MICROFLUDIC CHIP WITH THREE-DIMENSIONAL HYDRODYNAMIC FOCUSING FOR HIGH-THROUGHPUT SINGLE-CELL ANALYSIS WITH CONTINUOUS CELL INTRODUCTION AND RAPID DYNAMIC LYSIS

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ABSTRACT

A cross microchip with three sheath-flow channels located on both sides of and below the sampling channel was developed for high-throughput single-cell analysis. The sample stream containing labeled carcinoma cells is constrained by the underneath sheath-flow stream to an upper layer, thus avoiding channel blockage by adhered cells. With electric field applied on the separation channel, the aligned cells were continuously driven into the separation channel and rapidly lysed within 400 ms by sodium dodecylsulfate. Subsequently, L-Glutathione and reactive oxygen species in single cells were electrophoretic separated and detected by LIF. The maximum rate of 151 cells·min^{-1} was achieved.

KEYWORDS: Single-cell analysis, Microchip electrophoresis, Three-dimensional hydrodynamic focusing , High- throughput

INTRODUCTION

Single cell analysis has become one of the frontiers in analytical chemistry. However, most cells tend to adhere to the surface of plastic or glass materials [1,2], the cell throughput in most reported microfludic devices was rather low and therefore limited their practical applications. Cell manipulation has become a critical issue to realize high-throughput microfluidic cell analysis. Herein, a simple three-dimensional (3D) hydrodynamic focusing microfluidic device integrated with continuous sampling, rapid dynamic lysis, capillary electrophoretic separation and laser induced fluorescence (LIF) detection is reported for analysis of intracellular constituents in single carcinoma cells. In the proposed approach, the sample stream is initially compressed in the vertical direction by a sheath-flow below and then focused in the horizontal direction by two sheath-flows by hydrodynamic force. The blockage of the sampling channel by adhered cells is eliminated. It has been demonstrated that 3D hydrodynamic focusing produced by the sheath-flow buffer containing lysis solution not only enable the cells to-sequentially and smoothly steer into separation channel but also ensure rapid chemical and electric cell lysis at the entry of the separation channel. A high throughput single-cell analysis was achieved by the proposed method.

EXPERIMENTAL

The schematic diagram of the experimental setup is shown in Fig. 1. It consists of a microfludic chip, a home-built confocal microscope LIF system and a single high voltage power supply. The chip consisted of a double layer glass structure as shown in Fig.1a-b. After a photolithographic and wet chemical etching procedure described elsewhere[3], the chip was assembled by sealing the two glass layer together under microscope. The channel between the sample reservoir (S), sheath-flow 1 reservoir (SF_1), sheath-flow 2 reservoir (SF_2), sheath-flow3 reservoir (SF_3) and sample waste reservoir (SW) was used for sampling and 3D focusing of cells. The channel between the buffer reservoir (B) and the buffer waste reservoir (BW) was used for cytolysis and separation. Separation buffer with volumes of 100, 100 and 150 μL was added to reservoirs B, BW and SF_3 each. Then 130 μL 1% SDS-separation buffer was added to reservoirs SF_1 and SF_2, respectively. 130 μL of the labeled cell suspension was added to reservoir S. While reservoir SW was kept empty. Owing to the difference in the liquid levels of the reservoirs, the cell suspension would flow from S to SW, while focused by three pieces of sheath-flow SF_1, SF_2, SF_3 under hydrostatic pressure. A voltage was applied to the two reservoirs with B at 3500V and BW grounded. Electroosmotic flow (EOF) produced by the potential applied along the separation channel would drive the cells continuously into the separation channel from the sampling channel. At the same time, the cells would be lysed at the entry of the separation channel by electric force and SDS added in the sheath-flows. The labeled intracellular ROS and GSH immediately steered into the separation channel, separated by electrophoresis and detected by LIF. Simultaneously, the data acquisition and processing system was activated to record electropherograms.

