Selective single cell detachment and retrieval for downstream analyses using nanosecond laser pulses in cnt-coated microwell arrays

SELECTIVE SINGLE CELL DETACHMENT AND RETRIEVAL FOR DOWNSTREAM ANALYSES USING NANOSECOND LASER PULSES IN CNT-COATED MICROWELL ARRAYS

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ABSTRACT

Cellular heterogeneity is one of the key hallmarks in cancer biology, but conventional dish-based assays only report the average behavior of many cells. Microfluidics can facilitate manipulating and monitoring of individual cells, yet it is difficult to retrieve a specific target single cell from an enclosed microfluidic chip. In this work, we have successfully developed a selective cell detachment and retrieval scheme with a spatial resolution of around 10μm. The retrieved cells were proved to be viable, and the detachment process has negligible effect on membrane proteins and mRNA expression, providing an ideal tools for the downstream analysis of target cells.

KEYWORDS: Cell retrieval, Cell detachment, Single Cell, Microfluidics, CNT

INTRODUCTION

Due to the genomic instability, cancer cells are heterogeneous [1], and certain cells play critical roles in drug resistance, metastasis, and tumorgenesis [2-3]. Compared to conventional dish-based assays, which report only the average behaviors, microfluidic array chips have provided capability to monitor individual cell behaviors [4]. To correlate single-cell behavior and its related gene expressions, it is critical to retrieve a target cell for further analysis. Conventional cell detachment approaches, such as trypsinization or PNIPAAm-based detachment [5], have poor spatial resolution. Although there are a few publications reporting cell retrieval techniques using various mechanisms [6-10], these methods can be only applied to open substrate but not to the enclosed microfluidic chambers. We present a novel cell detaching mechanism that can retrieve target cells at single-cell resolution from the microfluidic array chip without affecting cell viability and gene expressions (mRNA).

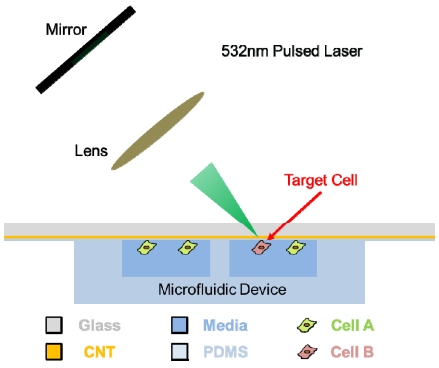

Fig. 1. Schematic diagram of single cell detachment setup. The cells were cultured in the microfluidic chamber coated with CNT in the substrate as shown in Fig. 2. A short pulse laser is used to detach the target cell. The attached cells can then be retrieved in the outlet.

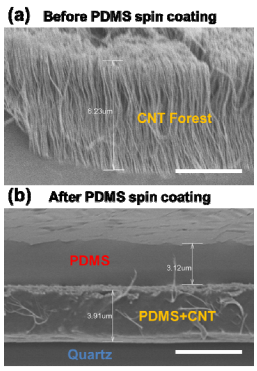

Fig. 2. SEM images of the substrate: (a) CNT forest on quartz substrate, (b) embedded CNTs in PDMS after PDMS spin coating. (scale bar: 5 μm)

EXPERIMENTAL

Fig. 1 shows schematic diagram of single cell detachment setup. Once a target cell is identified inside the microfluidic chamber, a short-pulse (6ns) of 532nm laser is applied for cell detachment. The substrate of microfluidic chambers have a CNT-PDMS composite layer (Fig. 2), so that laser energy (0.1 mJ) can be absorbed

by the CNTs, generating thermal deformation of a PDMS film where cells are attached. The shear stress caused by rapid deformation can detach a cell as illustrated in Fig. 3. Fig. 4 illustrates the precise spatial control of cell detachment. By focusing laser only on one end of the cell, we can even partially detach the cell, demonstrating subcellular spatial resolution. After detachment, the cells in the even rows and the odd rows can be retrieved separately using different flow schemes for further downstream analyses (Fig. 5(b, c)).

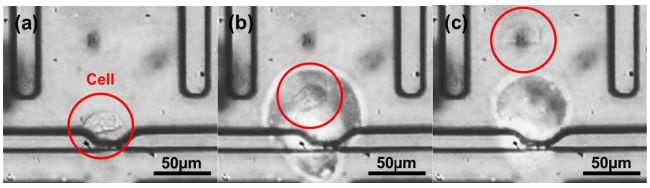

Fig. 3. Process of single cell detachment: (a) before detachment, (b) laser irradiation, and (c) cell detached and flowed away. (scale bar: 50 μm)

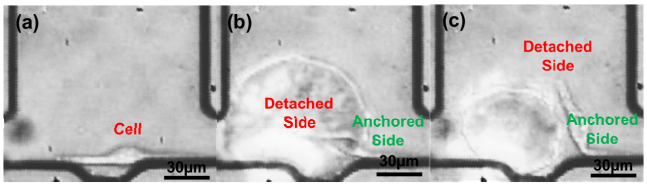

Fig. 4. Partial cell detachment: (a) before detachment, (b) laser irradiation, and (c) after detachment showing one end (left side) of the cell detached. (scale bar: 50 μm)

RESULTS AND DISCUSSION

Fig. 6 shows the recovery process of a MDA-MB-231 cell after detachment. Cells were detached one by one at single cell resolution and placed in a 96-well plate (Fig. 6(a - c)). 4 days after detachment, each single cell proliferated to ~20 cells, and all of them were proved to be viable using LIVE/DEAD staining (Fig. 6(d)). In Fig. 7, we compared the viability of laser-detached cells and trypsinized cells. Remarkably, laser detachment showed better cell viability than trypsinization. The laser detachment doesn't affect cell viability, because (1) the low thermal conductivity of PDMS provides an ideal thermal insulating layer, (2) heat can be dissipated quickly through the CNT composite layer laterally, and (3) a short laser pulse can minimize possible cell damage [7].

