3D Microstructures to Realize Single Cell Culture on Digital Microfluidic Chip for Precise Medicine

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Abstract

This paper reports a simple microfabrication method for single cell culture on a digital microfluidics chip. In the microfluidics field, single cell culture has been investigated with channel-based droplet microfluidics. Among the reported single cell analysis methods, microwell arrays, with high single cell capture efficiency, is the most popular method [1]. In addition, the microfluidic devices integrated with other devices, such as dielectrophoresis (DEP), optical tweezers, or acoustic waves, is also powerful to trap and manipulate single cells. However, in these studies, the cells were exposed to a limited number of stimuli and there are possibilities for products to diffuse away. With the advantage of each droplet isolated from its surroundings, simultaneous control of many reagents, and being easily integrated with other analytical techniques, digital microfluidics (DMF) has attracted attention from researchers in recent years [2]. Cell culture and cell based research have been explored [3], but there is still no report for single cell culture on a digital microfluidic (DMF) chip.

In this paper, a digital microfluidic system is designed for single cell culture on-chip for drug screening (Figure 1). Fabricated fences form virtual channels and virtual chambers restricting the droplets in certain place (Figure 2). Cells and drugs are introduced through the inlet holes and mixed on-chip with desired drug concentration before being transported to the virtual chambers for culturing. We tried four kinds of microstructures for single cell culture on the virtual chambers (Figure 4). The results suggested a high single cell trapping efficiency of the well-like array. Under well-like array conditions, we explored the effect of cell concentration on the single cell trapping efficiency. The results in Figure 5 reveal that the optimized cell concentration is $8*10^5$ cells/ml. Using Cisplatin, one kind of clinically established chemotherapeutic reagent, as model, we measured the cell toxicity to MDA-MB-231 cells on our chip and off-chip (96-well plate). The ID_{50} value (Figure 6) for off-chip is 22.6 μM, close to 5 μM on-chip with 24h as incubation time. The results suggest that the designed structures are effective for single cell trapping, and evaluation of drug toxicity over time.

Due to the simplicity of 3D microstructure fabrication on a DMF chip, droplet controllability, and high single-cell trapping efficiency, the developed method exhibits great potential for fundamental applications in precision medicine and drug response analysis at the single cell level.

References

[1] A. Khademhosseini, J. Yeh, S. Jon, G. Eng, K. Y. Suh, J. A. Burdick and R. Langer. Cell docking inside microwells within reversibly sealed microfluidic channels for fabricating multiphenotype cell arrays. Lab on a Chip, 4, 425–430, 2004.

[2] M. G. Pollack, A. D. Shenderov and R.B. Fair. Electrowetting-based actuation of droplets for integrated microfluidics. Lab on a Chip, 2, 96-101, 2002.

[3] Alphonsus H.C. Ng, Bingyu Betty Li, M. Dean Chamberlain and Aaron R. Wheeler. Digital Microfluidic Cell Culture. Annu. Rev. Biomed. Eng, 17, 91–112, 2015.

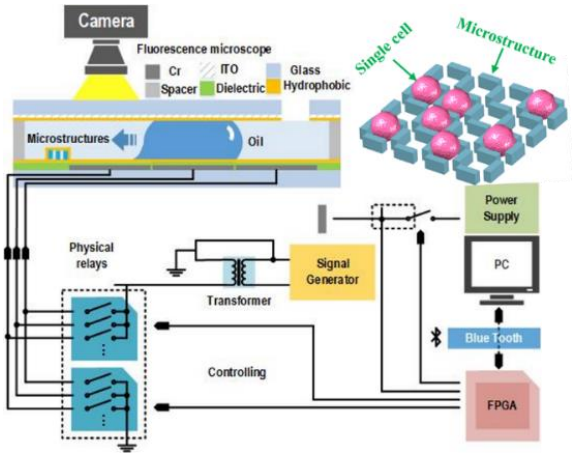

Figure 1. Side view schematic of the DMF chip (embedded with on-chip 3D microstructures for single cell culture and drug toxicity test) and electronical control system for droplet actuation. **[Enlarged Figure]**

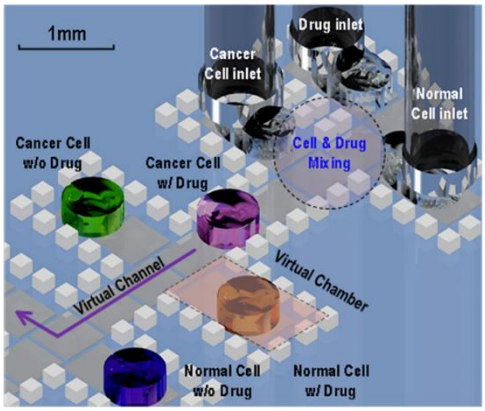

Figure 2 Schematic of the DMF chip with 3D microstructures for cell culture-based drug screening.

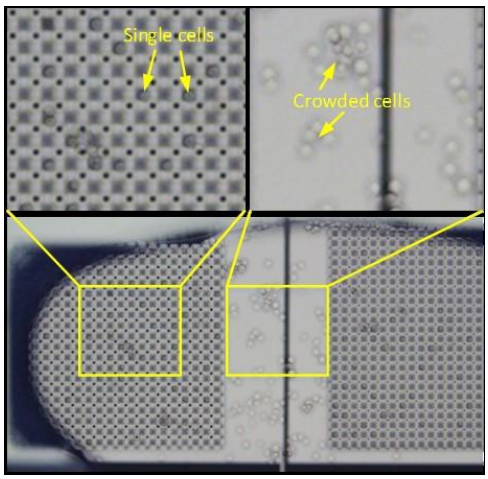

Figure 3 Optimized 3D microstructure for single cell culture on DMF chip. **[Not referred on text]**

Figure 4. The results of trapping cells under different conditions. (a) stage-like array, (b) wall-like array, (c) well array and (d) well-like array.

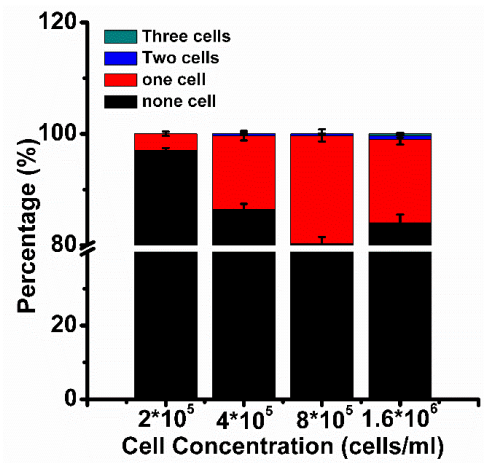

Figure 5. The results of trapping cells for (a) 2*105 cells/ml, (b) 4*105cells/ml, (c) 8*105cells/ml, (d) 1.6*106cells/ml, under the optimized 200 microstructures condition. (e) The percentage of trapping cells classified as none, one, two and three cells under each cell concentration. The statistical results include 900 microstructures.

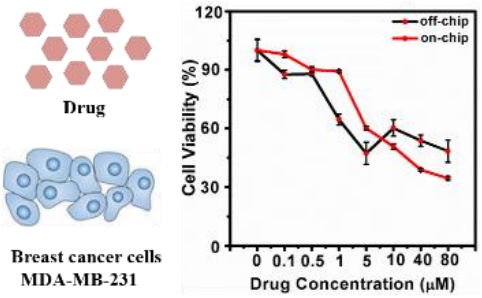

Figure 6 Cisplatin used as model for drug toxicity test to MDA-MB-231 cells for 24h on our chip assay and off-chip (96-well plate).