Anal Bioanal Chem (2007) 387:91–96
DOI 10.1007/s00216-006-0866-7

ORIGINAL PAPER

Cell lysis inside the capillary facilitated by transverse diffusion of laminar flow profiles (TDLFP)

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Received: 15 August 2006 / Revised: 12 September 2006 / Accepted: 15 September 2006 / Published online: 26 October 2006

Abstract Chemical cytometry studies the molecular composition of individual cells by means of capillary electrophoresis or capillary chromatography. In one of its realizations an intact cell is injected inside the capillary, the plasma membrane is disrupted to release the cellular contents into the separation buffer, and, finally, the molecules of interest are separated and detected. The solubilization of the plasma membrane with a surfactant is a simple and efficient way of achieving cell lysis inside the capillary. To facilitate cell lysis by a surfactant the cell has to be contacted with the surfactant inside the capillary. We recently introduced a generic method for mixing solutions inside the capillary termed transverse diffusion of laminar flow profiles (TDLFP). In this work, we propose that TDLFP can facilitate efficient cell lysis inside the capillary. Conceptually, a short plug of the surfactant is injected by pressure prior to cell injection. The cell is then injected by pressure wizthin a plug of the physiological buffer. Due to the parabolic profiles of pressure-driven laminar flows the interface between the plug of the surfactant and that of the physiological buffer is predominantly longitudinal. Transverse diffusion mixes the surfactant with the physiological buffer, which leads to surfactant's contact with the cell and subsequent cell lysis. Here, we demonstrate that the proposed concept is valid. TDLFP-facilitated cell lysis by a short plug of the surfactant allows us to exclude the surfactant from the run buffer, and, hence, facilitates modes of separation, which are incompatible with the surfactant's presence in the run buffer. In addition to cell lysis, TDLFP will be used to mix the cellular components with labeling reactants, affinity probes, inhibitors, etc. We foresee that the generic nature and enabling capabilities of TDLFP will speed up the maturation of chemical cytometry into a practical bioanalytical tool.

Keywords Single-cell analysis · Chemical cytometry · Cell lysis · Transverse diffusion of laminar flow profiles

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Introduction

Cytometry is a collective term for techniques used in studies of single cells. Three major cytometry techniques are image cytometry, flow cytometry, and chemical cytometry. Chemical cytometry uses highly efficient capillary separation tools of instrumental analytical chemistry to analyze the chemical composition of individual cells. The most widely practiced mode of chemical cytometry includes the following steps: (i) cell injection into a capillary, (ii) cell lysis inside the capillary with consequent solubilization of the cellular components, and (iii) the separation of molecules of interest by capillary electrophoresis and their detection by fluorescence. Dovichi and co-authors introduced the term of chemical cytometry in 2000 [1–3], but the approach of micro-separation of single-cell contents has been known for as long as a half a century [4]. Chemical cytometry started developing intensively along with fast development of capillary electrophoresis in late the 1980s to early 1990s. For a comprehensive overview on chemical cytometry the reader is referred to the review published by Dovichi in 2003 [5]. A few important works appeared after that review. In particular, Ramsey and Zare's groups developed microfluidic chips for

chemical cytometry [6, 7]. On-chip chemical cytometry can facilitate higher throughput of analyses. Our group introduced two-channel chemical cytometry that analyzes daughter cells simultaneously [8]. Two-channel chemical cytometry revealed asymmetry between sister cells in a cancer cell line. The latter approach makes it possible to study in detail molecular mechanisms of asymmetric cell division by chemical cytometry.

Two major modes of cell lysis in chemical cytometry are used: the cell is lysed prior to cell injection into the capillary [9] and the cell is lysed after cell injection while the cell is inside the capillary [3, 10]. Lysing before the injection is performed by a laser-induced shock wave. The laser pulse, which creates the wave, is synchronized with a pulse of suction applied to the distal end of the capillary. This facilitates the injection of cell lysate into the capillary immediately after the cell lysis. Cell lysis outside the capillary has the advantage of allowing one to work with adherent cells. At the downside this approach requires sophisticated and expensive laser equipment and leaves the possibility of incomplete injection of the cell lysate. Lysis inside the capillary is simpler methodologically and instrumentally; in addition, it guarantees no losses of cellular components. It is easily applicable to suspended cells, but can hardly be used for adherent cells. Although the surfaces can be modified to suppress cell adhesion [11, 12], interfering with cell adhesion can alter cells physiology and biochemistry [13].

