A CELLULAR MICROARRAY PERFUSION SYSTEM FOR CHEMO-DRUG SCREENINGS

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ABSTRACT

With low efficiency of chemotherapy for some patients, there is an increasing need for predicting the outcome of a treatment by performing *in vitro* cellular drug screenings. In order to promote a prediction of drug responses, cellular microarray—a powerful tool for high throughput screening—has been targeted to meet the need. Here, we provide a dielectrophoresis (DEP)-based cellular microarray perfusion system tailoring to uniform cell patterning and stable drug perfusion for anticancer drug screenings. The tailored collagen-coated *planar interdigitated ring electrode* (PIRE) arrays for dielectrophoretic cell patterning (DCP) resulted in uniform cell count on a PIRE (92±5 cells per PIRE) among chambers; after DCP, around 91% of patterned cells (84±5 cells per PIRE) remained for following drug perfusion. Besides, the corporative work of a linear concentration gradient generator (CGG) and an anti-crosstalk valve (ACV) was experimentally verified to prevent an unwanted crosstalk of drug concentrations for generating stable drug concentrations. Moreover, observations of cells during 24-hour drug perfusion showed: a viable microfluidic perfusion environment for drug screenings; and a good correlation between cell viability and drug concentration. Our cellular microarray perfusion system would supplement microfluidic perfusion model for building up a correlation to *in vivo* drug screenings for improving efficacy of chemotherapy.

KEYWORDS: Cellular Microarray, Perfusion Culture System, Uniform Cell Patterning, Concentration Gradient

INTRODUCTION

With low efficacy of chemotherapy for some patients, there is an increasing need for predicting the outcome of a treatment by performing *in vitro* drug screenings [1]. Cellular microarray, a powerful tool for high-throughput drug screening, has been targeted to meet this need [2].

In this paper, we provide a perfusion system of cellular microarray for a chemo-drug screening using microfluidics (Fig.1). Features of the system include: (1) applying pressurized air to eliminate bubbles inside PDMS microchannels (Fig.1a) for a bubble-free perfusion [3], (2) a PDMS anti-crosstalk valve (ACV) (Fig.1b-c) to prevent an unwanted crosstalk of drug concentrations during drug perfusion. During drug perfusion, ACV was driven with a hydraulic pressure of 20 psi to blocking the connection holes for preventing an unwanted crosstalk via the manifold channels. Bright-field images (middle panel) and green fluorescent images (lower panel) demonstrates the operation of ACV. (3) *planar interdigitated ring electrodes* (PIRE) to uniformly pattern cells [5] for the repeatability of drug screenings.

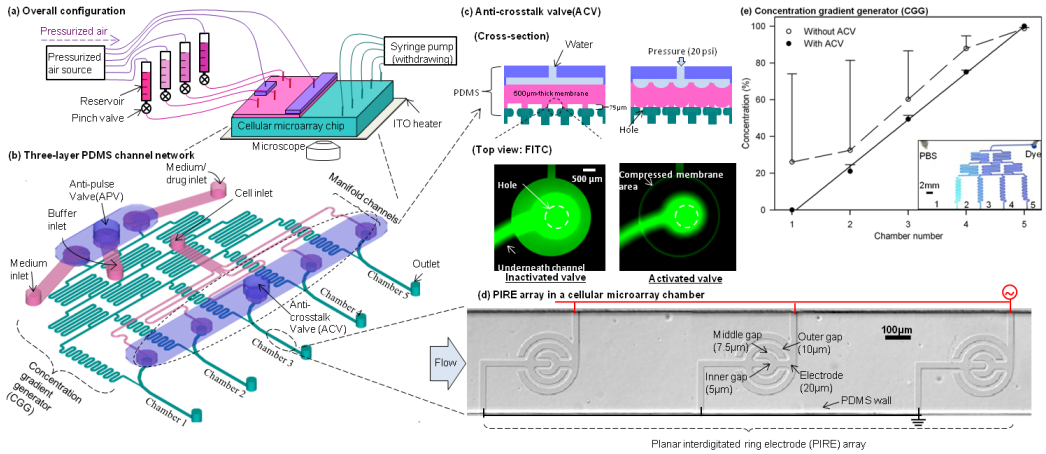

Figure 1. A cellular microarray perfusion system for chemo-drug screenings. (a) The overall perfusion system. (b)Solutions were introduced into the chip through inlet channels (pink color, in the left); cells were seeded into the chip through the manifold channels (pink color, in the middle). A hydraulic pulse was moderated by the pressure-driven anti-pulse valve (APV) (blue color, in the left) to keep flows in channels steady. A stable drug concentration gradient generated by concentration gradient generator (CGG) (green color, in the left) flowed to cellular microarray chambers (green color, in the bottom). The pressure-driven anti-crosstalk valve (ACV) (in the bottom, blue color) was used to preventing an unwanted fluidic crosstalk during drug perfusion. The right panel is the final product of the chip. (c) The working principal of the pressure-driven ACV. (d)The 1x3 planar interdigitated ring electrode (PIRE) designed for dielectrophoretic cell patterning (e) A characterization of concentration gradient profiles after 24 hours of perfusion. Without driving ACV, no significant concentration gradient was found; with driving ACV, a significant stable linear concentration gradient was acquired. Data were means ± standard deviations of three measurements.