After detachment, we visualized the detached cells for both laser and trypsinization processes under SEM (Fig. 8). Trypsinization digests all the membrane proteins (giving the cells a smooth surface (Fig. 8(b, d)), while the laser detachment preserves surface proteins (leaving the cells rough under the SEM (Fig. 8(a, c)) [11]. The preservation of these membrane proteins may enhance cell viability for laser-detached cells. We also characterized the mRNA expression of 96 genes by comparing 20 laser detached cells and 20 trypsinized cells (data not shown). The cells detached by both methods maintain typical T47D cell expressions, and no significant difference was found between the two populations.

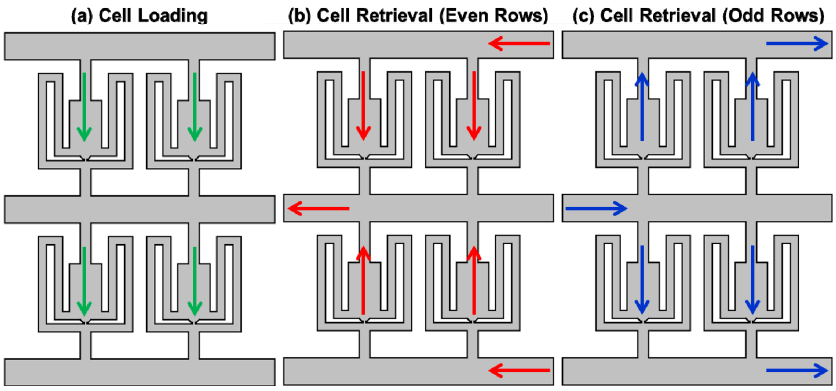

Fig. 5. Three different flow schemes of the microfluidic device: (a) cell loading into the chambers for culturing and monitoring, (b) retrieving cells in the even rows, and (c) retrieving cells in the odd rows.

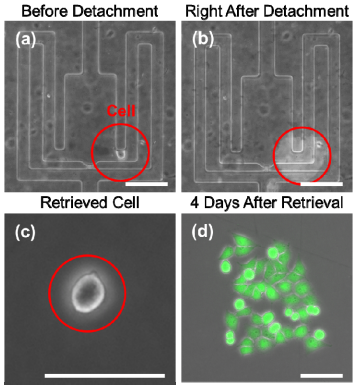

Fig. 6. The recovery of a MDA-MB-231 cell: (a) before detachment, (b) right after detachment, (c) cell retrieved (d) proliferation after 4 days. (scale bar: 100 µm)

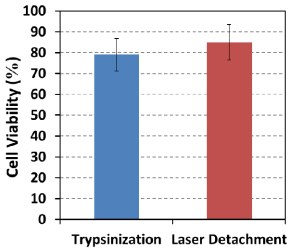

Fig. 7. Quantitative recovery data of MDA-MB-231 cells, 2 days after the detachment by laser and by trypsin for comparison. Laser detached cells have slightly higher viability than the trypsinized cells, showing the laser detachment does not affect cell viability. (N = 30)

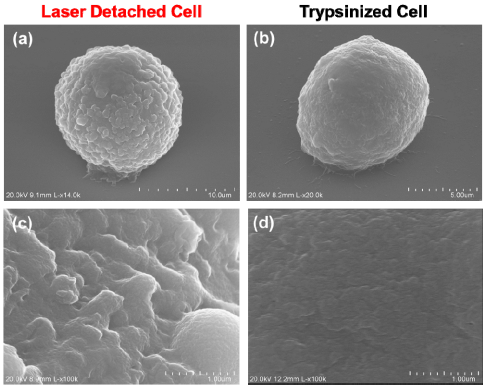

Fig. 8. Scanning electron microscope (SEM) images of laser detached and trypsinized cells: (a) a laser detached MDA-MB-231 cell, (b) a trypsinized MDA-MB-231 cell, (c) Enlarged view of a laser detached MDA-MB-231 cell, and (d) Enlarged view of a trypsinized MDA-MB-231 cell.

CONCLUSION

We have successfully demonstrated a selective single-cell retrieval technique. Utilizing the shear stress induced by photo-initiated PDMS deformation, single cells can be selectively detached. With the novel flow schemes, the detached cell can be retrieved for downstream analysis. The technique has been proved to neither affect viability nor alter mRNA expression of single cells, granting the capability to perform downstream analysis of a target single cell.

ACKNOWLEDGEMENTS

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