MICROFLUIDIC ALIGNMENT SYSTEM FOR SINGLE CELL MANIPULATION AND CULTURE

Takahiro. Arakawa[1,3], Naoya Takeda[2], Yoshikuni Edagawa[2], Yoshinori Yamaguchi[2], and S. Shoji[1]

[1]*Major in Nano-science and Nano-engineering, Waseda University, Japan*
[2]*Consolidated Research institute for Advanced Science and Medical Care, Waseda Uuniversity, Japan*
[3]*Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan*

ABSTRACT

Microfluidic alignment system for single cell manipulation and culture was developed to investigate the differences between individual cells in cell biology. We describe a microfluidic manipulation system that consists of a main channel, a pressure balancing channel (PBC), 3 μm drain channels and semicircle micropockets of 100 μm in diameter, for single cell capture, cell release and cell culture. Fundamental single cell manipulation was obtained by simple flow rate, the ratio between the cells that passed though the injected microchannel and captured cell were about 72%. Multiplication from the single cell in the micropocket was confirmed up to the third generation. This microfluidic system realized noninvasive capture of living single cells, and long-term culture up to 6 days. And this system contributes to the elucidation of cellular nature at the single cell level.

KEYWORDS: Single cell manipulation, Single cell culture, Cell rupture

INTRODUCTION

Lab-on-a-chip and μTAS provides an excellent platform to explore cell-cell signaling, genetic heterogeneity and heterotypic biological functions because its dimensions are comparable with the size of single cell. Recently several reports have been published on the mechanical cell trapping [1], seqiencial isolation of single cell [2], dielectric rupture, chemical lysis, cell-by-cell patch clamping.

We noninvasively isolated cells from a cell suspension, and then incubated the isolated cells by controlling the flow rate in the microchannel. A multiple microfluidic device to isolate cells from the cell suspension and then to culture the cells, with both actions taking place was also developed in the microchannel.

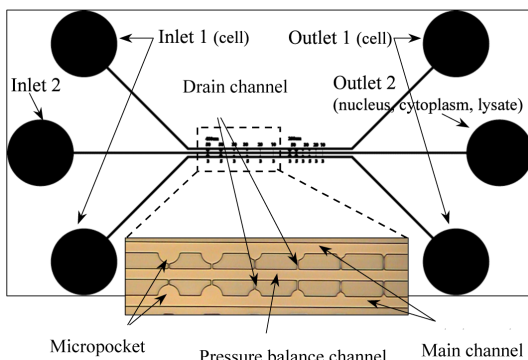

Figure 1. Individual cell capture microfluidic system for cell manipulation and culture.

EXPERIMENTAL

The PDMS microchannel has 20 ~ 50 semicircle micropockets

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for the single cell capture, whose diameter is varied from 20 μm to 100 μm correspond to the cell size, and 3 μm-width drain channel which connects main channel and PBC (Figure 1,2). Single cells were injected main channel, thread the channel and captured to each micropocket spot sequentially with the pressure difference between main channel and PBC. The cell suspension followed by the trypsin treatment to flat the cells was injected into main channel. The main channel was 50 μm wide to obtain constant flow of the cell suspension, while the diameter of the cell capture pocket was 100 μm wide to realize efficient capture of the cells. The height of the microchannel was 20 μm deep to ensure the cell capture in the cell pocket because the typical diameter of NIH3T3 cell was around 15 μm.

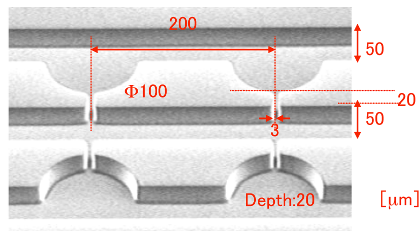
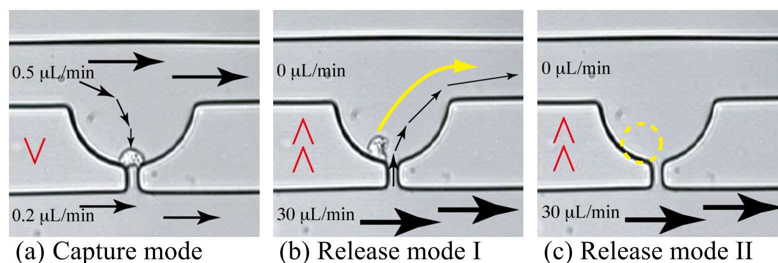


Figure 2. SEM image of the PDMS microfluidic device used for single cell isolation and culture in this study. The width of the drain channel through which cells were released from the micropockets was 3 μm.