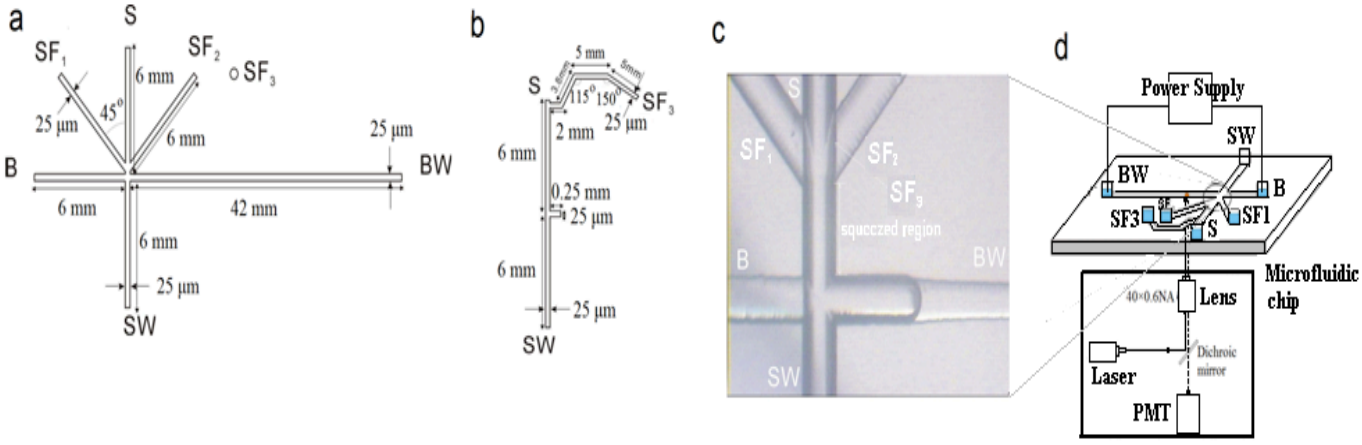

Figure 1 Schematics of (a) channel design of the top layer and (b) channel design of the bottom layer (c) photograph of the cross section of the microfluidic chip (d) experimental setup

RESULTS AND DISCUSSION

Under hydrostatic pressure generated by adjusting liquid levels in the reservoirs, the sample stream containing labeled cell suspension is constrained by the sheath-flow stream below (SF3) to an upper layer isolate from the channel bottom, thus avoiding channel blockage by adhered cells. Moreover, the cells were hydrodynamically focused by the two horizontal sheath-flow streams (SF1 and SF2) and sequentially introduced into the cross section of the microchip as shown in Fig.2a. With electric field applied on the separation channel B-BW, the aligned cells were driven into the separation channel (Fig. 2b). As shown in Fig.2b, the electrophoresis medium in the separation channel was composed of a mixture of sheath-flow solution and phosphate buffered saline (PBS) in cell suspension and only a part of the sample stream was driven into the separation channel. The PBS concentration in the electrophoresis medium could be greatly diluted by the sheath-flow solution, thus reducing Joule heating during electrophoresis[4].

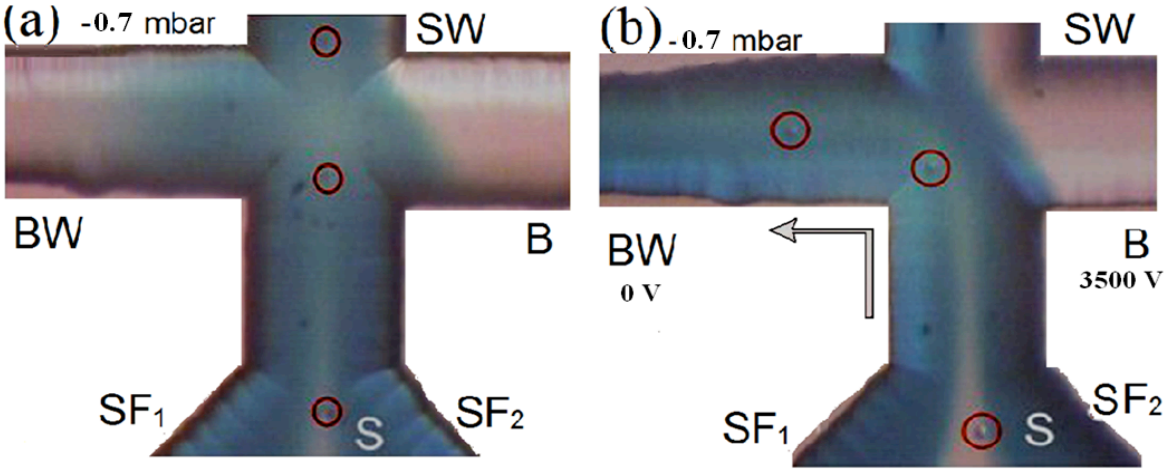

Figure 2 CCD images showing sample flow hydrodynamically focused by sheath flow streams (a) a hydrostatic pressure of-0.4 mbar applied on SW (b) -0.4 mbar on SW combined with an electric field of 729 V/cm applied between B and BW. Sheath flow stream is visualized by dissolving 2% brilliant blue in a borate buffer solution.

