Moving Single Cells Into Low Shear Stress PEG-Based C-Shape Microwells By OET Force

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Abstract

In microfluidic, the dead zone was brought by poly (ethylene glycol)-based C-shaped microwells. In this paper, we used optoelectronic tweezers (OET) to move single cell for low shear stress culture microenvironment. Based on our experiment, we provided a suited cell culture chamber for studying the interaction of nature killer (NK) cells and target cells in biomedical microdevice.

1. Introduction

Trapping single cell is not only to access the immune system of uncultivated organisms, but also for comparing the immune activity of individual cells sequenced from a population. Recently, the manipulations of single cells based on OET with the operation AC frequency have been first reported and successfully demonstrated by Chiou's group [I]. Sunghoon Kwon's group using railed microfluidic channels via PEG-DA microstructure, which created a movable part in a microchannel [2]. Furthermore, it will lead to a better understanding of the function and regulation of the immune system at the single cell level [3]. However, it is important for trapping single cell and keeping cell in a stationary area. By the way, we had used the OET force to trap single cell which was by the PEG-based microstructures to keep cell into C-shaped microwells.

In our paper, we wanted to understand the study of the immune system about the nature killer cell and the target cell. Microfluidic dead zones were manufactured by the array of the C-shaped microwells for low shear stress culture microenvi-ronment. For virus-infected or tumor cell, the MHC class-1 disappeared on the surface of the cell as shown in figure 1. The inhibitory receptor of the NK cell was able to detect the protein and the vi-rus-infected or tumor cell was killed by the NK cell.



Fig. 1 Schematic diagram of the target cell is killed by natural killer (NK) cell. The NK cell and target cell need contact to interact for the study of the immune system.



Fig.2 (a) Schematic diagram of the PEG-based OET chip. (b Using OET force to trap single cell into the microwells for the problem of the dead zone in TiOPC-based OET chip.

2. Experiments and Discussion

In the microfluidic system or chip, the dead zone is an important issue in a microchannel. We have de-veloped a low shear stress culture microenvironment in our PEG-based OET chip. In figure 2, it is a sche-matic diagram of the PEG-based OET chip. These dead zones are produced by PEG-based C-shape microwells as shown in the upper of the figure 2. In the dynamical microflow, the cells were moved by OET force, passing the dead zone in microfluidic channels. The yellow donut shape is the manipulation of the light for moving the target cell into the PEG-based C-shape microwells.

The PEG-based OET chip consists of four layers : the PDMS layer of the inlet and out let, ITO glass, tape layer, and TiOPc-based ITO glass as shown in figure 3.



Fig.3 Schematic diagram of the PEG-based OET chip. The PEG-based OET chip consists of four layers for a chip and three channels for three repeated experiments. The OET force is produced by the TiOPc layer.



Fig. 4 (a) This mask of the pattern of the C-shape. (b)The opening angle of PEG-DA microwell is 55^0 and the internal diameter is 90 micrometer. (c) The microscope photo shows the array of the PEG-based C-shaped microwells. The scale bar is 100um.

In tissue engineering field, hydrogel has been widely used because of their biocompatibility. The mask was used for the pattern of the C-shape as shown in figure 4(a). For the purpose of the low share stress culture microenvironment, the opening angle of C-shape was set 55^0 and the internal diameter is 90 micrometer in figure 4(b). And then, the array pattern of the PEG-based C-shaped microwells were formed by a single ultraviolet exposure. In figure 4(c), the photo shows that the opening angle is 22.5^0 by the absorption of the PEG-DA.

For the diffusing experiment of PEG-based C-shape array in figure5 (a)~(c), these serial photos have shown the fluorescence of internal PEG-based C-shaped microwells using fluorescence green dye (FITC). Figure5 (d) has shown the array of the C-shaped microwells by loading the fluorescent solution. After loading the PBS solution without fluorescence, the fluorescent solution still has flown into the C-shaped microwells in figure 5(e).

In figure6, we demonstrated the manipulation of the target cells by the OET force. The color of the TiOPc material is the blue appeared on figure 6. The yellow donut shape marked the manipulation of the light. The orange arrowhead was shown a target cell that had trapped into PEG-based C-shaped microwells by the donut shape of the OET force.

3. Conclusions

In order to improving current dead zone in micro-fluidic chip to a low shear stress microenvironment in which we could demonstrate trapping different target cells for cell-cell interaction in our PEG-based OET chip. Single cells had trapped by the donut shape of the OET force and PEG-based C-shaped microwells.

The purposes of this research are not only to increase the efficiency of trapping immune cells but also to regulate these immune cells to achieve mass culture. We conclude that trapping and culturing live cells in the low shear stress microenvironment are possible.



Fig. 5 The diffusing experiment of PEG-based OET chip. (a) \sim (c) These serial photos have shown the fluorescence of internal PEG-based C-shaped microwells using FITC dye (green) (d) this photo have shown the array of the C-shaped microwells by loading the fluorescent solution. (e)After loading the PBS solution without fluorescence, the fluorescent solution still has flown into the C-shaped microwell.



Fig. 6 The photo of manipulations of target cells in the PEG-based OET chip. Based on the OET method with the operation AC frequency, it has been controlled and successfully demonstrated. The color of the TiOPc material is the blue appeared. The yellow donut shape marked the manipulation of the light. The orange arrowhead was shown a target cell that had trapped into PEG-based C-shaped microwells by the donut shape of the OET force.

References

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