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Laminar flow mediated continuous single-cell analysis on a novel poly(dimethylsiloxane) microfluidic chip

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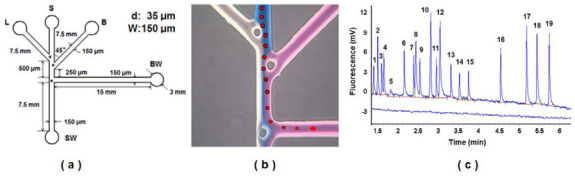
HIGHLIGHTS

- Laminar flows were used to align and introduce single cells in the microfluidic chip.
- Microcylinders in channel were used to mix laminar flows to rapidly lyse moving cells.
- DOX uptake in single cells was continuously detected in flowing streams.
- Membrane P-gp in single cells was continuously detected in flowing streams.
- The designed microfluidic chip suits to high-throughput single-cell analysis.

GRAPHICAL ABSTRACT

A novel asymmetric microfluidic device was developed for simple, convenient and high-throughput single-cell analysis based on dynamic cell manipulation in flowing streams. Single cells could be continuously introduced and rapidly lysed in microchannels with the mediation of laminar flows.



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ABSTRACT

A novel microfluidic chip with simple design, easy fabrication and low cost, coupled with high-sensitive laser induced fluorescence detection, was developed to provide continuous single-cell analysis based on dynamic cell manipulation in flowing streams. Making use of laminar flows, which formed in microchannels, single cells were aligned and continuously introduced into the sample channel and then detection channel in the chip. In order to rapidly lyse the moving cells and completely transport cellular contents into the detection channel, the angle of the side-flow channels, the asymmetric design of the channels, and the number, shape and layout of micro-obstacles were optimized for effectively redistributing and mixing the laminar flows of single cells suspension, cell lysing reagent and detection buffer. The optimized microfluidic chip was an asymmetric structure of three microchannels, with three microcylinders at the proper positions in the intersections of channels. The microchip was evaluated by detection of anticancer drug doxorubicin (DOX) uptake and membrane surface P-glycoprotein (P-gp) expression in single leukemia K562 cells. An average throughput of 6–8 cells min^{-1} was achieved. The

Abbreviations: CE, capillary electrophoresis; DOX, doxorubicin; FITC, fluorescein isothiocyanate; FWHM, full width at half maximum; LIF, laser-induced fluorescence; LOD, limit of detection; PBS, physiological buffer saline; PDMS, poly(dimethylsiloxane); P-gp, P-glycoprotein; PMT, photomultiplier tube; Re, Reynolds number; RSD, relative standard deviation; SDS, sodium dodecyl sulfate.

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detection results showed the cellular heterogeneity in DOX uptake and surface P-gp expression within K562 cells. Our researches demonstrated the feasibility and simplicity of the newly developed microfluidic chip for chemical single-cell analysis.

1. Introduction

Cells are the building blocks of life. It has been demonstrated that marked heterogeneity exists in individual phenotype, gene and protein expression in cell populations [1–4]. The traditional batch approaches report average results that would mask the difference within cells and could not reveal the real structure of cell populations [5,6]. Therefore, single-cell analysis shows special significance to uncover the heterogeneity within cell populations in various fields including system biology, biochemistry, medicine and pathology [7,8]. However, trace amount of analytes and complicated manipulation of single cells present the analysis challenging.

Several methods, such as flow cytometry [9], chemical cytometry [10–12], mass cytometry [13,14], image cytometry [15] and nanotechnology [16], were rapidly developed for single-cell analysis since 1990s. Recently, microfluidic chips [17,18] with micrometer channel showed high integration and compatibility for chemical analysis of single cells. Nevertheless, manipulation of one cell is complicated using electrokinetics on simple cross [19] or double-T [20] microfluidic chips. It also needs multi-path power supply and rewriteable manipulation software to carry out chip experiments. To simplify the manipulation, valves [21] and pumps [22] are involved within chips. However, it makes the design and fabrication of microchips more complex. Furthermore, cell immobilization and lysis under static condition greatly reduce the throughput to typical 25 cells h^{-1} or less [19,23,24], though it is much higher than that of capillary electrophoresis (CE) as 3–5 cells h^{-1} [25]. Thus, simplification and high-throughput are required to realize practical applications of single-cell analysis on microfluidic chips.

