Application Note MMI-CE-001 – Adherent Single Cells

Selective Isolation of Adherent Single Cells

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Abstract

The isolation of single cells is an essential pre-requisite for many research projects in pathology, oncology or forensics.

The MMI CellEctor is designed to facilitate selective isolation of single cells in suspensions. This study illustrates the development of the novel Shake Mode feature for the efficient uptake of adherent cells. The unique module has been integrated into the MMI CellTools software to promote gentle detachment of adherent cells. Thus, the Shake Mode enables efficient transfer even of living adherent cells.

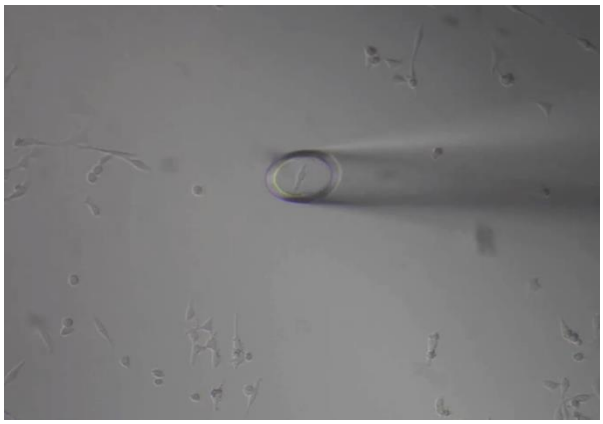

Figure 1: HeLa cell being picked by the MMI CellEctor micro-capillary

Introduction

There are many reasons why it is important to study single cells rather than doing bulk analysis. These reasons are very much dependent on the research focus. In case of forensics, DNA profiles are studied to assign samples at a crime scene to victim and perpetrator. In oncology, different mutations of tumor suppressor genes or oncogenes in circulating tumor cells (CTCs) or in metastases are sought to be identified, since these sub-tumor species are the major factor contributing to the patients' survival and they may react differently to therapy than the primary tumor.

Even though a cell population has the exact same DNA, individual cells often vary in their expression profile. Therefore, many researchers are interested in RNA and protein levels of cells in different tissues or under different conditions such as time, stress, toxins, growth factors, etc.

In the recent years, many methods to isolate single cells have been developed, both for tissue sections and for cells in suspension. However, most of these methods lack the ability to specifically select individual cells, such as fluorescence-activated cell sorting (FACS). The MMI CellEctor has been shown

to be a suitable tool to selectively isolate single cells in solution [1–7]. Basically, the instrument consists of a microscope and a pump connected to a software-controlled micro-capillary. This allows researchers to optically select a single cell which is then taken up by the micro-capillary with a defined volume. The pump volume can be adjusted to generate the forces required for cell aspiration and dispensing. However, it has been difficult to aspirate living adherent cells since the adhesive forces prevent detachment and uptake of the cells.

Living adherent cells can typically be detached by trypsinization, but the cells' RNA and protein expression profile will be altered by this treatment [8] leading to biased transcriptomics or proteomics data.

The aim of this work was therefore to establish optimal conditions for the isolation of single adherent cells, avoiding trypsinization but at the same time ensuring that the isolated cells are viable for further cultivation and downstream analysis. Thus, the parameters needed to be tested and adjusted to selectively and sensitively aspire one cell without any contamination by additional cells, and to effectively deposit this cell.

Material and Methods

HeLa cells were cultivated in Dulbecco's Modified Eagle's medium (DMEM), supplemented with FBS, non-essential amino acids, L-Glutamin and Penicillin-Streptomycin at 37 °C and 10 % CO_2. 300 µl of a cell suspension with $1x10^4$ Cells/ml were transferred into an 8-well Slide (Glass Bottom or treated polyethylene, Ibidi) and incubated overnight.

Single cells were selected, aspired and deposited using the MMI CellEctor mounted on a Nikon Ti2 inverted microscope. The MMI CellEctor was equipped with an 80 µm capillary pre-filled with cell culture medium. The capillary was rinsed with MMI Capillary Clean and cell culture medium after 10 steps of cell acquisition and deposition. Cells were deposited into a selected well on a second 8-well Slide, which has been prepared with 300 µl cell culture medium and pre-incubated at 37 °C, 10 % CO_2.

Results

In order to identify optimal conditions for the selective isolation of single HeLa cells and their transfer into a separate slide, different parameters, such as pump volumes and pump speed as well as dwell times and the micro-capillary angle were varied. However, the flow force generated by the pump turned out not to be sufficiently strong to detach adherent cells. Since the microscope stage can be precisely moved using the MMI CellTools software, we started to slightly rotate the stage in order to push the selected cell towards the capillary walls (Figure 2).

