INTEGRATED MICROFLUIDIC SYSTEMS BIOLOGY PLATFORM: CELL CULTURE, DRUG TREATMENT, LYSIS, SEPARATION, AND DETECTION

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ABSTRACT

We are developing an integrated microfluidic system capable of performing online cell culturing, phenotype analysis, lysing, and analyses of the lysate for protein expression profiling in response to various drug treatments for quantitative medical analysis and systems biology.

KEYWORDS: microfluidic, cell culture, drug treatment, capillary electrophoresis (CE), protein separation.

INTRODUCTION

Microfluidic devices are playing an important role in analytical biology and medical research with the promise of high throughput, efficiency, automation, consumption of small volumes of reagents/samples, and on-chip integration of various operational modules for quantitative medicine and systems biology [1, 2]. In the post-genomic era, attention is focusing increasingly on the elements which determine cellular architecture and function to address future personalized medicine and molecular diagnostics. We are developing a microfluidic platform for monitoring changes in cellular responses to physiologically relevant perturbations and various drugs.

EXPERIMENTAL RESULTS

The system, which includes cell culture, drug exposure, lysing, reagent/label mixing, and separation channels, was fabricated using standard photolithographic techniques in polydimethylsiloxane (PDMS) substrates bonded to PDMS-coated or bare glass wafers (100 mm diameter). A linear array (1 × 4) of the system is shown in Fig. 1A with the insets (B-E) depicting the details of the various compartments. The system was fabricated with the potential of directly interfacing to an electrospray ionization mass spectrometer for detection, as shown in Fig. 1C.

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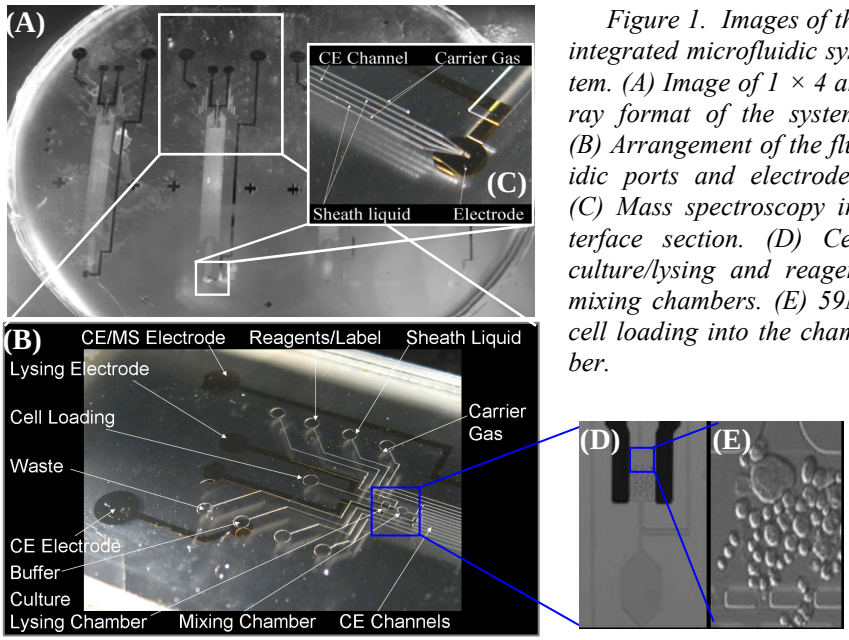

Figure 1. Images of the integrated microfluidic system. (A) Image of 1 × 4 array format of the system, (B) Arrangement of the fluidic ports and electrodes. (C) Mass spectroscopy interface section. (D) Cell culture/lysing and reagent mixing chambers. (E) 59M cell loading into the chamber.

Functionalities of the components were successfully tested. Cells were loaded hydrodynamically in poly-L-Lysine-coated culture chambers. After loading, the cells were allowed to adhere/grow for 24 hours while medium was perfused continuously through the chamber (Fig. 2) and cell viability was tested using Calcein AM probe. The cultured cells were treated with the desired drug. After dose-dependent pre-treatment, morphological variations in the cells were recorded by optical imaging.

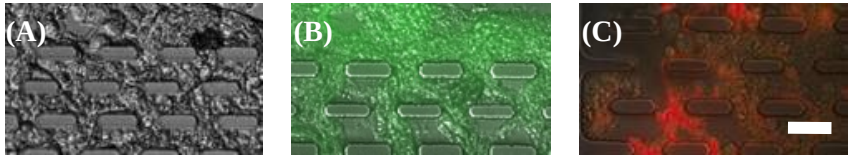

Figure 2. Cell culturing and drug treatment of HeLa cells. (A) 24 h after loading the cells. (B) Calcein AM viability test after 24 hours. (C) Propidium iodide-stained HeLa cells showing the effect of anti-cancer drug after 24 hours of incubation with 34 μg/mL Paclitaxel in minimum essential medium (Eagle media) at 37 C. Scale bar: 40 μm.

Lysis of the pretreated cells was done either chemically or electrochemically. For electrochemical lysis, a pair of Au/Pt electrodes was provided in the chamber to generate hydroxide ions through electrolysis using low-voltage

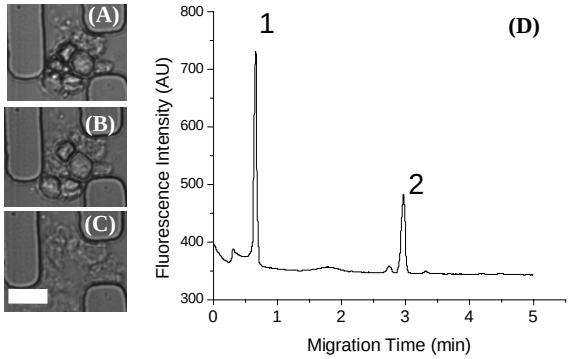

Figure 3. Lysis of M59 cells. Exposure to 0.1 M NaOH, images after (A) 5 s, (B) 10 s, (C) 25 s. Scale bar: 20 μm. (D) An electropherogram of FITC and fluorescein (each 10 μM). Run buffer: 20 mM boric acid/NaOH, 0.05% Sodium dodecyl sulfate, 0.1% n-dodecyl-β-D-maltoside, pH 10. Electrokinetic injection: 5 s, anodic end 60 V/cm, applied voltage 300 V/cm, detection at 5 cm. Fluorescence detection: ANDOR CCD camera. Electropherogram extracted from the images (100 ms). Peak numbering: 1, FITC; 2, Fluorescein.

(2 V) DC power [2]. Cells exposed to the hydroxide ion flux showed disintegration of the cell membrane and release of their cellular contents (Fig. 3A-C).

Online sample preparation was achieved by mixing standard solutions in a mixing chamber. The sample was transferred to a subsequent electrophoresis channel and separation of the analyte mixture was performed with capillary electrophoresis (CE, 300 V/cm) in zone-electrophoresis mode. The separated zones were optically detected and their concentration profiles were obtained as electropherograms (Fig. 3D).

CONCLUSIONS

An integrated microfluidic systems biology platform has been fabricated for high-throughput drug treatment and molecular diagnostics of cells. Work is in progress to fully functionalize the integrated system in order to study the effect of K^+ channel blockers on ovarian cancer cells. The aim is to quantify dose-dependent expression of proteins such as p53, p21, and Bax in response to K^+ channel blockers such as tetraethylammonium ion, tetrandrine, and 4-aminopyridine [3].

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