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FABRICATION

Microfabrication of the cellular microarray chip utilized a combination of photolithography, reactive ion etching (RIE), soft lithography, and collagen coating (Fig. 2). The procedures were reported in our previous work [3]. After the fabrication, the chip was stored at 4℃ prior to use.

EXPERIMENTAL

The operations of the system were divided into four stages (Fig.3). In stage I, a pressure (7 psi) was applied to eliminating bubbles inside the PDMS chip [3]. In stage II, MCF-7 (a breast carcinoma cell line) suspended in a low-conductive buffer (0.5mM HEPES, 301.3mM sucrose, 1.5% FBS; pH7.0, 341.5mOsm, 2.29×10^{-2}S/m) was introduced into microchambers (Fig.1d). An AC signal (5 Vpp, 10 MHz) was applied to PIRE arrays to generate dielectrophoretic (DEP) forces to pattern cells on collagen-coated PIRE arrays. After cell patterning, the buffer was exchanged for culture medium (DMEM/F12, 10% FBS, 1% penicillin/streptomycin). During the solution exchange, a hydraulic pulse would be caused to deteriorate cellular patterns. Therefore, a pressure-driven anti-pulse valve (APV) (Fig.1b) was gradually activated to preserve cellular patterns. Then, cells were kept static for 3 hours. In stage III, a linear concentration gradient (0, 10.5, 24.7, 37.5, and 50 μg ml^{-1}) of cisplatin (a chemotherapeutic drug) was generated by a concentration gradient generator (Fig.1b), then introduced at 0.1μg min^{-1} to treat cells for 24 hours. During drug perfusion, a pressure-driven ACV (Fig.1c) was applied to prevent unwanted crosstalk for sustaining stable drug concentrations. In stage IV, the viability of treated cells was quantified by a dual staining.

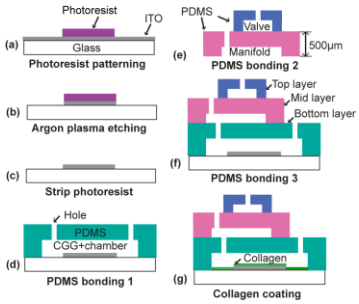

Figure 2.Chip microfabrication process with collagen coating.

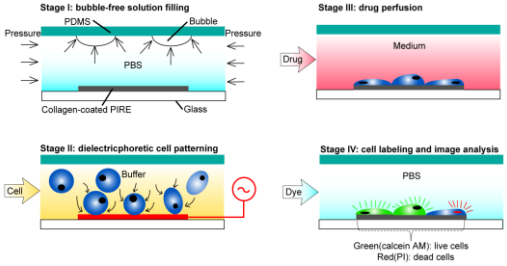

Figure 3.Operating process of the system.

RESULTS AND DISCUSSION

Anti-crosstalk valve (ACV) and concentration gradient generator (CGG) for a stable drug perfusion is shown in Figure 1e. The pressure-driven ACV, locating atop of the connection holes between the manifold channels and CGG, was mainly featured with a 500 μm-thick PDMS membrane (see the cross-sectional schematic in upper panel of Fig. 1c).

Figure 4 shows cells uniformly patterned on a 1×3 collagen-coated PIRE array in a chamber. Cells covered an entire PIRE, not only the edges. About the distribution of patterned cells in chambers (cell-seeding density was about 1×10^{7} cells ml^{-1}) (Fig. 4a), the mean cell count on a PIRE showed a good overall uniformity, 92±5 cells (coefficient of variation (CV): 5.4%), among chambers(Fig. 4b). Morphological observations of cells during 24-hour drug perfusion are shown in Figure 5a. After solution exchange, most of patterned cells well attached to the substrate. Some cells migrated from PIREs to surrounding areas. Cells were treated with a linear concentration gradient (0, 12.5, 25, 37.5, 50 μg ml^{-1}) of cisplatin with a perfusion rate of 0.1 μl ml^{-1} (channel width: 300 μm, height: 60 μm) for 24 hours. At t=24hr of drug perfusion, a polygonal cell morphology and a significant cell proliferation were found in chambers with lower drug concentrations (i.e. 0, 12.5 and 25 μg ml^{-1}); while a spherical cell morphology and a significant decrease in cell amount found in chambers with higher concentrations (i.e. 37.5 and 50 μg ml^{-1}). After staining, live cells and dead cells were indicated by a green dye (calcein AM) and a red dye (propidium iodide), respectively. The composites of fluorescent and bright-field images showed a good correlation between cell viability and drug concentration. Results proved a viable microfluidic perfusion environment for drug screenings.

