Fuzzy Systems and Data Mining IX
A.J. Tallón-Ballesteros and R. Beltrán-Barba (Eds.)

doi:10.3233/FAIA231106

Dual-Function Microfluidic Chip for Identification and Drug Response Testing of Lung Cancer Organoids

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Abstract. Organoids, as a novel in vitro drug screening platform, have received widespread attention due to their relevance to clinical research as they are derived from patient samples. Lung cancer organoids are categorized into two subtypes: EGFR mutation type and EGFR wild type. EGFR mutation lung cancer organoids exhibit better therapeutic effects with tyrosine kinase inhibitors (TKIs), whereas EGFR wild type lung cancer organoids have better responses to chemotherapy drugs. However, the current lack of a microfluidic chip capable of identifying EGFR mutations in lung cancer organoids and testing their drug response poses a challenge. To address this, we have developed a dual-functional chip that streamlines EGFR mutation identification and drug testing in lung cancer organoids. This method not only reduces time and cost but also enhances drug screening efficiency.

Keywords. microfluidic chip, drug response, organoids

1. Introduction

Organoids, which preserve the specific matrix of pathological patients and retain the cellular structures and molecular characteristics of tumor tissues, are widely recognized as reliable drug platforms. [1,2] They can be maintained long-term, making them useful for effective drug testing and individualized drug development. [3]

However, there are limitations in terms of variability and scalability in organoid culture on traditional platforms such as Petri dishes, including lack of vascularization[4], limited scalability[5], and technical difficulties in handling[6], making high-throughput drug screening for lung cancer organoids challenging. To address these issues, researchers have turned to microfluidic technology, which offers precise control over organoids[7-9], dynamic physical conditions[10,11], and homogeneous culture within microchambers[12-15]. Although some high-throughput microfluidic platforms have been developed for organ-on-a-chip drug response assays, there are still limitations that need to be addressed. These include the lack of identification functional areas resulting in a longer and more labor-intensive drug response testing process, the single drug administration mode on these platforms, and the lack of integration with subtype identification for drug screening.

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In our study, the identification and drug response testing of lung cancer organoids on a chip were achieved. The organoids' single cells were captured and identified in situ using a single-cell trapping module. Based on the identification results, corresponding drugs were added to the drug injection module to complete the final drug response testing of lung cancer organoids. This study has enhanced the efficiency of drug response testing, reducing the required time and workload.

2. Methods

2.1. Fabrication Process of DF-Chip

The DF-Chip is composed of three tightly bonded layers of PDMS. Through traditional photolithography technology, the mold of the drug injecting layer with a thickness of 200 μm is obtained by photolithography on the SU-2075 negative photoresist mask. The template for the cell loading layer is prepared using ICP technology and two overlay mask lithography techniques, with PDMS molds used for fabrication. Finally, the three layers are tightly bonded together through plasma treatment.

2.2. Establishment of PDOs

The postoperative clinical specimen was cut into 1-3 mm^3 pieces, digested with a digestion solution at 37 ℃ for 1.5 h, and then the digestion was stopped with FBS. The cell suspension was obtained by filtering it through a 70 μm filter, and the red blood cells were lysed using a red blood cell lysis solution. The cells were then suspended in a mixture of PDO medium and an ECM solution with double volume and injected into the cell loading layer, where they solidified for 30 minutes. PDO culture medium was added to the drug injecting layer. After a period of cultivation, the establishment of a PDO is completed.

3. Results and Discussion

To achieve the identification of EGFR mutations in lung cancer organs and drug response testing, a dual-function microfluidic chip (named DF-chip) was invented as shown in Figure 1. Figure 1a shows the three-dimensional image of the DF-chip, which consists of three layers: the drug injecting layer, the cell loading layer and the glass substrate. The drug injecting layer is used for injecting organ culture medium, drugs, and cell dyes; the cell loading layer is used for cell capture and cultivation; and the glass substrate supports the entire chip. The physical photo of the chip is shown in Figure 1b, with the drug injecting layer filled with blue and the cell loading layer filled with red. The DF-chip mainly consists of two functional modules: the single-cell trapping module (Figure 1c) and the drug injecting module (Figure 1d). The single-cell trapping module consists of 6 rows, with 100 trapping sites in each row, totaling 600 trapping sites. It is used to capture single cells from the organ and perform in-situ staining and EGFR mutation identification. The drug injecting module is composed of 7 spiral channels and can be controlled by valves for the addition of drugs in a fixed proportion or without mixing.

