroic mirror (XF2077,505 DRLP; Omega) and focused onto the center of the microchannel by a 20 × microscope objective. Fluorescence was collected by the same objective and filtered by a band-pass emission filter (XF3084, 535AF45; Omega); then it was

focused by a convex lens onto a pinhole (0.4 mm) and detected by a PMT module (H7711-04; Hamamatsu, Japan). Controls of the high-voltage system, translation stages, and PMT data collection were achieved through a computer with program written in house.

2.5 Experimental procedures

Prior to single-cell analysis, 10 μL of BGE solutions were added to the buffer and buffer waste reservoirs, respectively. Then 10 μL of cell suspension were added to the sample reservoir S with the sample waste reservoir SW empty. Under hydrostatic pressure created by the differences in liquid levels in the reservoirs, the cell suspension flowed from S to SW. High voltage was applied to BW reservoirs and with B reservoirs grounded. When moving into the cross section of the channels, the negatively charged cells would turn toward the BW by electric force and then were rapidly lysed and analyzed.

3 Results and discussions

3.1 Sampling of single cells

For microchip-based cell studies, cells were often transported by electrical fields [23]. Unfortunately, the high current and Joule heating tend to damage the cell viability. Laser-trap and dielectrophoretic forces were also used to sample single cell but these methods need specialized and sophisticated equipment [24]. In our preliminary studies [25], hydrostatic pressure generated by the difference of liquid level was used to transport cells and it appeared to be a gentle and easy means. One major factor for consideration in single-cell sampling was cell adherence on microchannel surface. It was found that cell adhered strongly on untreated glass or PDMS surface. Cell adhesion can be reduced after the surface was modified with an anti-adhesive coating. In this work, epoxy-modified polymer was selected as an effect way to generate hydrophilic and protein-resistant surface coating. On PDMS microchip coated by poly(dimethylacrylamide-co-glycidyl methacrylate), cells flowed smoothly one by one without adhering problem (video not shown).

Another major factor for consideration in single-cell sampling was the density of cell population. It was investigated that when the cell density exceeded $5 \times 10^6\ mL^{-1}$, the chance of multi-cell loading and cell agglomeration occurred increasingly within the channels. Contrarily, cell density less than $1 \times 10^6\ mL^{-1}$ would result in long intervals between cells and low analysis throughput. For less component analysis, such as only one or two components, a cell density of $\sim 5 \times 10^6$ cells mL^{-1} was better. Although the density of cell population was the same; the cell analysis throughput in every minute was different. It decreased with time because cells would sedimentate gradually in PBS under gravity, which decreased the cell density in suspension. Because the movement of a cell is of relatively stochastic, the cell throughputs were not consistent every time.

3.2 Single-cell lysis on microfluidic chip

Cell lysis is especially critical in the process of cell analysis. Several methods have been developed for cell lysis on microfluidic chip such as mechanical force [26], minisonicator [27, 28], AC electrical or laser pulse [15, 29] detergent [30–32], and so on. Among these methods, AC electrical pulse and laser pulse can lyse cell in subsecond but its device is complicated. The detergent method is a simple and efficient way but the lysis speed and analysis throughput are sufficiently slow. Krylov and Dovichi [30] designed a single-cell injector to reduce the volume of cell medium plug and a carcinoma cell was lysed by SDS within 30 s. Ocvirk *et al.* [31] designed a Y-shaped microchannel at which cells mixed with lytic agents at junction and individual cells lysed on-chip within 30 s using Triton X-100 and 2 s using SDS, respectively. Sun and Yin [32] fabricated a weir-structure microfluidic chip; thus the loaded cell was stopped at the weir and trapped cell was lysed by SDS in 20 s. Kleparnik and Horky [33] designed a planar-structure microfluidic chip and the cell was injected by a vacuum-driven flow and lysed by alkaline in 3–5 min. Hellmich *et al.* [34] designed a micropillar-structure microfluidic chip where the cell was trapped at a predefined position and lysed by 0.5%SDS within 6 s. Thus, we try to develop a method for simple, fast, and high-throughput cell lysis.