Toxicity profiles of cisplatin in the cellular microarray perfusion chip and 96-well plates obtained after 24-hour drug treatment are presented in Figure 5b. Cell viabilities in the chip after perfusion with different concentrations were normalized against that of untreated cells. IC_{50} from the chip (37.5μg ml^{-1}) was higher than that from 96-well plates (7.0μg ml^{-1}) (Fig.5b) which may be caused by an unwanted PDMS absorption of cisplatin in the chip resulting in a lower effective drug concentration [5].

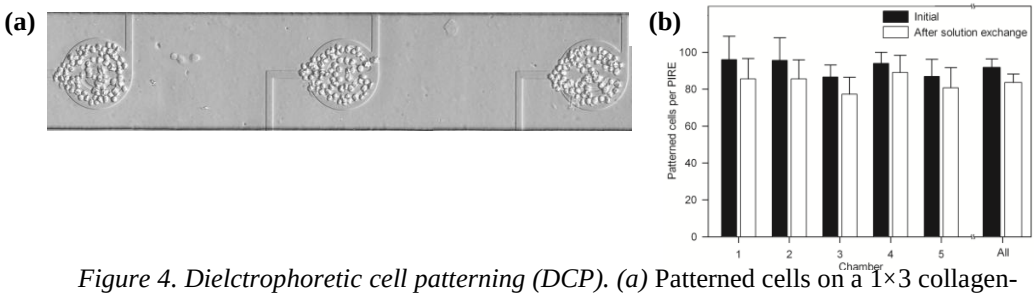

Figure 4. Dielctrophoretic cell patterning (DCP). (a) Patterned cells on a 1×3 collagen-coated PIRE array. *(b) Cell count of a PIRE.*

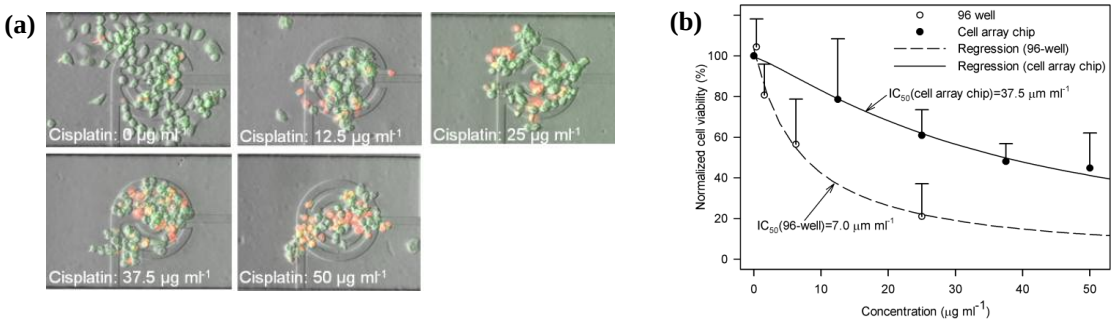

Figure 5.Chemo-drug screenings. (a) Cells were treated with a linear concentration gradient for 24 hours. (b) IC_{50} from the chip(37.5μg ml^{-1}) and from 96-well plate(7μg ml^{-1}).

CONCLUSION

A DEP-based cellular microarray perfusion system tailoring to uniform cell patterning and stable drug perfusion was put forth for anticancer drug screenings. The tailored integration of PIRE arrays and a microfluidic perfusion configuration resulted in a uniform cellular microarray and stable drug perfusion. During drug perfusion, a good correlation between cell viability and drug concentration was affirmed; furthermore, the difference in IC_{50} value between the chip and 96-well plates may be caused by an unwanted PDMS absorption of cisplatin in the chip. The combinatory work of moderate electric fields of DCP and a stable drug perfusion may be the key to the success. However, this microfluidic perfusion system cannot work for drug cocktails screening. Our cellular microarray perfusion system would supplement microfluidic perfusion model for building up a correlation to *in vivo* drug screenings for improving efficacy of chemotherapy in the years to come.

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