DIAGNOSIS-ON-A-CHIP: A MICROFLUIDIC PLATFORM FOR CELL CULTURE AND VIRUS ASSAYS

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ABSTRACT

A high-throughput microfluidic chip for cell culture and immunofluorescence virus assays is presented in this paper. The chip is fabricated by laser engraving technology with the polymer material polymethylmethacrylate (PMMA). Madin-Darby canine kidney (MDCK) cell line has been successfully loaded, cultured and passaged in culturing chambers with 1mm diameter and 1.5 µl volume; and rapid growth of influenza A virus (H3N2) in MDCK cells cultured has been observed and proved by immunofluorescence assay. This kind of cell culture array could offer a platform for virus growth testing for vaccine production and also is promising for early diagnosis and drug screening in drug screening, bioinformatics, and quantitative cell biology.

Keywords: MDCK cells, influenza A, in situ observation, lab on a chip

1. INTRODUCTION

The field of cell culture is developing much more sophisticated abilities based on microfabrication techniques. Compared with conventional cell culture technology which recruits instruments of large size and manual manipulation, the microfluidic system features high throughput, low cost and portability allows integration with other functions and provides static microscale cultures more similar to in vivo microenvironment. Some microfabricated eukaryotic cell culture devices have previously been demonstrated with various kinds of cells. However, little work has been done to simulate virus assays onto chip. In this paper a high throughput chip designed for long-term cell culture and virus growth is presented, combined with real-time optical monitoring.

2. DEVICE DESIGN AND FABRICATION

The culture and assays chip is developed as shown in Fig. 1, which consists of two pairs (could be more) of cell culturing chambers. One is connected to a virus inlet while the other has no virus connection for comparison purpose. Culturing medium, antibody and other reagents are loaded through the major inlet into the branch channels designed as a symmetric arc with binary splitting so as to provide equal flow to all the chambers.
The array chip is fabricated by a CO2 laser engraving system on polymethylmethacrylate (PMMA) which, compared with conventional PDMS lithography technology, is cost effective and are especially useful in microfluidic prototyping due to the very short cycle time of production [1]. During the experiment, Madin-Darby canine kidney (MDCK) cell line, which has long been known to successfully support influenza growth [2], were loaded into to the chip and cultured. After the cell formed a confluent monolayer, influenza A (flu A) virus was introduced and incubated. After obvious lysis was observed, monoclonal flu A antibody-FITC was loaded for the immunofluorescence.

3. RESULTS AND DISCUSSIONS

Figure 2 shows pictures of the in situ morphology of MDCK cells cultured in a chamber (diameter 600 µm, volume 0.56 µl). (a) The cells were suspended right after cell loaded; (b) the cells have attached and divided after 24 hr culturing; (c) The cells formed a confluent monolayer after 48 hr culturing and then were inoculated with flu A. The other three pictures were taken: (d) 1 hr after inoculation; (e) 24 hr later; (f) 48 hr later, and show the gradually lysed cells. Before the flu A inoculation, the cells are cultured in DMEM (Dulbecco’s modified eagle media, invitrogen) with 10% FBS (fetal bovine serum, invitrogen), and DMEM with 1% FBS after that. In these assays MDCK cell line exhibits super reliability to support influenza growth.

![Figure 1. Schematic diagram and the photograph of the microchip for cell culture and virus assays](image)

![Figure 2. Photograph of MDCK cells. (a) Right after being loaded; (b) cultured for 24 hr; (c) cultured for 48 hr; (d) 1 hr after flu A virus being loaded; (e) 24 hr later; and (f) 48 hr later.](image)
Figure 3 shows the immunofluorescence of MDCK cells. The bright spots are positive cells (infected), and the dark ones are negative (uninfected). This is, to our best knowledge, the first demonstration of virus infection and immunofluorescence assay in micro chip.

Figure 3. Immunofluorescence of MDCK cells cultured for two days and then inoculated with flu A virus, followed by culturing for another two days.

Multiple parallel experiments such as culture of different cell line and virus infections can be done on the chip with outside optical system for real-time monitoring. This kind of cell culture array could offer a platform for virus growth testing for vaccine production which is determined by how well the virus is able to replicate and how easily the cell line can be maintained [2], it also has potential applications in drug screening, bioinformatics, and quantitative cell biology [3]. With all these capabilities, it is possible to realize the diagnosis of disease on a single chip.

4. CONCLUSIONS

In conclusion, a high-throughput microfluidic for long-term cell culture and immunofluorescence virus assays is proposed. Madin-Darby canine kidney (MDCK) cell line has been successfully loaded, cultured and passaged in culturing chambers with 1 mm diameter and 1.5 µl volume; and rapid growth of influenza A virus (H3N2) in MDCK cells cultured has been observed and approved by the immunofluorescence assay.

REFERENCES