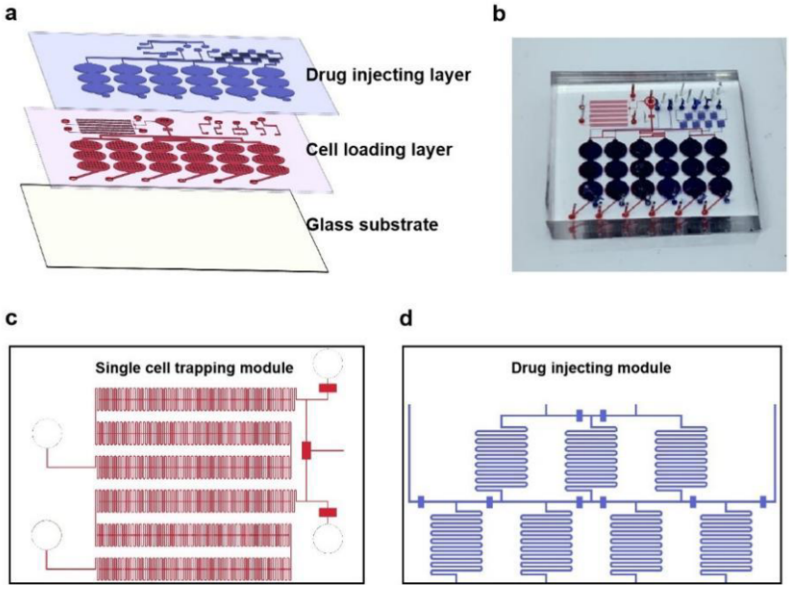

Figure 1. Design of the DF-chip. a 3D display diagram of the chip. b Photograph of the physical chip. c Overall display diagram of single cell trapping module. d Overall display diagram of drug injecting module.

To achieve the true capture of single cells in Organoids, the dimensions of the single-cell trapping module need to be precisely designed, as shown in Figure 2a. The width of the single-cell flow channel and the width of the single-cell trapping sites are both 30 μm, while the length and width of the cell trapping site are both 5 μm. The actual photo

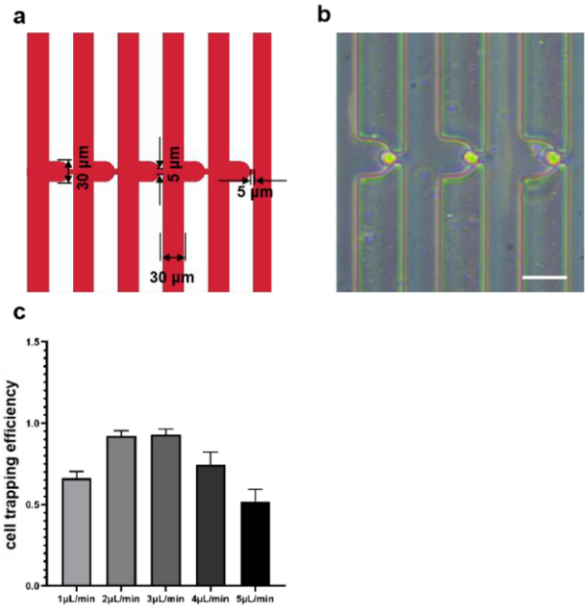

Figure 2. Design of the single cell trapping module. a The photo of single cell trapping. b Detailed dimensions of the single cell trapping module. c The relationship between the cell trapping efficiency and the flow rate. Scale bars: 30 μm.

of cell trapping is shown in Figure 2b, where cells are successfully trapped by the cell. Figure 2c shows the relationship between cell trapping efficiency and flow rate. As the flow rate increases from 1 μl/min to 3 μl/min, the cell trapping efficiency improves. However, when the flow rate exceeds 4 μl/min, the cells deform and pass through the trapping site, resulting in a decrease in cell trapping efficiency.

