TRAPPING SINGLE CELLS IN MICROFLUIDIC DEAD ZONE BY USING PEG-BASED OPTOELECTRONIC TWEEZERS FOR IMMUNE ACTIVITY

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ABSTRACT

In this paper, we have demonstrated the trapping single cell into the microfluidic dead zone by using optoelectronic tweezers (OET) in the poly (ethylene glycol)-based U-shaped microwells for the study of the immune activity. Using light-induced dielectrophoresis (DEP) on a photoconductor, virtual electrode pattern was generated by projecting an LED light through a DMD spatial light modulator. We have shown a target cell had trapped by the donut shape of the OET force and PEG-based U-shaped microwells. Based on our experiment, we provided an easily performed method for study the interaction of NK cells and target cells. By the way, we had evaluated the NK cell activity in single cell level by the PEG-based OET chip.

KEYWORDS

Single cell, Hydrogel, Dead zone, Optoelectronic tweezers, Immune activity.

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. To understand the movement exhibited by cellular aggregates, we must understand how the local interactions between moving cells affect the collective motion. 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 [l]. 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]. Microfluidic chips are also useful for automated single cell isolation and allow for more efficient RNA purification and amplification. Some years ago, in Quake's group designed a microfluidic chip that employed microscopic bead columns for extracting total mRNA from individual single-cells and for synthesizing cDNA from them [4]. However, it is important for trapping single cell and keeping cell in a stationary area. By the way, we had used the PEG-based OET chip to trap single cell which was by the PEG-based microstructures to keep cell into U-shaped microwells. Indeed, immune activity drives some aspects of NK and target cells observed among closely related cells.

METHODS

In microfluidic system or chip, the dead zone is an important issue in a microchannel. In figure 1(a), we show the schematic diagram of PEG-based U-shaped microwells. The orange dotted lines are dead zone in U-shaped microwells. In the dynamical microflow, the cells were moved by pass the dead zone in microfluidic channels. In our design, we had conquered the problem of the dead zone by using OET force to trap single cell into the microwell in TiOPC-based OET chip in figure 1(b). We had also developed the immune cell activity regulated by the cell trapping as showed in figure 1(c).

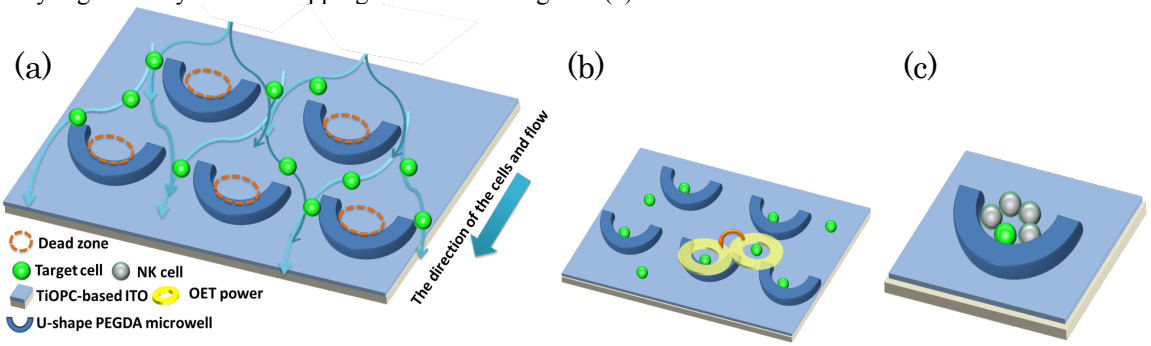

Figure 1: (A) Schematic diagram of the PEG-based OET chip with PEG-based U-shaped microwells. The microflow bypass the dead zone in microfluidic channels moved the cells. (B) We had conquered the problem of the dead zone by using OET force to trap single cell into the miceowell in TiOPC-based OET chip. (C) We had also developed the immune cell activity regulated by the cell trapping.