As we know, the speed of cell lysis by SDS was mainly depended on the concentration of SDS molecules diffusing to cells surface. There were several methods to quickly enhance the concentration of SDS surrounding the cell, such as reducing diffuse distance by reducing the volume of cell medium plug [30] or increasing the chance of contact by holding up a cell by weir [32] or micropillar. In experiments, it was observed that cells suspended in PBS on microscope slide were lysed quickly by 0.2% SDS because enough SDS molecule could diffuse to cell surface under static condition; however, it was difficult to lyse a cell in microchannel even within 20–30 s after it was electrophoretically dispensed and traveled 10–20 mm downstream in the separation channel. This fact suggested that few SDS molecules diffused to cell membrane when the cell buffer and background buffer were moving simultaneously under electroosmosis. Interestingly, we found that, if the electroosmosis in the separation channel was totally suppressed, the cell could be rapidly lysed within 0.5 s after it was dispensed into the separation channel under electrical field. Figure 2 shows a time series of CCD images, which demonstrated the lysis of an individual K562 cell by 0.2% SDS. Each image was captured at an interval of 100 ms. The first image corresponded to the starting point of lysis; the cell was found to be intact. The cell was partly damaged after 300 ms and completely lysed within 500 ms. A video clip showing the cell sampling and lysis process is provided in Supporting Information.

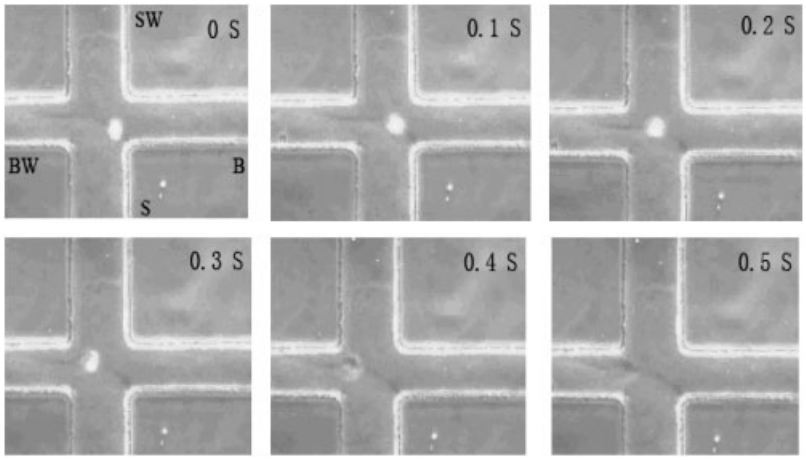

Figure 2. CCD images showing the process of cell lysis. It indicated that the cell was lysed within 500 ms. Separate images were taken at intervals of 100 ms. Buffer 20 mM borate (pH 9.2), 0.2% SDS; electric field: 400 V/cm.

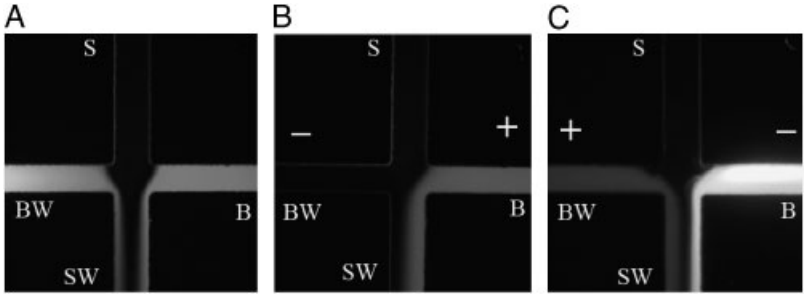

Figure 3. Fluorescent imaging of the flowing profile of Rh-123 in microchannel. (A) The flowing profile of fluorescent dye under hydrodynamic force. (B–C) The flowing profile of fluorescent dye in the presence and absence of electroosmosis, respectively, under an electric field and hydrodynamic force.

