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Editorial

Micro/Nanofluidic Devices for Single Cells Analysis

Tuhin Subhra Santra [1] and Fan Gang Tseng [1,2,3,*]

[1] Institute of Nano Engineering and Microsystems, National Tsing Hua University, Kuang Fu Road, No. 101, Section 2, Hsinchu 30013, Taiwan; E-Mail: santra.tuhin@gmail.com

[2] Department of Engineering and System Science, National Tsing Hua University, Kuang Fu Road, No. 101, Section 2, Hsinchu 30013, Taiwan

[3] Division of Mechanics, Research Center for Applied Sciences, Academia Sinica, Nankang, 11574, Taipei, Taiwan

***** Author to whom correspondence should be addressed; E-Mail: fangang@ess.nthu.edu.tw; Tel.: +886-3-571-5131 (ext. 34270); Fax: +886-3-573-3054.

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The Special Issue of *Micromachines* entitled "Micro/Nanofluidic Devices for Single Cell Analysis" covers recent advancements regarding the analysis of single cells by different microfluidic approaches. To understand cell to cell behavior with their organelles and their intracellular biochemical effect, single cell analysis (SCA) can provide much more detailed information from small groups of cells or even single cells, compared to conventional approaches, which only provide ensemble-average information of millions of cells together. Earlier reviews provided single cell analysis using different approaches [1–3]. The author demonstrates invasive and noninvasive with time and non-time resolved SCA [1]; whereas some other literature provided destructive (with dyes, DNA, RNA, proteins and amino acids) and nondestructive (electroporation, impedance measurement and fluorescence based methods) cellular content analysis using microfluidic devices [3]. Further literature also suggest that single cell analysis is possible with capillary electrophoresis (CE) combined with a detection method such as electrochemical detection (ED), laser induced fluorescence (LIF) detection and mass spectrometry (MS) [4,5].

This special issue mainly focuses on the recent development of SCA with different microfluidic devices based technologies. Wiklund *et al.* [6] reviewed the cell to cell interaction in multi-well microplates combined with live cell fluorescence microcopy by using an ultrasound method. They describe the interaction between natural killer (NK) cells and cancer cells at an individual level. This review not only elucidates on the heterogeneity in cytotoxicity of NK cells and their ability to form one or several immune synapses simultaneously, but also on the impact of ultrasound exposure for cell viability, proliferation rate and their function. Another author, Kin Fong Lei, in this special issue, reviewed the impedance detection of cellular response in a micro/nano environment [7]. The author

reviewed the impact of a microfluidic system combined with the impedance measurement technique, which can provide non-invasive and label-free monitoring of cellular responses in 2D and 3D culture systems. Santra *et al.* [8] reviewed recent progress of micro/nanaofluidic single cell electroporation for intracellular and extracellular delivery. The electroporation technique is not only useful for cell lysis, cell to cell fusion or separation, insertion of drugs, DNA and antibodies inside single cell, but also it can control biochemical, electrical and mechanical parameters. The single cell electroporation technique can provide high transfection efficiency, higher cell viability, lower sample contamination, lower joule heating effect, and low toxicity during experiments compared to bulk measurement. As a result, single cells with their organelles can be measured more precisely by using micro/nanaofluidic devices. The authors describe in detail recent single cell and localized single cell electroporation techniques and their impact on single cell analysis.

Maorshed and coworkers [9], provided theoretical and experimental information on single cell electrical lysis in a microfluidic device and effects in a microchannel. They suggested that the generation of an electric field in a microchannel can provide sufficient energy with low current requirements. Single cell lysis in a microfluidic device was found to apply an external applied voltage approximately 700 V to 900 V within seven seconds and used less than 300 mW power consumption. For this single cell lysis, they used an 8 mm long microchannel with dimensions of 100 μm × 20 μm.

Feng *et al.* [10] proposed on-chip enucleation of bovine oocytes by using magnetically driven microrobot flow control. The microrobot can control the flow speed with fluid resistance adjustment in a microfluidic device and was specifically designed for enucleation. Their device can: (a) promote fluid flow control; (b) the volume of the oocyte can be adjusted resulting in less damage of the oocyte; (c) and to control microrobot and hydrodynamic forces, the nucleus can be removed. To use this device, they achieved minimally invasive enucleation with 2.5 s average enucleation time and 20% average removal volume ratio.

Probst *et al.* [11] suggested PDMS based submicron traps for single cell analysis of bacteria. The authors presented a hundred submicron size-based trapped barrier structure in a microfluidic device for immobilization and cultivation of individual bacteria. However, the study of prokaryotic cells, such as E. *coli,* encountered some challenges because of their small size and fast growth rates. They cultivated E. *Coli* for several hours within their microfluidic trapped structure, and it showed constant division times with rod-shaped morphology, indicating excellent cell growth with high cell viability.

Banaeiyan *et al.* [12] presents a specific microfluidic device as a cell comb, which is capable of high throughput single cell experiments. The microfluidic device can trap at least six cells in each V shape structure by using hydrodynamic forces giving a cellular response, such as protein migration followed by bright field and fluorescence imaging. They monitored arsenite (As (III)) uptaken in Saccharomyces cerevisiae cells with different flow rates (low = 25 nL min^{-1}, moderate = 50 nL min^{-1}, and high = 100 nL min^{-1}). The device might be applicable for cell signaling pathways and to their modes of function and regulation.

Blomqvist *et al.* [13] studied single yeast cells with highly effective Hog1 inhibitor and the use of osmotic stress. They have used four channel microfluidic systems to enable multiple signal inputs (Hog 1 and sorbitol) to a yeast signal transduction pathway for studying single cell response. To activate the Hog 1 signaling pathways for the presence or absence of the cellular response,

was monitored by the imaging of the nuclear translocation of the cytosolic MAPK, Hog1 on a single-cell level.

Hall *et al.* [14] described single cyanobacterium lysis for whole genome amplification. They present a lysis protocol, which can extract genomic information from single cyanobacterium of Synechocystis sp.PCC 6803, which have multilayer cell wall structures usually preventing the use of a conventional lysis mechanism. The high-fidelity genome sequencing of single cells of Synechocystis can be achieved by performing microfluidic MDA (Multiple Displacement Amplification) reactions with selected genes (15 loci nearly equally spaced throughout the main chromosome).

In conclusion, this special issue of *Micromachines*, "Micro/Nanofluidic Devices for Single Cell Analysis" not only emphasizes the new microfluidic devices for SCA, but also reviews recent advancements of SCA with different techniques such as electroporation, ultrasound, and impedance measurement. In the last couple of years, SCA has not only been at the forefront of biological cell studies and therapeutic research, but also it has been in close collaboration with human health. Recently microfabricated devices called "Laboratory on a chip" (LOC) perform a tremendous role in single cell analysis. Micro/nanofluidic devices are not only useful for cell manipulation, cell lysis, and cell separation, but also can easily control biochemical, electrical, mechanical parameters for single cell analysis. By miniaturizing the device such as in micro total analysis (μTAS) systems, analysis of single cell organelles with precise biochemical control can be achieved inside the single cell. SCA for system biology, genomics, transcriptomics, proteomics, metabolomics and fluxomics is not only a broad research area, but also a challenging task for application in biology, medicine, pathology and clinical trials, *etc.* With the continuous progress of single cell analysis, development of biomedical technologies which are essential to our daily life could be extended to offer many possibilities in the future.

Author Contributions

Tuhin Subhra Santra wrote this editorial and Fan Gang Tseng provided the concept to write an editorial and finally corrected it.

Conflicts of Interest

The authors declare no conflict of interest.

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