Four means of cell lysis inside the capillary have been described: (i) ultrasonic treatment [14], (ii) high electric field [7, 14, 15], (iii) hypotonic solution [16], and (iv) a surfactant [8, 10, 17]. Using a surfactant is a simple and efficient way as surfactants represent the best way to solubilize cellular membranes. Because a plug of physiological solution always accompanies the cell injected into a capillary, a surfactant has to be either present in the run buffer [8, 10] or injected as a separate plug immediately before or after the cell [17]. The only means of surfactant reaching the cell is its diffusion through the plug of the physiological buffer, which surrounds the cell. Although surfactant-induced cell lysis has been widely used, the diffusion of surfactant towards the cell has never been studied or optimized. Cell lysis was demonstrated in a number of works mainly through introducing the surfactants in the run buffer. The presence of the surfactant in the run buffer limits the mode of separation to micellar electrokinetic capillary chromatography (MECC). Therefore, it is important to develop a robust method of cell lysis by short plugs of a surfactant injected prior or after the cell.

Transverse diffusion of laminar flow profiles (TDLFP) has been recently introduced as a generic approach for diffusion-controlled mixing of two or more separately injected solutions inside a capillary [18]. In contrast to typical vortex-based mixing in larger-scale containers, which is impossible to accurately model, the diffusion-based mixing is simple and can be easily modeled to optimize the mixing process. Due to the laminar nature of the flow inside a capillary, the non-diffused plugs of solutions injected into a capillary by pressure have parabolic profiles with long and thin tails along the inner surface of the capillary. Therefore, there are predominantly longitudinal interfaces between the plugs, and the solutes in the serial plugs will be mixed inside capillary mainly by transverse diffusion rather than longitudinal diffusion, owing to the large ratios of the length versus diameter of the plugs (Fig. 1).

In this paper, we studied the contribution of TDLFP to cell lysis. A mild surfactant, Triton X-100, was used as a lysing agent. It was dissolved in the run buffer and introduced into the capillary in a number of ways. The results prove that TDLFP facilitates the diffusion of the surfactant towards the cell and leads to cell lysis. TDLFP is a process that can be accurately modeled, which makes TDLFP-based cell lysis predictable and suitable for rational optimization.

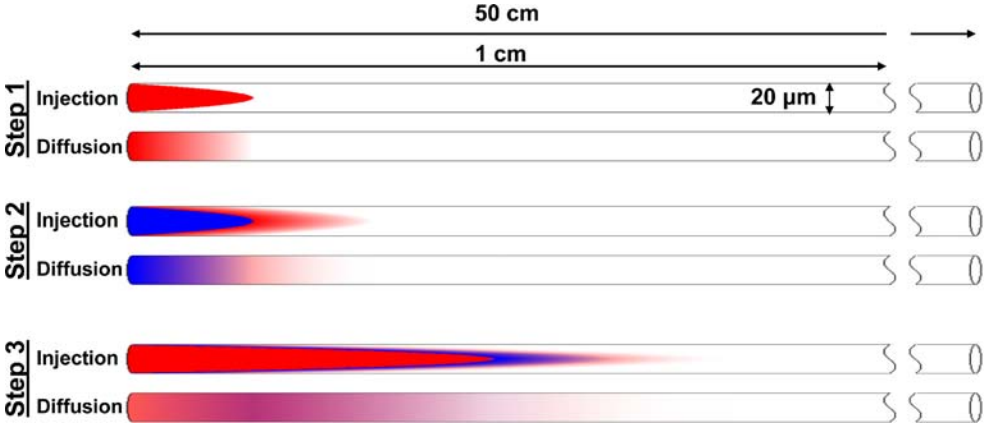

Fig. 1 Simulated mixing of two components, *blue* and *red*, inside the capillary by transverse diffusion of laminar flow profiles. *White color* inside the capillary represents the blank buffer. The volume of the last red plug in *step 3* is equal to the sum volume of the three blue plugs

Experimental

Materials

BODIPY FL hydrazide (D2371) was purchased from Molecular Probes (Eugene, OR, USA). Fluorescein sodium salt was purchased from Sigma-Aldrich (Oakville, ON, Canada). Triton X-100 and other reagents were analytical grade chemicals from Sigma-Aldrich (Oakville, ON, Canada) unless otherwise stated. All the solutions were filtered through a 0.22-μm filter (Millipore, Nepean, ON, Canada) before use.