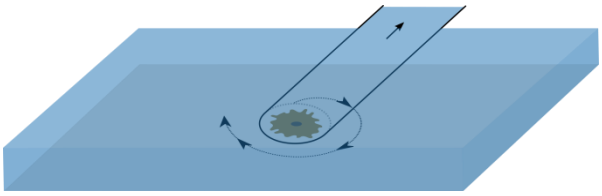

Figure 2: Schematic representation of the Shake Mode.

This gentle mechanical force was sufficient to detach the cell enabling subsequent aspiration by the micro-capillary. From this observation, the so-called "Shake Mode" has been developed as a novel software feature within the MMI CellTools package. The Shake Mode can be adapted regarding its rotation dimensions (defined by the x- and y-distances from the rotation center), the rotation speed, and the number of repetitions. Here, the Shake Mode was applied with a radius of 60 µm (x = y = 60 µm) at a speed of 100 µm/s and 2 repetitions. These conditions

allowed for effective HeLa cell detachment and successful aspiration (Figure 3).

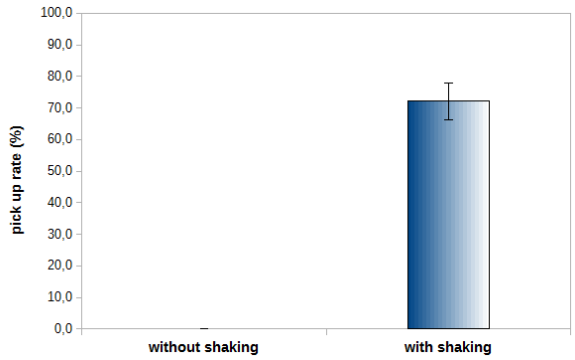

Figure 3: Pick up rates of HeLa Cells with and without the application of the Shake Mode. Adherent single cells can be aspirated with more than 70 % success rate using the Shake Mode. Using the same conditions but not applying the Shake Mode, cells cannot be aspirated at all.

Applying the Shake Mode, we then found effective and reproducible cell transfer conditions with an aspiration volume of 50 nl and a deposition volume of 60 nl, both at 6 nl/s. The capillary angle has been adjusted to 90 °. In addition, the capillary was allowed to dwell for 3 s after each cell deposition cycle. A typical cycle of single cell aspiration and deposition is depicted in Figure 4.

Furthermore, the transfer procedure was shown to be very gentle enabling HeLa cells to stay vital. After transfer into a new cell culture dish, the cells were able to grow and divide, and thus to form the basis for a new culture (Figure 5) [9].