EXPERIMENT

In fabricated process as shown in figure 2, the PEG-based OET chip is fabricated by the TiOPC, which is used to define the pattern of the light by the conductivity. And then, square PEG-based U-shaped microwells were formed by a single ultraviolet exposure. In our design, microfluidic force was made by pass the dead zone and the single cell was trapped by the donut shape of the OET force and PEG-based U-shaped microwells.

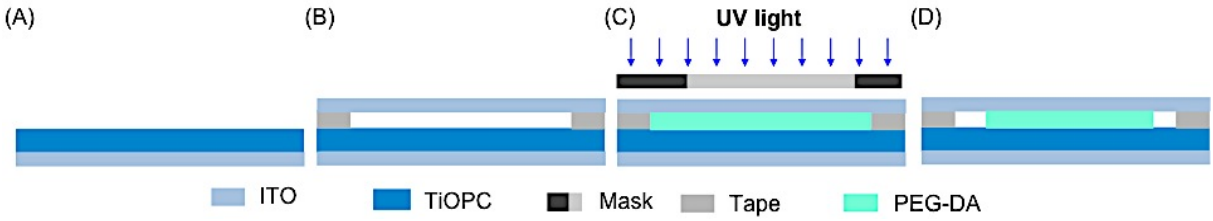

Figure 2: The PEG-based OET chip are fabricated by the TiOPC, which is used to define the pattern of the light by the conductivity. And then, square PEG-based U-shaped microwells were formed by a single ultraviolet exposure.

RESULTS AND DISCUSSION

Two microscope photos were shown the array of the PEG-based U-shaped microwells in figure 3. In these two photos, PEG-based U-shaped microwells were exposed by the UV-light and swelled by the buffer. The scale is 100um. We used a DMD projector to project the donut shape of the pattern in the PEG-based OET chip as shown in figure 4. In the PEG-based OET chip, we had demonstrated the manipulations of single cell by the donut shape of the OET force with the operation AC frequency. It had been controlled and successfully demonstrated by the light-induced negative dielectrophoresis.

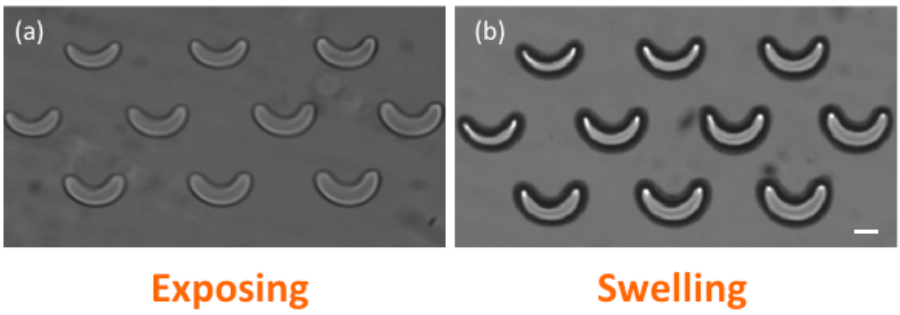

Figure 3: The microscope photo shows the array of the PEG-based U-shaped microwells. The scale is 100um.

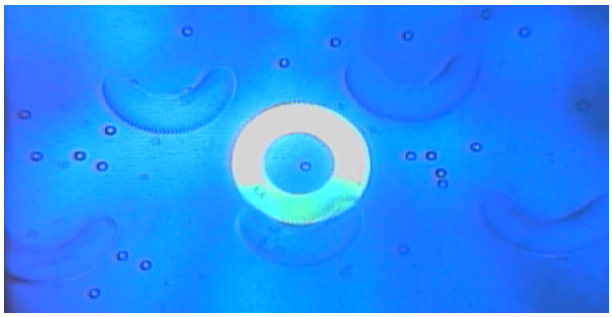

Figure 4: The manipulations of single cell based on the OET method with the operation AC frequency have been controlled and successfully demonstrated.