Green fluorescence protein (GFP) stably expressing cells (Madin Darby Canine Kidney cell line, MDCK) were grown in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Burlington, ON, Canada), supplemented with 10% fetal bovine serum (FBS) (Hyclone, Toronto, ON, Canada), 100 units/mL penicillin, and 100 units/mL streptomycin (Invitrogen, Burlington, ON, Canada) in tissue culture dishes, maintained in an incubator at 37 °C with 5% CO_2 in a humidified atmosphere. When grown to 80–90% confluence, cells were detached with 0.25% Trypsin (Invitrogen, Burlington, ON, Canada) and EDTA (0.02%), washed, and single-cell suspension (10^6 cells/mL) was prepared in phosphate-buffered saline (PBS).

Instrumentation

Cell injection into the capillary, cell lysis inside the capillary, and the following CE analysis were performed with a custom-made CE instrument described in detail elsewhere [3]. The cell injection was carried out under an inverted IX-70 Olympus microscope (Carsen Group, Markham, ON). Twenty microliters of cell suspension in PBS was deposited on a microscope slide coated with PVA to reduce cell adhesion to the surface [12], and the cells were allowed to settle down. A fused silica capillary of 50 cm×20 μm ID×150 μm OD (Polymicro, Phoenix, AZ) was pre-filled with the electrophoresis run buffer: 50 mM Tris-acetate at pH 8.2 with or without 0.1% (v/v) Triton X-100. The capillary was held vertically over the microscope slide by means of a multi-functional capillary holder (Scitomix, Concord, ON), which was mounted on a long-travel 3-dimensional micromanipulator (Scitomix, Concord, ON). The base of the manipulator was fixed to the microscope body. The capillary was located in the center of the field of view of the microscope, with the tip of the capillary at a distance of 10–20 μm from the surface. A cell chosen for the analysis was superimposed with the orifice of the capillary by moving a microscope stage and injected into the capillary by a timer-controlled suction pulse or by an electroosmotic flow. After TDLFP mixing or electroosmotic injection, the capillary was immersed into the run buffer and a high voltage of 400 V/cm was applied across the capillary (positive electrode at the injection end and grounded electrode at the distal end). An intact cell or lysed cell was electrophoresed and detected with laser-induced fluorescence (LIF) at the distal end of the capillary. Fluorescence was excited by a 488-nm line of an Ar-ion laser (Melles Griot, Nepean, ON) and detected at 90° to incident light by a PMT (Hamamatsu, Japan). Fluorescence was filtered from stray and scattered laser light with a band pass filter centered at 520 nm (Omega Optical, Brattleboro, VT). The capillary was rinsed with the run buffer solution for 2 min before each run. At the end of each run, the capillary was rinsed with 100 mM HCl and 100 mM NaOH for 2 min, followed by a rinse with deionized water for 2 min.

TDLFP-based mixing

Several TDLFP mixing modes were used (Fig. 2). One-step mixing included the injection of a single cell (suspended in PBS) by a suction pulse (9.1 kPa×1 s) followed by 1-min incubation to facilitate mixing (Fig. 2a,b). Two-step mixing was realized in two ways (Fig. 2d,e). In the first way, a plug of 0.1% Triton X-100 (dissolved in the run buffer) was injected by a suction pulse (9.1 kPa×1 s) and followed by the injection of a single cell (suspended in PBS) by a suction pulse (9.1 kPa×3 s) and 1-min incubation to facilitate mixing (Fig. 2d). In the second way, a single cell (suspended in PBS) was injected by a suction pulse (9.1 kPa×1 s) followed by the injection of 0.1% Triton X-100 (dissolved in the run buffer) by a suction pulse (9.1 kPa×3 s) and 1-min incubation to facilitate mixing (Fig. 2e). For three-step mixing (Fig. 2f), the following sequence of injections was used: (i) 0.1% Triton X-100 (dissolved in the run buffer) by a suction pulse (9.1 kPa×1 s), (ii) a single cell (suspended in PBS) by a suction pulse (9.1 kPa×1 s), and (iii) 0.1% Triton X-100 (dissolved in the run buffer) by a suction pulse (9.1 kPa×3 s) followed by 1-min incubation to facilitate mixing. After each injection of Triton X-100, the capillary tip and the electrode were dipped in the run buffer to prevent the contamination of solutions, from which the next plugs were to be injected. Sixty cells in total were injected and analyzed.