The above results indicated that the concentration of SDS surrounding the cell membrane was totally different in the microchannel in the presence or absence of electroosmosis. In order to visualize these differences, Rh-123 fluorescent dye, as negative small molecule as SDS in pH 9.2 borate buffer, was used to simulate the change of the concentration of SDS molecules in separation channel. A solution of 10 μL of 20 mM borate buffer spiked with 5×10^{-6} M Rh-123 was added to B and BW. PBS buffer was added to S, and SW was kept empty. Figure 3A simulates the flow profile of cell loading under hydrodynamic pressure, in which PBS buffer, pinched by the bilateral fluorescent dye flows, was continuously flowing from S into SW. Figure 3B and C shows the change in fluorescence intensity in the separation channel in the presence and absence of electroosmosis, respectively, under electric field. It could be observed that the dyes in separation channel were quickly displaced by PBS under EOF. In this case, the concentration of SDS surrounding cells was very low, resulting in a delayed or failed cell lysis. When the electroosmosis was inhibited, the fluorescent dyes could be retained in the separation channel and mixed with the PBS buffer, although the intensity of fluorescence faded gradually. In this condition, the cells in the PBS buffer could interact sufficiently with SDS; thus, the cell could be lysed rapidly as demonstrated in Fig. 2. In addition, it could be observed that the fluorescence intensity in the right-hand side channel of the cross section is much brighter than the left-hand side channel as shown in Fig. 3C. This could be explained by the fact that sample stacking occurred at the boundary of two different concentrations of buffers. Owing to the absence of electroosmosis and encountering of two different concentrations of buffer in the cross section (the concentrations of PBS and borate buffers were 100 and 20 mM, respectively), the fluorescent dyes were continuously stacked at the boundary of two different buffers, which suggested high concentration of SDS in intersection. It was another reason why cell lysed quickly nearby the intersection after it was electrically turned to separation channel. Contrarily, in the presence of electroosmosis the sample stacking was weak because the fluorescence molecules continuously moved to cathode under EOF. According to the fluorescence experiment result, it was presumed that the concentration of SDS surrounding the cell at the cross section was much higher in the absence of electroosmosis than in the presence of electroosmosis; thus, higher concentration of SDS resulted in quick cell lysis in half a second. It was a simple and fast way to speed cell lysis by whole restraining electroosmosis compared with laser pulse or AC electrical pulse.

3.3 Analysis of GSH and Rh-123 in single K562 cells

Rh-123 is a sensitive dye for mitochondrial membrane potential collapse. It can penetrate cell membrane and bind to the inner and outer membranes of mitochondria. It was selected to confirm the reliability and quality of high-throughput single-cell analysis on this PDMS chip. Figure 4 shows the electropherograms of Rh-123 released from

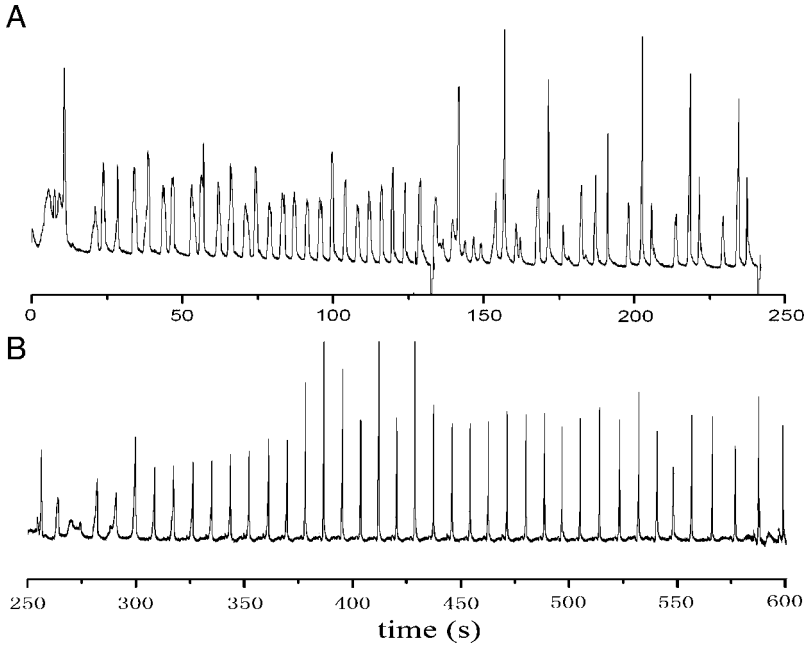

Figure 4. Electropherograms of Rh-123 released from individual K562 cells. In the first 4 min and in the following 6 min the analysis throughputs were about 15 cells/min (A) and 7 cells/min (B), respectively. Buffer: 20 mM borate (pH 9.2), 0.2% SDS; electric field: 400 V/cm; detection distance: 1 cm from intersection.

individual K562 cells. As shown in Fig. 4A and B, the cell analysis throughputs were ~15 cells/min in the first 4 min and 7 cells/min in the following 6 min, respectively. The averaged cell analysis throughput was about 10 cells/min in 10 min at a density of $\sim 5 \times 10^6$ cells/mL. The cell analysis throughput decreased with time because cells would sedimentate gradually in PBS under gravity, which decreased the cell density in suspension. It was reported that gravity-dependent sedimentation could be alleviated effectively by adding a certain concentration of hydroxyprophylmethylcellulose (HPMC) into the cell suspension [32, 35]. In experiment, it was found that the nonionic-HPMC, and anionic surfactant-SDS interacted with each other. The speed of cell lysis was influenced after adding HPMC; thus, the cells were suspended in free PBS solution. Although the gravity-dependent sedimentation existed, the analysis throughput of more than 100 cells in each run in about 10 min was acceptable and was much higher than previously reported [18, 19, 32].

