INTEGRATED PERFUSION CULTURE MICRO-CHAMBER ARRAY CHIP FOR HIGH-THROUGHPUT DRUG DOSE RESPONSE ASSAY

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ABSTRACT

We report a microplate-sized integrated perfusion culture microchamber array chip for high throughput drug dose response assays. The microchamber array chip was equipped with a pressure-driven interface, in which solutions were delivered into the microfluidic network by pneumatic pressure, and with a serial dilution microfluidic network, which generated a logarithmic concentration profile. The microchamber array chip is composed of an array of 384 microchambers for parallel quadruplicate assays using 12 drugs with 8 varying concentrations. The perfusion culture microchamber array chip, with its simple interface and well-designed microfluidic network, will likely become an advantageous platform for future cell-based assay.

KEYWORDS: Cell-based assay, Perfusion culture microchamber array, Serial dilution, Dose response assay

INTRODUCTION

In the drug discovery process, a cell-based assay is one of the key assay technologies. Espescially, a cell-based drug dose response assay is often used to analyze the 50% growth inhibitory concentration (IC_{50}) or 50% effective concentration (EC_{50}). Needless to say, preparation of a large number of solutions with different concentrations is tedious and time-consuming work. In addition, the dilution process sometimes causes experimental errors, since serial dilution using a micropipette can induce accumulation of dilution errors. Microfluidic technologies are espected as promising candidates to miniaturize assays and to increase experimental throughput and reliability in drug discovery applications [1].

Recently, several research groups have reported the use of perfusion culture chips equipped with a microfluidic concentration profile generator. However, most of the generated concentration profiles are linear, and there are no reports about the use of such a generator to generate a wide concentration range, e.g., 6 orders of magnitude, which is indispensable for the pharmacological drug dose response assay. We have reported the perfusion culture microchamber array chip for high-throughput cell-based assay [2], and serial dilution microfluidic network for generating a arbitraly monotonic concentration profile [3]. More recently, we combined these technologies and developed the perfusion culture microchamber array chip equipped with 12 microchambers for IC_{50} assay with 12 varying concentrations, and with a serial dilution microfluidic network for generating a logarithmic concentration profile spanning 6 orders of magnitude [4].

In this paper, we report the microplate-sized integrated perfusion culture microchamber array chip, which is equipped with an array of 384 microchambers for parallel quadruplicate assays using 12 drugs with 8 varying concentrations.

EXPERIMENTAL

The integrated perfusion culture microchamber array chip with specification of the standard microplate was equipped with an array of 384 microchambers for parallel quadruplicate assays using 12 drugs with 8 varying concentrations (Fig. 1a), and with the serial dilution microfluidic network (Fig. 1b). The serial dilution microfluidic network used in this study has a structure similar to that designed in our previous study, in which the drug solution was diluted at a ratio of $10^{0.5}$ in each dilution step to create a concentration profile spanning 3 orders of magnitude in 6 dilution steps.

Perfusion culture microchamber arrays were made of polydimethylsiloxane (PDMS) and were fabricated by photolithography and replica molding using photoresist patterns as master templates [2, 3]. The macroscopic medium and drag solution reservoirs and cell-inlet/medium-outlet reservoir were fabricated in the same manner, using acrylic resin plates and rods as templates. After surface oxidization by O2 plasma using a plasma reactor, the PDMS plates and reservoirs were bonded.

Fig. 2 shows the experimental procedure for a dose response assay using the integrated perfusion culture microchamber array chip with the serial dilution microfluidic network. Cell suspension was manually introduced into the liquid reservoirs with micropippet (Fig. 2a). Microchamber array chip is fixed in the holder and cell suspension was loaded into each microchamber by applying pressure

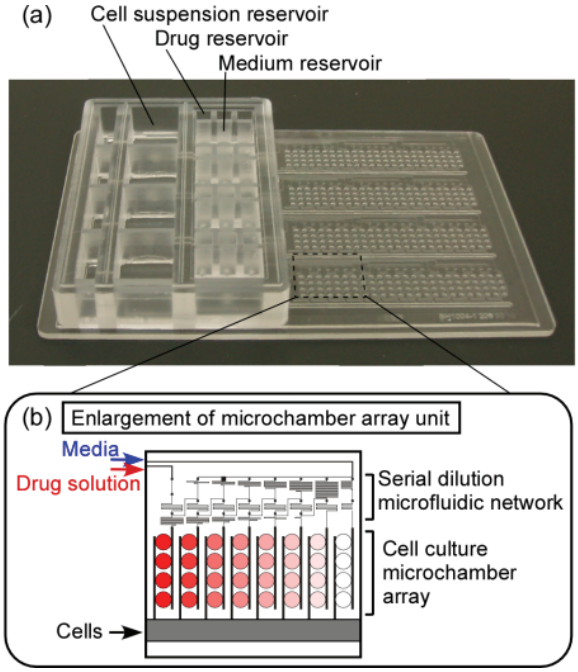

Fig. 1: Microplate-sized integrated perfusion culture microchamber array chip equipped with serial dilution microfluidic network (a) and the enlargement of a microchamber array unit (b).