By adding 1% sodium dodecylsulfate (SDS) in sheath-flow solution, PBS surrounding the cell was quickly replaced by SDS-containing sheath-flow solution, inducing rapidly dynamic lysis within 400 ms. By optimizing the squeezed region length, individual cells could be lysed in the stream to BW immediately after entering the separation channel to achieve a complete injection of intracellular components into the separation channel (Fig.3).

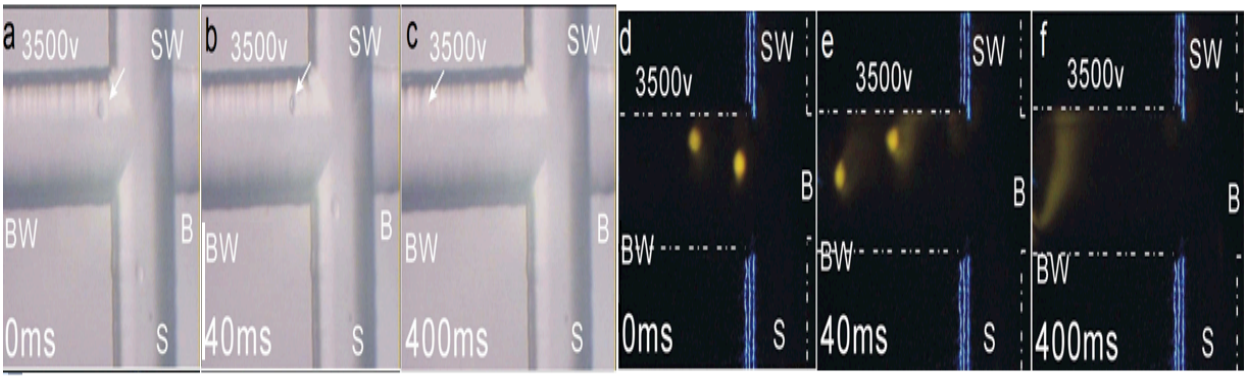

Figure 3 CCD images of cell lysing in the microchip with the squeezed region length of 0.25 mm. (a-c): light field images of cell lysing process; (d-f): fluorescent images of lysing process for cells labeled with 2 mmol·L^{-1} acridine orange*. The channels are marked by dotted lines.*

After intracellular derivatization as described early[3], ROS and GSH in single HepG2 cells were analyzed by the proposed method. Although the maximum rate for introduction of individual cells into separation channel was about 150 cells/min , the actual rate for continuous analysis of single cell was limited by the difference between migration times of ROS and GSH. To avoid peak overlaps between ROS and GSH from cells close to one another, the average analysis throughput was 16-18 cells·min^{-1} as shown in Fig.4, which is much fast than that reported previously.

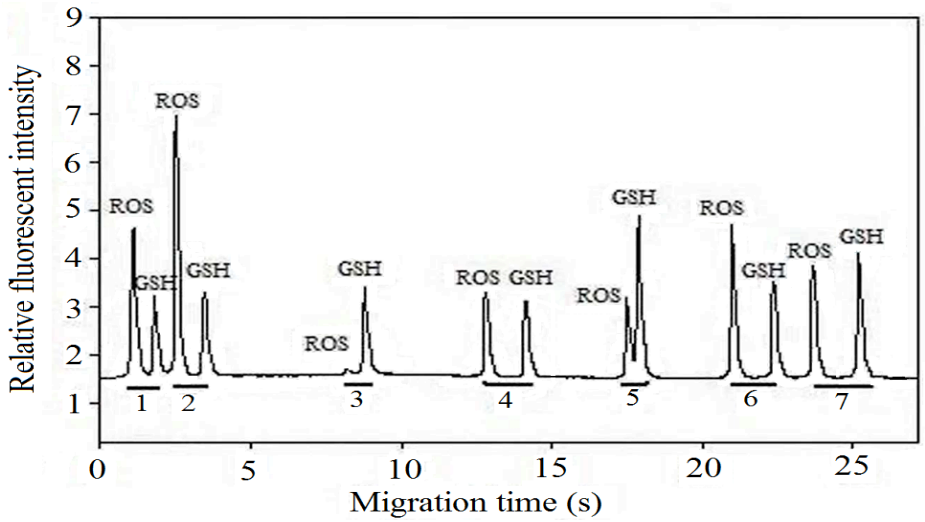

Figure 4 Electrophoretic separation of ROS and GSH released from individual HepG2 cells; E=729 V·cm^{-1}; the effective separation length is 1.0 cm; hydrostatic pressure on SW -0.7 mbar.

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