ON-CHIP FLOW CYTOMETRY AND SINGLE-CELL IMAGING IN TANDEM: INTEGRATION OF A μFACS WITH A SINGLE-CELL ARRAY
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ABSTRACT
Single-cell analysis, a key approach for future biological discoveries, requires revolutionary tools to transport, sort, immobilize, and image individual cells efficiently. In this paper, we demonstrate the tandem integration of micro fluorescence-activated cell sorter (μFACS) with single-cell traps to enable the multiplexed analysis of a selected subpopulation of macrophage cells for understanding signaling pathways associated with host-pathogen response. Our approach couples a novel optical-force cell sorter with an array of micropores to sequentially and reproducibly trap cells for high-resolution imaging of cellular events.

KEYWORDS: flow cytometry, cell sorting, optical tweezers, single-cell

INTRODUCTION
Lab-on-a-chip platforms are in concept capable of performing multiplexed assays on the same sample, a key enabler for microfluidic-based system biology. Among the desired functionalities, on-chip flow cytometry is capable of rapid, multi-parametric measurements on large cell populations; whereas, microscopy imaging can provide spatial information at high-resolution, but only on a limited number of cells. These two techniques are fundamentally different, but the integration of the two into a single multiplexed assay would be powerful approach to studying cellular system. In this paper, we present our work on the tandem integration of these two functionalities for studying host-pathogen interactions and its associated signal transduction pathways. To achieve this, on-chip flow cytometry is coupled with noninvasive optical cell sorting [1] to select representative cells and register them into downstream single-cell traps for high-resolution imaging.

Figure 1. Principle and illustration of sorting based on optical forces. Hydrodynamically focused macrophage is: A) initially detected by forward scattering; B) enters the near-IR laser sorting region; C) is deflected by optical gradient forces D) and finally released in a different laminar flow stream.
EXPERIMENTAL

For flow cytometry, the center sample stream entrained with RAW 264.7 mouse macrophage cells are focused by two neighboring sheath flows (velocity of 20 mm/s). Macrophages are detected and characterized by forward-scattering and laser-induced fluorescence (red and green). These fluorescence signals trigger a powerful (9.6 W at the sample) 1064-nm laser, which optically deflects the cell of interest from the center of the channel into a neighboring flow stream (Figure 1). This sorting mechanism is based on optical tweezers, where strong gradient forces laterally displace the cell by 30 μm into a neighboring flow stream. The laminar nature of microfluidic flow ensures that the sorted cell will stay on the same flow path and be directed into a collection channel, where the auxiliary flow additionally focuses the cells against the inner channel wall. Cells are then individually trapped in a series of fabricated micropores. The viability of the cells trapped at the micropore interface was assessed by epifluorescent imaging using 1 μM calcein AM fluorescent stain.

RESULTS AND DISCUSSION

The design for the tandem integration of the µFACS and a 12-trap single-cell array is shown in Figure 2. Each trap consists of a 26-μm-wide by 7-μm-deep micropore formed at each junction of the folded channel. Corners are distanced such that during the wet-etch fabrication, they partially etch through to form a micropore at the junction.[2] Since the pore height is smaller than the 15-μm diameter macrophage, it acts as a trapping site and immobilizes the cell for microscopy imaging (Figure 3). Furthermore, the channel geometry is designed such that the initial flow path...
for the fluid in the channel is through the pore because it has the lowest fluidic resistance. Once the pore is occupied by a cell, the partial plug dramatically increases the fluidic resistance and redirects the next sorted cell around the bend and towards the next trap. In this configuration, each cell is trapped sequentially (starting from the closest trap to the sorter) with a near 100% trapping efficiency. The use of the auxiliary channel dramatically improve trapping efficiency because the sorted cells are positioned near the inside wall of the microfluidic channel. Macrophage cells trapped at the micropore remain viable and show no change in morphology for up to 5 hours, enabling long time course measurements of cellular events (Figure 4). To achieve long-term viability, constant perfusion of nutrient media is required to maintain cell health. Additionally, cells can be easily removed by increasing the pressure drop across the pore, clearing the array for another experiment.

CONCLUSIONS

We have successfully demonstrated the tandem integration of a µFACS and a single cell array. This multiplexed capability provides a novel approach to combine different, but complementary, techniques for on-chip cell analysis.

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REFERENCES


Figure 4. Calcein-AM-stained macrophage trapped at a micropore in the array (position #6). Images taken at three time points: 0, 133, 273 min). To remain viable, cells require constant perfusion of nutrient media.