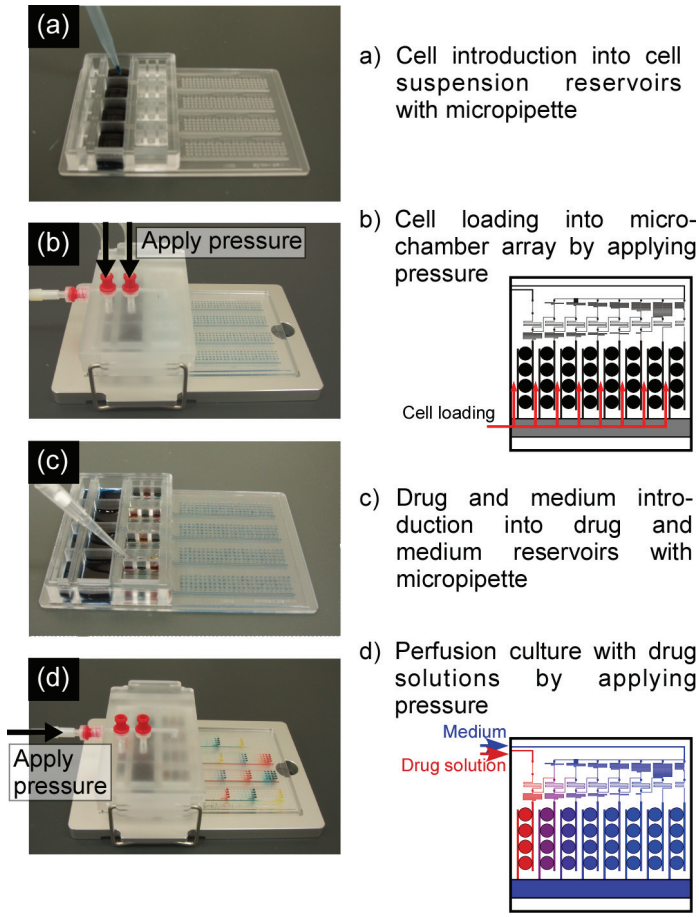

Fig. 2: Experimental procedure for dose response assay using the integrated perfusion culture microchamber array with the serial dilution microfluidic network. (a) Cell introduction into the reservoirs. (b) Cell loading into the microchambers. (c) Drug and medium introduction into the reservoirs. (d) Perfusion culture.

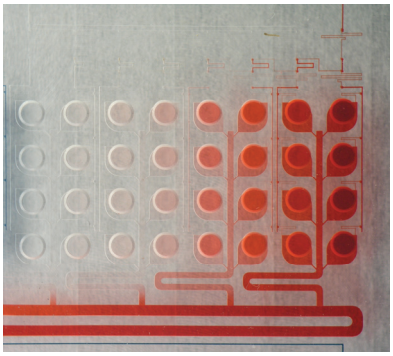

Fig. 3 Microscope photograph of the generated concentration profile in the microchamber.

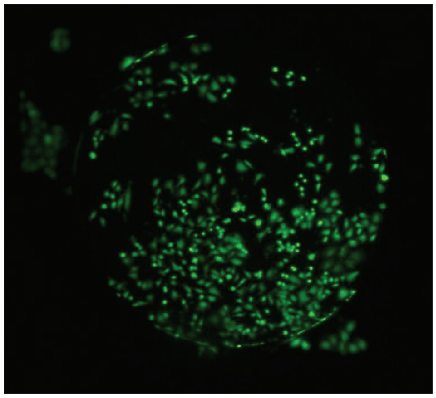

Fig. 4 Fluorescent microscope images of cell growth in the microchamber.

through air vent filter (Fig. 2b). After cell loading, drug solutions and media were manually introduced into the liquid reservoirs with a micropipette (Fig. 2c). Microchamber array chip is again fixed in the holder and perfusion culture was carried by applying pressure in the CO_2 incubator (Fig. 2d).

Human cervical carcinoma cell line HeLa was maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg of streptomycin and glutamine at 37 °C in a humidified atmosphere containing 5% CO2. The HeLa cells were harvested by trypsin and suspended in the culture medium. The cell suspension (3.5×10^5 cells/mL) was added to the cell suspension reservoir, and cells were loaded into each of the microchambers by applying 20 kPa pressure to the reservoir. The perfusion culture was carried out by applying 8 kPa pressure to the reservoir.

RESULTS AND DISCUSSION

To visualize the serial dilution microfluidic network we introduced 5 wt% new coccine (red dye) solution into the microfluidic network from the drug reservoir and water into the medium reservoir. The solutions were introduced into microchambers at an applied pressure of 20 kPa. The logalismic concentration profile is automatically generated by the serial dilution network (Fig. 3).

HeLa cells were introduced into the cell culture microchamber array from the cell suspension reservoir. After 6 hours of static culture, the HeLa cells were adhered to the bottom of the microchamber. The microfluidic network then was filled with medium, and a perfusion culture was carried out in the absence of the drug for 48 hours. The evaluation of viability of the cells in the microchamber was possible by the fluorescent image analysis by staining living cells with Calcein-AM (Fig. 4). These result indicate that the perfusion culture microchamber array chip could be useful for cell growth analysis applications, since the chip provides oxygen through the gas permeable PDMS wall and provides nutrients to the cells by means of perfusion.

The integrated perfusion culture microchamber array chip, with its simple interface and well-designed microfluidic network, will likely become an advantageous platform for future cell-based assay on microchips.

CONCLUSION

We have developed the microplate-sized microchamber array chip composed of an array of 384 microchambers for parallel quadruplicate assays using 12 drugs with 8 varying concentrations. The microchamber array chip was equipped with the pressure-driven interface and the serial dilution microfluidic network. We demonstrated the generation of concentration profile and cultivation of cancer cells using the microchamber array chip. The perfusion culture microchamber array chip, with its simple interface and well-designed microfluidic network, will likely become an advantageous platform for future cell-based assay.

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