DRUG RESISTANCE OF A SINGLE MOUSE MELANOMA CELL AND ITS INTERACTION WITH A MOUSE DENDRITIC CELL STUDIED USING THE MICROFLUIDIC SINGLE CELL BIOANALYZER

ABSTRACT
The melanoma cell is relatively drug resistant and one of the mechanisms is drug efflux from the cancer cell. We have developed a single cell bioanalyzer to confirm the reversal of drug efflux by an inhibitor compound, leading to the enhancement of drug accumulation, on the murine melanoma cell (B16OVA). A second therapeutic approach is the tumor-targeted immunotherapy, based on the use of the dendritic cell (DC), which has been used to treat melanoma patients. Here, the B16OVA cell has been used as a tumor cell model to determine in what way the murine dendritic cells (DC2114) interact with it.

INRODUCTION
Queensland in Australia has the highest incidence rate of melanoma in the world and novel treatment approaches are needed [1]. Melanoma is highly drug resistant, and the drug efflux from the cancer cells leads to low drug accumulation, a phenomenon termed as multidrug resistance (MDR). The efflux is due to the drug-pumping action of transporter proteins on cell membranes, and MDR inhibitor compounds have been applied to reverse efflux, thus enhancing the drug accumulation [2]. Another therapeutic strategy is to make use of dendritic cells (DC), which have a unique antigen presenting ability, for presenting tumor-specific antigens to the patient’s immune system so that specifically activated T cells can kill the cancer cells [3].

MATERIALS AND METHODS
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Fabrication of the microfluidic biochip
The biochip was made of a polydimethylsiloxane (PDMS) slab (Fig. 1) that was sealed to a 0.17-mm glass cover slip [6]. Reservoirs 1 & 2 serve as the inlet & waste ports, respectively; whereas, reservoirs
3-5 are used for drug delivery. The cells were selected by hydrodynamic liquid flow [7].

**Cell lines and reagents**

B16OVA is a murine melanoma cell line developed to include the artificial tumour antigen ovalbumin [8]. The murine DC2114 cell line was developed and used as the dendritic cell model [9]. Daunorubicin (DNR) and cyclosporine A (CsA) were obtained from Sigma-Aldrich (St Louis, MO).

**On-Chip Drug Accumulation Study**

After the retention of a B16OVA cell, simultaneous optical observation & fluorescent measurement were conducted [10]. During this, the chip was moved up and down across the detection aperture window to get the signals for the cell and the background (when inside the detection window, the cell’s total fluorescence was measured; the background signal was measured when it was outside the window) [11].

**RESULTS AND DISCUSSION**

**Drug accumulation study**

Drug accumulation of DNR in a single B16OVA cell was measured. In order to overcome the issue of cellular variations by measuring many cells, the drug accumulation study was performed in which the same cell was used as both the control and test cell using SASCA-A. Fig. 2a shows DNR accumulation measured in a single B16OVA cell treated with the drug in the absence and presence of CsA. The initial drug accumulation after adding DNR alone was observed in a low fluorescent intensity, an obvious slope transition in the curve occurred after adding DNR solution containing CsA to the same single cell, causing the single cell fluorescence to increase by 2.2 fold. The morphologies of this MDR cell were shown, indicating some changes in the cell membrane integrity (Fig. 2b). Such an experiment had been repeated a few more times.

**Cell interaction study**

After the DC2114 cell was trapped, the melanoma cell B16OVA was brought in close proximity to it. Fig. 3 shows the B16OVA stained in red by the PKH26 membrane dye. It was observed B16OVA interacted with multiple DC2114 cells (green). The time-course changes of the interactions of an attached DC2114 cell and a stained B16OVA cell have been followed.

**CONCLUSION**

The microfluidic SASCA-A method has provided time-dependent drug transport in single B16OVA cells as well as the cell morphological information. The B16OVA cell was found to be multidrug resistant, with low initial daunorubicin (DNR) accumulation. Treatment of the melanoma cell with DNR as the anti-cancer drug in the presence of CsA enhanced drug accumulation notably. Furthermore, only a small amount of cells and reagents are needed to confirm the findings. Selection and attachment of mouse melanoma (B16OVA) cells was achieved in a
Selection and attachment of mouse dendritic cells (DC2114) was observed. Initial interaction between two cells were observed. Such a cell interaction study may provide useful information about the antigen-presentation of dendritic cells.

**Fig. 2.** Drug accumulation on a single B16OVA cell. (a) Slope transition occurred after using CsA as an inhibitor. (b) Cell images before experiment (b1) and after treating cell with only DNR (b2), with DNR and CsA (b3), after experiment (b4).

**Fig. 3.** Images of B16OVA cells (stained by PKH26 in red) interacting with DC2114 cells, which constitutively expressed green fluorescent protein (GFP).

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**References**