RESULTS AND DISCUSSION

The sequential 'capture and release' of a living cell was achieved by introduced NIH3T3 cell in serum-free DMEM in the micropocket (Figure 3,4). After isolation of a single cell, the cell culture was performed. The cells were repeatedly subcultured in four passage numbers in the single cell micropocket. Those single cell manipulations were demonstrated by the only controlling the flow rate, that is essentially the pressure on each channel, and the shapes of the microfluidic device. The captured single cells were observed to adhere on the surface of the device, and cultured under the condition of 37 degrees Celsius, 5% CO_2.

The trapped cell in the micropoket was growing and devided into two at 14 hours latter. Then the next cell division were obtained at 33 hours 20 min and 33 hours 30 min. The 10 min difference was clearly observed with this system (Figure 5). On the other hand, the rupture of single cell was also achieved successfully after single cell isolation. The lysate of each single cell, especially the nuclei and mitochondria, were observed after the cell breakage.



(a) Capture mode (b) Release mode I (c) Release mode II

Figure 3. Release of the captured cell within the micropocket in the fluidic devices. NIH3T3 cells in the serum-free DMEM were applied into the micropocket.

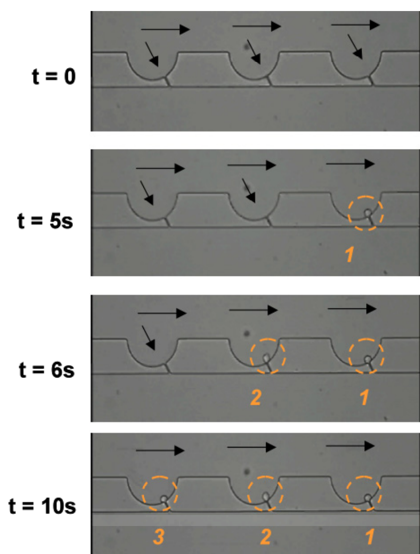


Figure 4. 3T3 Cell capture of single cells in the Micropocket. The trapping ratio was approximately 72%. The ratio was calculated by the folllowing fomula: (the number of captured cells) / (the number of injected cells) x 100.

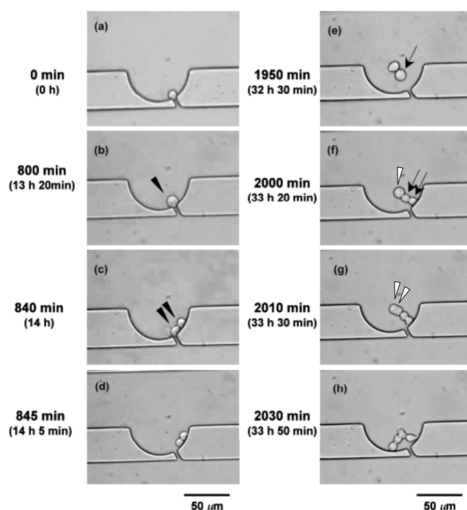


Figure 5. The 3T3 cell culture of the captured single cells was successfully realized for at least 34 h.

CONCLUSIONS

We developed a microfluidic alignment system for single cell manipulation and cell culture. Cells from the suspension were isolated and captured in single-cell micropockets by adjusting the micro-flow through the drain channel. Since the cells were captured noninvasively, both cell isolation and then cell division were achieved in the same pocket.

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REFERENCES

[1] A. R. Wheeler, W. R. Throndset, R. J. Whelan, A. M. Leach, R. N. Zare, Y. H. Liao, K. Farrell, I. D. Manger, A. Daridon, Anal. Chem. 75, 3581-3586. 2003

[2] H. K. Wu, A. Wheeler, R. N. Zare, Proc. Natl. Acad. Sci. U.S.A. 101, 12809-12813. 2004,