Estimation of a plug size for the injection by suction

The fluorescein solution (2 nM) was continuously sucked into the 50-cm-long capillary by a differential pressure of 9.1 kPa until the capillary was fully filled (when the fluorescein solution reached the capillary outlet), and the time was recorded. Then, the suction speed was calculated by dividing the length of the capillary by the time of filling the capillary. Finally, the length of the plug injected into the capillary was calculated as the suction speed multiplied by the injection time (1 or 3 s).

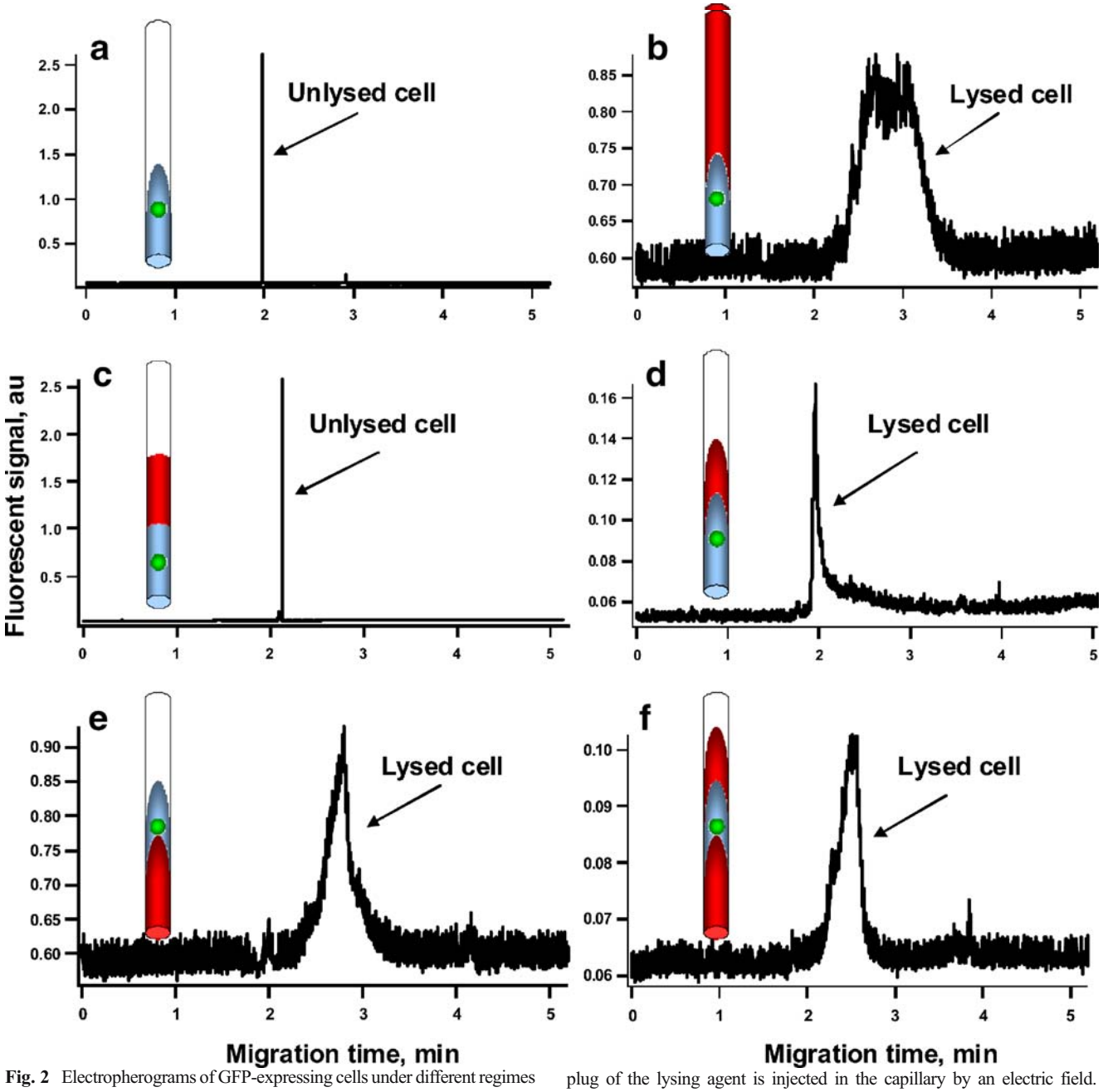

Fig. 2 Electropherograms of GFP-expressing cells under different regimes of cell injections/lysis. *Inserts* show the corresponding schemes of injection. The *white color* corresponds to the bare run buffer. The *red color* corresponds to the lysing agent (Triton X-100) dissolved in the run buffer. The *blue color* corresponds to the physiological buffer (PBS) in which the cells are suspended. The cell is shown as a *green circle*. **a** The inlet and outlet reservoirs and the capillary contain the bare run buffer. A cell is injected by suction. **b** The inlet and outlet reservoirs and the capillary contain the lysing agent. A cell is injected by suction. **c** The inlet and outlet reservoirs and the capillary contain the bare run buffer. First, a plug of the lysing agent is injected in the capillary by an electric field. Second, a cell is injected by an electric field. **d** The inlet and outlet reservoirs and the capillary contain the bare run buffer. First, a plug of the lysing agent is injected in the capillary by suction. Second, a cell is injected by suction. **e** The inlet and outlet reservoirs and the capillary contain the bare run buffer. First, a cell is injected by suction. Second, a plug of the lysing agent is injected by suction. **f** The inlet and outlet reservoirs and the capillary contain the bare run buffer. First, a plug of the lysing agent is injected by suction. Second, a cell is injected by suction. Third, another plug of the lysing agent is injected by suction