A few microfluidic devices have been reported for high-throughput chemical single-cell analysis thus far [26–29]. The throughput was greatly improved based on continuous cell introduction, lysis and detection of intracellular contents in a flowing stream. However, expensive accessories were required, such as automatic cell targeting and laser lysis system [27] which increased the cost and hindered its application. In Yin's study [28,29], two-steps etching were used to fabricate the multi-depth glass microfluidic chip, which complicated the manufacturing process.

In this study, we propose a novel polydimethylsiloxane (PDMS) microfluidic chip with simple design, easy fabrication and low cost. Single cells were aligned and continuously introduced by laminar flow into detection channels. Fast lysis of individual moving cells and cellular contents movement into the detection channel were realized through redistribution and passive mixing of laminar flows caused by the optimized design of channels and microcylinders in channels. The microfluidic system was evaluated by analysis of anticancer drug doxorubicin (DOX) uptake and membrane surface P-glycoprotein (P-gp) expression in single K562 cells. The obtained results are useful to understand the cellular pharmacokinetics and multidrug resistance mechanism in tumor cells.

2. Experimental

2.1. Chemicals

Doxorubicin hydrochloride (98.5%) and verapamil hydrochloride (99.5%) were purchased from Meilun Biology Technology (Dalian, China). Their stock solutions were prepared as 1 mg mL^{-1} in water, stored at −20 °C in the dark. Sodium borate decahydrate (Borate), NaCl, KCl, KH_2PO_4, Na_2HPO_4, $CaCl_2$ and $MgSO_4$ were purchased from Bodi Chemical Holding (Tianjin, China). SDS was purchased from Aladdin Chemistry (Shanghai, China). Eosin Y disodium salt (China National Medicines Co., Ltd, Shanghai, China) and trypan blue (Biosharp, Hefei, China) were used as dyes. The physiological buffer saline (PBS) solution for cells washing and preserving was composed of 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 and 1.47 mM KH_2PO_4 at pH 7.4. The BS10 buffer was composed of 10 mM borate and 10 mM SDS at pH 9.3. The S50 cell lysis solution was 50 mM SDS in water. All chemicals were of analytical grade unless otherwise indicated. Water was deionized water from a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Instruments

The schematic diagram of the home-built system of microfluidic chip stage coupled with laser-induced fluorescence (LIF) detection for continuous single-cell analysis is shown in Fig. S1. The system was constructed with optical apparatuses purchased from Zolix Instruments (Beijing, China). The microfluidic chip was put into an invented chip holder and stabilized on an X–Y translational platform. The laser beam was focused by a lens into the chip detection channel with an incident angle of 35°. The emitted fluorescence was collected by an objective lens and detected by a photomultiplier tube.

2.3. Chip fabrication

Microfluidic chips were fabricated with PDMS using soft lithography method [30]. The master mold used for channels was a silicon wafer fabricated in the cleanroom facility using conventional photolithography with an AZ-50XT photoresist (AZ Electronic Materials Corp., NJ, USA). A 10:1 v/v mixture of PDMS (RTV615A/RTV615B, Momentive Performance Material Inc., Waterford, NY, USA) prepolymer and curing agent were casted against the master and the PDMS was cured in an oven at 78 °C for 3 h. The negative replica of the master was peeled off carefully from the master. After liquid receivers in the PDMS chip were punched, the PDMS chip and a microscope slide (Cltoglas, Citotest Labware Manufacturing Co., Ltd, Haimen, China) were all pretreated by an oxygen plasma for 60 s in a plasma cleaner (PDC-M, Mingheng Technology Co., Ltd, Chengdu, China). After that, the chip and the slide were brought together immediately for irreversible sealing.

2.4. Chip structure optimization

Designs for channels and micro-obstacles in channels were created using CorelDraw 12.0 software (Corel, Ottawa, Canada). The cell loading channel, the side-flow channels and the detection channel were 150 μm in width and 35 μm in depth. Liquid receivers with 3 mm diameter were drilled at the terminal of the channels. The arrangement of channels and the shape, number and layout of micro-obstacles in channels were optimized. Trypan blue in PBS solution and Eosin Y disodium in BS10 buffer were used to visualize flow streams in the chip. Light images were obtained from a CCD camera (A620, Canon, Tokyo, Japan) mounted on an inverted microscope (CKX41, Olympus, Tokyo, Japan).