In order to validate the reliability of simultaneous analysis of two components, GSH and Rh-123 in single K562 cells were analyzed on the microfluidic chip. GSH is the most abundant intracellular nonprotein thiol, existing mainly in its reduced form under steady-state conditions [36]. The probe NDA can react quickly with GSH to produce a fluorescent adduct. Both the negatively charged dyes NDA-GSH and Rh-123 were electrophoretically transported toward the anode in microchannel without electroosmosis. The effects of the applied electric field on the separation of the two analytes were investigated. The GSH and Rh-123 were not separated at an electrical field of 720 V/cm (figure not shown). With the decrease in electrical field to 600 V/cm, the separation efficient gradually improved and the two molecules were basically resolved at an electrical field of 500 V/cm. The representative electropherogram of Rh-123 and GSH from continuous and individual cells is shown in Fig. 5. The cell analysis throughputs were decreased from ~8 to ~3 cells/min in 10 min. The ability of multi-components separation and detection such as RNA in single cell on the same microfluidic chip is under investigation.

4 Conclusions

In this paper, we demonstrated high-throughput chemical analysis of single cells on a cross PDMS microfluidic device. The cells were hydrodynamically transported, rapidly lysed in 500 ms by 0.2% SDS, and subsequently the lysates were detected by LIF. The fast cell lysis might be due to the absence of electroosmosis after surface coating, which resulted in high concentration of SDS surrounding the cell and making cell lyse quick and complete. This microfluidic system provided a simple, fast, and high-throughput platform for the analysis of intracellular constituents, which might be of significance in the measurement of biological molecules involved in fast biochemical processes and studying heterogenous cells.

This research was supported by the National Nature Science Foundation of China (Project nos. 20635030, 90713014, and 30570479) and Key Project of National Programs for Fundamental Research and Development (973 program No. 2007CB714505, 2007CB714507 and 863 program No. 2006AA020201, 2006AA02Z305). In addition, we thank Luoyong for helping in writing this paper.

The authors declare no financial or commercial conflict of interest.

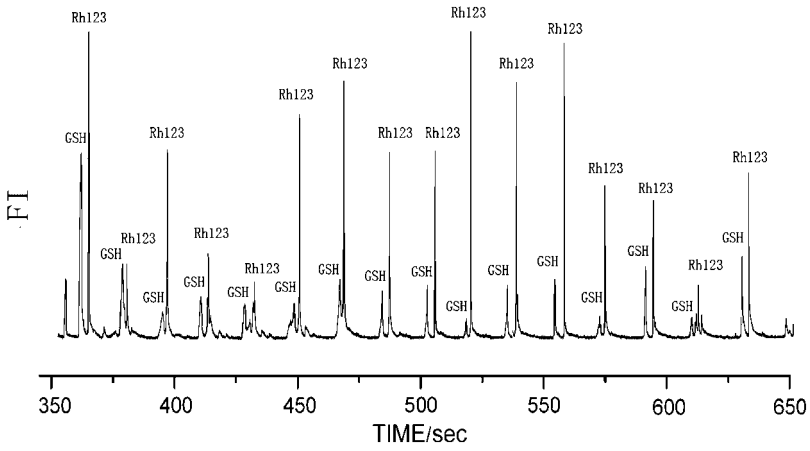

Figure 5. Typical electropherogram of Rh-123 and GSH released from individual K562 cells. Buffer: 20 mM borate (pH 9.2), 0.2% SDS; electric field: 500 V/cm; detection distance: 2.2 cm from intersection.

5 References

[1] Dolmetsch, R. E., Xu, K. L., Lewis, R. S., *Nature* 1998, *392*, 933–936.

[2] Meredith, G. D., Sims, C. E., Soughayer, J. S., Allbritton, N. L., *Nat. Biotechnol.* 2000, *18*, 309–312.

[3] Li, H. N., Sims, C. E., Wu, H. Y., Allbritton, N. L., *Anal. Chem.* 2001, *73*, 4625–4631.

[4] Han, F. T., Lillard, S. J., *Anal. Chem.* 2000, *72*, 4073–4079.

[5] Krylov, S. N., Starke, D. A., Arriaga, E. A., Zhang, Z. R. *et al.*, *Anal. Chem.* 2000, *72*, 872–877.

[6] Zhang, Z. R., Krylov, S., Arriaga, E. A., Polakowski, R., Dovichi, N. J., *Anal. Chem.* 2000, *72*, 318–322.