Electroosmotic injection

The inlet tip of the capillary was immersed into a solution, and an electric field of 50 V/cm×5 s was applied; the electrode was placed at approximately 5 mm from the capillary tip. A plug of 0.1% Triton X-100 (dissolved in the run buffer) was first injected at 50 V/cm×5 s. Then, a single cell (suspended in PBS) was injected under identical

conditions and incubated for 1 min to facilitate mixing (Fig. 2c). The electroosmotic mobility (μ_{eo}) was calculated from the following equation:

$$\mu_{eo} = L_d/(Et_m)$$

Here L_d is the length of the capillary, E is the strength of the field, and t_m is the migration time measured by electroosmotically pumping a neutral fluorescent marker (BODIPY FL hydrazide, 10 nM) through the capillary. The voltage and injection time were adjusted to ensure that the volumes of the fluid plugs were similar for electroosmotic and suction-driven injections.

Results and discussion

Electropherograms of intact and lysed cells

In the absence of a lysing agent, a GFP-expressing MDCK cell was not lysed in the capillary. In such a case, a very sharp spike-like peak with a migration time of 2–3 min was observed in the electropherogram (Fig. 2a). When a run buffer was supplemented with a lysing agent, the cell was efficiently lysed, and we observed a wide peak corresponding to the GFP molecules solubilized in the run buffer (Fig. 2b). These results confirmed our previous observations [3]. Thus, a spike-like peak was used as a criterion of the cell not being lysed, whereas a wide peak of GFP was used as an indication of successful cell lysis inside the capillary. If we observed a wide peak of GFP with a sharp spike on it we interpreted the result as a partial cell lysis with a part of the cell remaining intact.

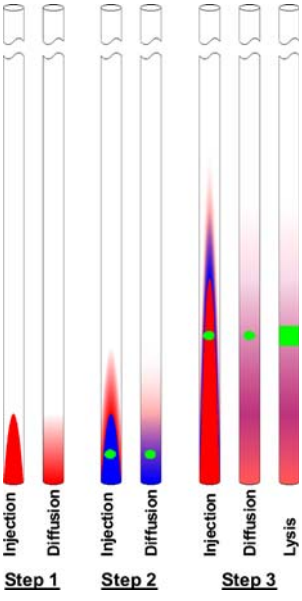

Fig. 3 Three-step mixing of a lysing agent (*red*) with a cell (*blue*) and lysis inside the capillary by TDLFP. The volume of the last red plug in *step 3* is equal to the sum volume of the three blue plugs