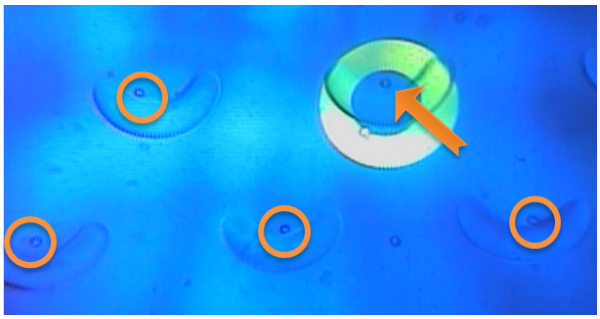

Figure 5: Orange circles show five target cells were trapped in the PEG-based U-shaped microwells by using the OET method.

These orange circles were shown four target cells had trapped in the PEG-based U-shaped microwells based on the PEG-based OET chip in figure 5. The orange arrowhead was shown a target cell had trapped by the donut shape of the OET force and PEG-based U-shaped microwells. In Figure 6, the photo as shown in single target cell with difference proportional NK cell to analysis for immune activity. Finally, Due to cell death in some well, the number of empty wells increased slightly in figure 7.

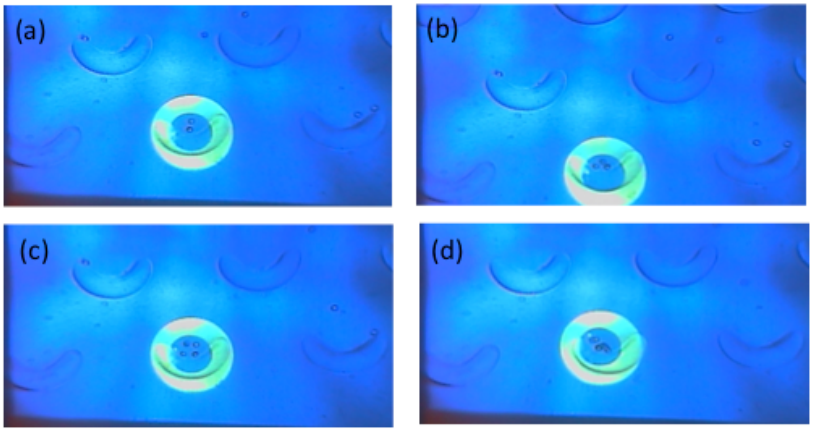

Figure 6: The photo as shown in single target cell with difference proportional NK cell to analysis for immune activity. (a) The ratio the target cell and NK cell is 1:1. (b) 1:2 (c) 1:3 (d) 1:4.

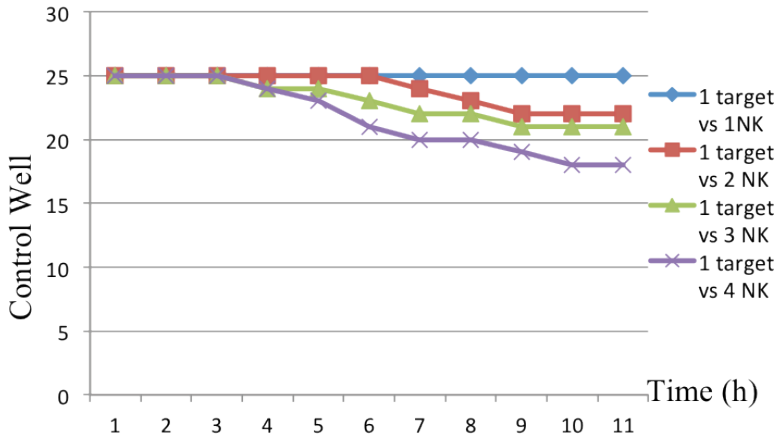

Figure 7: Due to cell death in some well, the number of empty wells increased slightly. We demonstrated the trapped different proportional NK-target cell and cell-cell interaction in our PEG-based OET chip.

CONCLUSION

We have demonstrated trapping different proportional NK-target cell and cell-cell interaction in our PEG-based OET chip. And single cells had trapped by the donut shape of the OET force and PEG-based U-shaped microwells. The purposes of this research are not only to develop the immune cell trapping but also to regulate by cell mass culture. We conclude that live cell by the imaging of NK-target cell interactions in U-shaped microwells are possible.

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