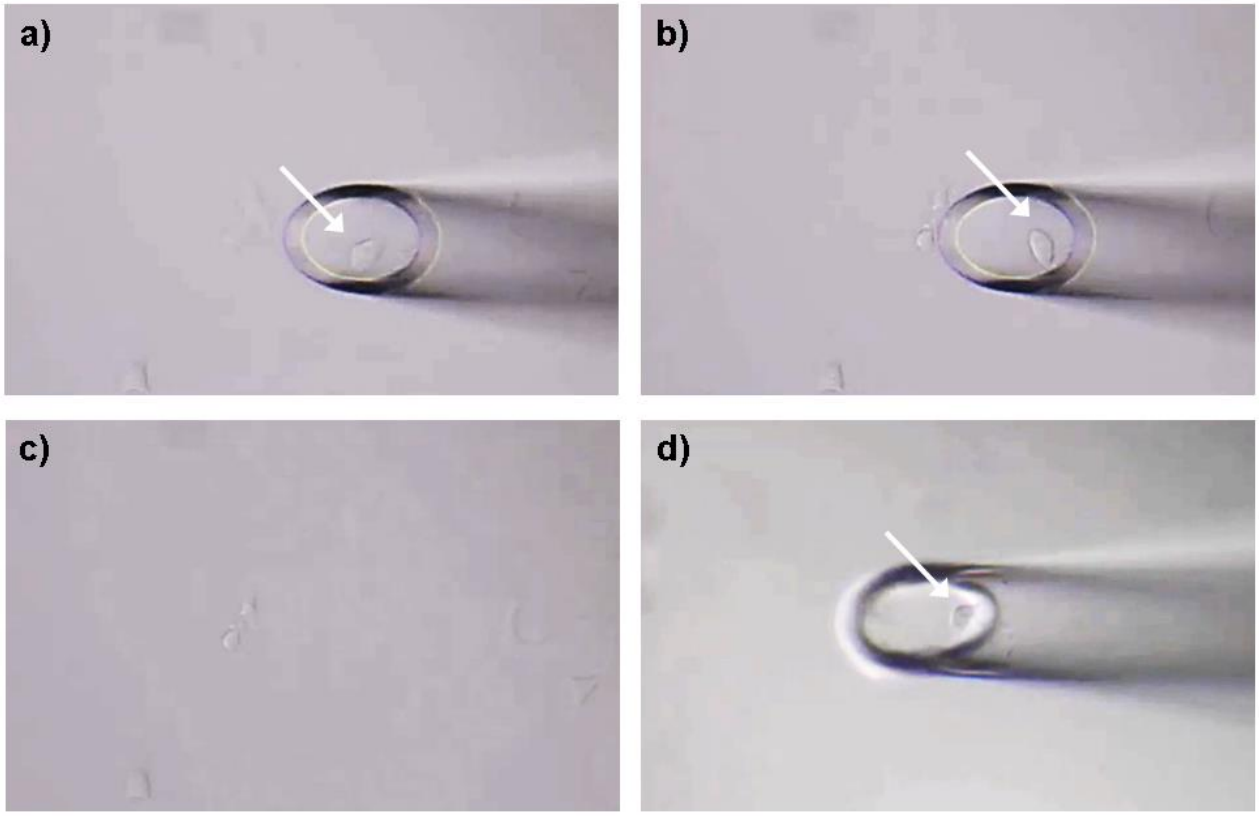

Figure 4: HeLa cells can be selectively isolated by the MMI CellEctor. a) A single cell is chosen and approached by the micro-capillary. b). Using the Shake Mode, the microscope stage rotates and pushes the cell towards the micro-capillary, which leads to a detachment of the cell from the cell chamber. The cell can then selectively be aspirated, c) not affecting any surrounding cells, and d) finally be deposited in the target well.

The full video is available on YouTube: https://www.youtube.com/watch?v=wLgd4CBhDwY

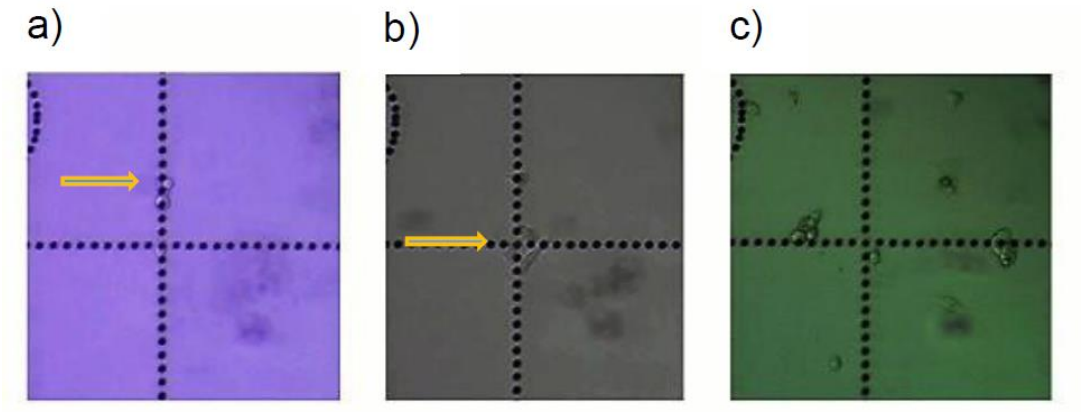

Figure 5: HeLa cells in cell culture (a) 1 day, (b) 2 days and (c) 5 days after transfer with the MMI CellEctor

Discussion

Applying the novel MMI CellTools Shake Mode software feature, this study demonstrates that a single cell is taken up by a micro-capillary without aspiration of additional, unwanted cells. In addition, the conditions were optimized to efficiently release aspirated cells at the target position.

For different cells types, the protocol might need some adjustment since the cells might have slightly different properties regarding their adhesion, size and shape. However, the parameters described above can be applied as a valuable starting point for subsequent fine tuning. With the Shake Mode, we offer a new tool to overcome the critical point of gentle cell detachment.

This work reveals that, using the novel Shake Mode, adherent cells can be detached from surfaces without compromising their viability or integrity. Thus, single cells can now be selectively isolated and utilized for any downstream applications, even for sensitive RNA expression analysis.

Watch the video illustrating adherent single cell isolation here:

References

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