Table 1 Cell lysis efficiency

Mixing method setup as shown in	Cell lysis efficiency		
	Non-lysed	Partially lysed	Completely lysed
Fig. 2A	10/10	0/10	0/10
Fig. 2B	0/10	0/10	10/10
Fig. 2C	8/10	2/10	0/10
Fig. 2D	0/10	2/10	8/10
Fig. 2E	0/10	1/10	9/10
Fig. 2F	10/10	0/10	10/10

TDLFP-based mixing for cell injection

Solution plugs, which are consecutively injected into a capillary by pressure or vacuum, have parabolic rather than rectangular profiles [19]. We hypothesized that transverse diffusion of such parabolic profiles can facilitate mixing of the surfactant with the physiological buffer and facilitate cell lysis. We studied two- and three-step injections in which the plug of a lysing agent was introduced before, after, or before and after a plug of a physiological buffer with a cell. All three injection approaches facilitated cell lysis as confirmed by observing wide peaks of solubilized GFP in corresponding electropherograms.

Comparison of lysing efficiency for injection by suction and electroosmosis

The importance of TDLFP for cell lysis was confirmed by comparing suction-driven and electroosmotic injections. It is known that an electroosmotically injected plug has a rectangular profile [19]. TDLFP is, therefore, not applicable to electroosmotically injected plugs. Electroosmotically injected plugs can be mixed only by longitudinal diffusion, which should be much less efficient than TDLFP due to the high length to diameter ratios of the plugs. The following experiments were carried out to compare the contribution of TDLFP and longitudinal diffusion to cell lysis. The capillary was pre-filled with the bare run buffer. A plug of 0.1% Triton (dissolved in the run buffer) was injected either by suction or by electroosmosis prior to cell injection. A single cell was injected by suction for the TDLFP experiment and by electroosmosis for the longitudinal diffusion experiment. We found that when the surfactant and the cell were injected into the capillary by electroosmosis the cell was not lysed (only a spike was observed) in 80% of experiments (8 cells out of 10) (Fig. 2c). In 20% (2 cells out of 10) of experiments we observed partial cell lysis (wide peak of GFP with a spike on it). Thus, the longitudinal diffusion cannot efficiently "deliver" the

surfactant to the cell. In the case of suction-driven injection of the lysing agent and a cell, the cell was completely lysed in 80% of experiments (Fig. 2d). Twenty percent of experiments led to partial cell lysis. We conclude that TDLFP facilitates efficient diffusion of the lysing agent towards the cell and promotes cell lysis.

The 80% efficiency of TDLFP-driven cell lysis can be explained by the geometry of the injection device. The capillary is mounted in a vertical position, which leads to the cell's moving to the capillary inlet under the influence of the gravity. Since the plug of the lysing agent is injected prior to the cell, the gravity moves the cell away from the lysing agent and reduces the efficiency of cell lysis. To solve this problem, we injected an additional plug of the lysing agent after the cell. In such a case, if the cell goes down in the direction of the capillary inlet under the influence of gravity, it will encounter the second plug of the lysing agent (Fig. 3). Our experiments showed that this "sandwich" of the lysing agent ensured 100% efficiency of cell lysis without introducing the lysing agent into the run buffer. Table 1 summarizes the results of this work with respect to efficiencies of cell lysis by different methods of injection/mixing.

Conclusion

A single cell can be rapidly lysed inside capillary not only by adding of a surfactant in the run buffer, but also by injecting small plugs of the surfactant solution. The TDLFP-based manner of "delivering" a lysing solution to the cell facilitates fast and efficient cell lysis inside the capillary. The method is applicable for different types of cells. Just the concentration and the nature of a lysing component with lysis time need to be optimized. Additionally, this technique opens an opportunity to create a "nanoliter-volume" reactor in which a cell is not only lysed but also its contents are mixed and reacted with different types of probes or reagents (antibodies, aptamers, enzymatic substrates and inhibitors, hybridization probes, etc.). Through the following CE separation, the cellular contents can be studied quantitatively and in great detail. Potentially, protein and mRNA contents can be quantitated, enzymatic activities of proteins can be determined, and biomolecular interactions can be studied. We are currently building a high-throughput chemical cytometer which will facilitate such comprehensive analyses.

Acknowledgment The authors thank Victor Okhonin, Zhenchuan Miao, and Zhulin Pang for technical assistance. This work was supported by the Natural Sciences and Engineering Research Council of Canada: Discovery and Strategic Projects grants for SNK and an NSERC Postdoctoral Fellowship for MVB.

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