In order to ensure the stable operation of the DF-chip, the design and arrangement of the valve structure should be reasonable. Figure 3 shows the valve arrangement of the DF-chip, with the valve-controlled channels located in the single cell trapping module on the Drug injecting layer, and the opposite arrangement in the Drug injecting module. The opening and closing of the valves respectively control the flow and interruption of the channels, thereby achieving the programmable operation of the DF-chip.

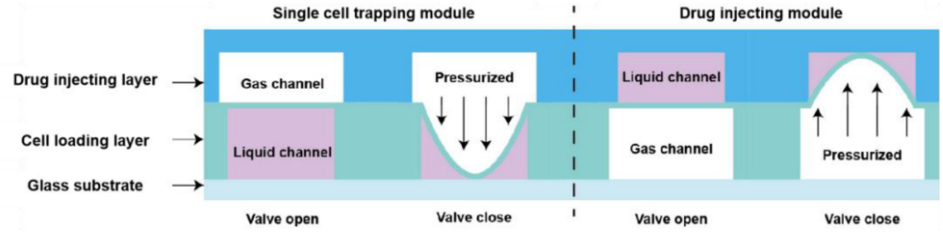

Figure 3. The valve arrangement of the DF-chip.

The functional status of the DF-chip is demonstrated by the patient-derived organoids (PDOs). The PDOs were obtained from clinical samples and cultured in the DF-chip. The molecular characterization of the organoids is shown in Figure 4. Unstained images displayed the growth status of the organoid. Blue fluorescent images showed the cell count of the organoid cluster, and green fluorescent images demonstrated the successful establishment of lung cancer organoids by indicating the staining of Cytokeratin 7.

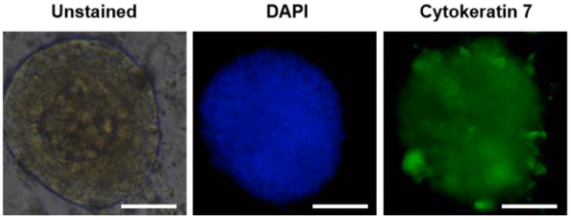

Figure 4. Staining image of PDOs. Scale bars: 100 μm.

Based on current methods, organoids are primarily identified through organoid staining. Although organoid identification results have not been obtained yet, single-cell trapping of organoids has already been achieved from the current experimental results, allowing us to obtain identification results through in situ staining. In the current stage, lung cancer organoids have been cultured on chips to lay the foundation for obtaining drug response test results. In the upcoming experiments, the focus will be on in situ staining identification of organoids and drug response tests, which holds great promise for completing the entire study based on the current experimental results.

4. Conclusions

In our study, the identification and drug response testing of lung cancer organoids on a chip were achieved. The study introduces a dual-function microfluidic chip for the identification and drug response testing of lung cancer organoids, which has the following advantages: 1) It enables the capture and identification of lung cancer organoid single cells within the chip; 2) It includes two drug administration modes for switching and adding different drugs; 3) It integrates the identification and drug response testing of lung cancer organoids, reducing time consumption and enhancing drug screening efficiency.

Acknowledgements

This work was supported by the Quancheng-5150 Talent Supporting Project of Jinan (Laureate, Zewen Wei) and the Industrial Talent Supporting Project of Huaiying (Laureate, Zewen Wei) and the Beijing Institute of Technology Research Fund Program for Young Scholars (No. 3160011181907). We thank the Biological & Medical Engineering Core Facilities of the Beijing Institute of Technology and the Analysis & Testing Center of the Beijing Institute of Technology for characterization services.

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