[7] Han, F. T., Lillard, S. J., *Anal. Biochem.* 2002, *302*, 136–143.

[8] Page, J. S., Rubakhin, S. S., Sweedler, J. V., *Anal. Chem.* 2002, *74*, 497–503.

[9] Roddy, T. P., Cannon, D. M., Meserole, C. A., Winograd, N., Ewing, A. G., *Anal. Chem.* 2002, *74*, 4011–4019.

[10] Roddy, T. P., Cannon, D. M., Ostrowski, S. G., Winograd, N., Ewing, A. G., *Anal. Chem.* 2002, *74*, 4020–4026.

[11] Ewing, A. G., Chen, G., *Abstr. Pap. Am. Chem. Soc.* 1994, *208*, 120–ANYL.

[12] Jin, W. R., Li, W., Xu, Q., *Electrophoresis* 2000, *21*, 774–779.

[13] Hogan, B. L., Yeung, E. S., *Anal. Chem.* 1992, *64*, 2841–2845.

[14] Vilkner, T., Janasek, D., Manz, A., *Anal. Chem.* 2004, *76*, 3373–3385.

[15] McClain, M. A., Culbertson, C. T., Jacobson, S. C., Allbritton, N. L. *et al.*, *Anal. Chem.* 2003, *75*, 5646–5655.

[16] Wu, H. K., Wheeler, A., Zare, R. N., *Proc. Natl. Acad. Sci. USA* 2004, *101*, 12809–12813.

[17] Hong, J. W., Studer, V., Hang, G., Anderson, W. F., Quake, S. R., *Nat. Biotechnol.* 2004, *22*, 435–439.

[18] Gao, J., Yin, X. F., Fang, Z. L., *Lab Chip* 2004, *4*, 47–52.

[19] Sun, Y., Yin, X. F., Ling, Y. Y., Fang, Z. L., *Anal. Bioanal. Chem.* 2005, *382*, 1472–1476.

[20] Qin, J. H., Ye, N. N., Yu, L. F., Liu, D. Y. *et al.*, *Electrophoresis* 2005, *26*, 1155–1162.

[21] Duffy, D. C., McDonald, J. C., Schueller, O. J. A., Whitesides, G. M., *Anal. Chem.* 1998, *70*, 4974–4984.

[22] Wu, D. P., Zhao, B. X., Dai, Z. P., Qin, J. H., Lin, B. C., *Lab Chip* 2006, *6*, 942–947.

[23] Li, P. C. H., Harrison, D. J., *Anal. Chem.* 1997, *69*, 1564–1568.

[24] Arai, F., Ichikawa, A., Ogawa, M., Fukuda, T. *et al.*, *Electrophoresis* 2001, *22*, 283–288.

[25] Gai, H. W., Yu, L. F., Dai, Z. P., Ma, Y. F., Lin, B. C., *Electrophoresis* 2004, *25*, 1888–1894.

[26] Di Carlo, D., Jeong, K. H., Lee, L. P., *Lab Chip* 2003, *3*, 287–291.

[27] Taylor, M. T., Belgrader, P., Furman, B. J., Pourahmadi, F. *et al.*, *Anal. Chem.* 2001, *73*, 492–496.

[28] Belgrader, P., Hansford, D., Kovacs, G. T. A., Venkateswaran, K. *et al.*, *Anal. Chem.* 1999, *71*, 4232–4236.

[29] Soughayer, J. S., Krasieva, T., Jacobson, S. C., Ramsey, J. M. *et al.*, *Anal. Chem.* 2000, *72*, 1342–1347.

[30] Krylov, S. N., Dovichi, N. J., *Electrophoresis* 2000, *21*, 767–773.

[31] Ocvirk, G., Salimi-Moosavi, H., Szarka, R. J., Arriaga, E. A. *et al.*, *Proc. IEEE* 2004, *92*, 115–125.

[32] Sun, Y., Yin, X. F., *J. Chromatogr. A* 2006, *1117*, 228–233.

[33] Kleparnik, K., Horky, M., *Electrophoresis* 2003, *24*, 3778–3783.

[34] Hellmich, W., Pelargus, C., Leffhalm, K., Ros, A., Anselmetti, D., *Electrophoresis* 2005, *26*, 3689–3696.

[35] Yao, B., Luo, G. A., Feng, X., Wang, W. *et al.*, *Lab Chip* 2004, *4*, 603–607.

[36] Davison, K., Cote, S., Mader, S., Miller, W. H., *Leukemia* 2003, *17*, 931–940.

