High-precision Acoustic Cell Sorting in BioMEMS

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Biological cells in human bodies, as the fundamental unit of life, are heterogeneous in nature. It is essential to identify and sort specific cell populations from highly heterogeneous biological samples for a variety of applications in biology, diagnostics, and medicine. As acoustic waves can easily propagate through solids and fluids, acoustic technique readily enables non-contact cell manipulation in relatively long operating distances. It has been found that acoustic technique has no or minor negative impact on the viability and functionality of biological cells, which is a significant advantage compared to existing cell sorting technologies. Herein we will present high-precision single cell sorting in bioMEMS using highly focused surface acoustic waves (SAW). I will discuss a unique design of a focused interdigital transducer (FIDT) structure, which is able to generate a highly localized SAW field on the order of 25 μm wide that is comparable to individual biological cells. We have further integrated single cell analysis (i.e., single cell fluorescence detection and single cell biophysical phenotyping) with the acoustic cell manipulation to implement detection activated single-cell sorting. This new type of acoustic manipulation platform has enabled high-precision and on-demand single cell sorting in diverse biomedical applications.

1. Introduction

Biological samples are heterogeneous in nature. It is essential to identify and sort specific cell populations from highly heterogeneous biological samples for a variety of applications in biology, diagnostics, and medicine. Existing cell sorting techniques mainly depends on specific cellular molecular biomarkers that can be fluorescently labeled or conjugated with magnetic beads. Labeling these biomarkers requires complex and costly sample preparation steps and possibly damage or even kill cells.

Biophysical markers of cells such as cellular electrical and mechanical properties have been proven as promising label-free biomarkers for studying, characterizing, and classifying different cell types. We have demonstrated that mechanical properties of single cells can be inferred from electrical impedance sensing that measures the transit time of single cells squeezing through a smaller constriction [1]. We have also developed single-cell level manipulation technology using a highly focused surface acoustic wave (SAW) [2]. Here, we discuss the integration of biophysical cytometry with acoustic cell manipulation to enable biophysical phenotyping activated sorting of cells and droplets for different biomedical applications

2. Results and discussion

2.1 Experimental setup

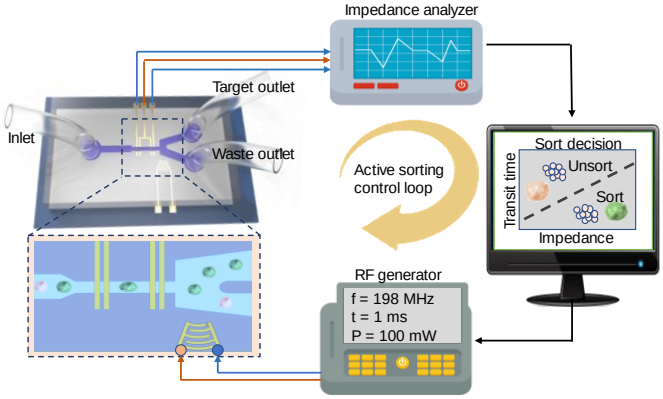

Fig. 1 Schematic setup of biophysical phenotyping activated microfluidic sorting system.

Fig. 1 shows the schematic experimental setup of the proposed biophysical phenotyping activated sorting platform. Electrical phenotyping is implemented by single-cell electrical impedance characterization with two pairs of differential sensing electrodes, while

mechanical phenotyping is performed by extracting the transit time for the single cell to pass through a micro-constriction from the impedance signals. A real-time impedance signal processing and triggering algorithm is used to identify the target population and activate a focused interdigital transducer (FIDT) to produce a pulsed highly focused surface acoustic wave (SAW) for single-particle level sorting.

2.2 Electro-phenotyping activated sorting of live cells

Cryopreserved cell samples after thawing can generate a large population of dead cells and cell debris. Removal of dead cells and cell debris is important to improve single cell sequencing data quality. Fig. 2 shows electrical phenotyping activated sorting of live cells from dead cells and cell debris. Dead cells have compromised cell membrane, which make them more permeable to electric current than live cells. As a result, an impedance characterization at a medium probing frequency around 8 MHz can accurately differentiate live cells from dead cells. Upon the electrical phenotyping for cell viability assessment, a pulsed SAW is generated to sort live cells from other bioparticles. Our platform can sort live peripheral blood mononuclear cells (PBMCs) without any labelling with a purity increase from 53.3% to 90.6%.

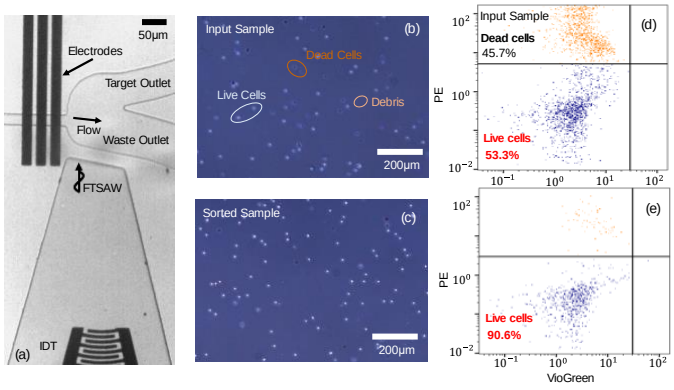

Fig. 2. Electrical impedance activated sorting of live cells from dead cells and cell debris. (a) Microscopic image of the sorting device. Microscopic images of input sample (b) and sorted live cells (c). Flow cytometric analysis of input sample (d) and sorted sample (e).

2.3 Electro-mechano-phenotyping Activated Sorting of One-cell One-gel-bead Droplets

Co-encapsulation of barcode gel-beads and biological cells has shown promising applications in single-cell genomics. However, under the dominance of Poisson distribution, the yield of one-cell one-gel-bead droplets is very low in existing droplet microfluidic platforms (i.e., 0.1%-10%). With electrical phenotyping alone, our biophysical cytometry can sort single-cell droplets from droplets with random cell encapsulation, as shown in Fig. 3. With electrical phenotyping of cells and mechanical phenotyping of gel-beads, our biophysical cytometry further allows accurate identification of different combinations of cells and gel-beads in microdroplets. Accordingly, one-cell one-gel-bead droplets can be effectively sorted out from other droplets with different encapsulation contents. We have demonstrated that our platform can increase the purity of one-cell one-gel-bead droplets to over 80% that is more than 8-fold higher than current co-encapsulation techniques.

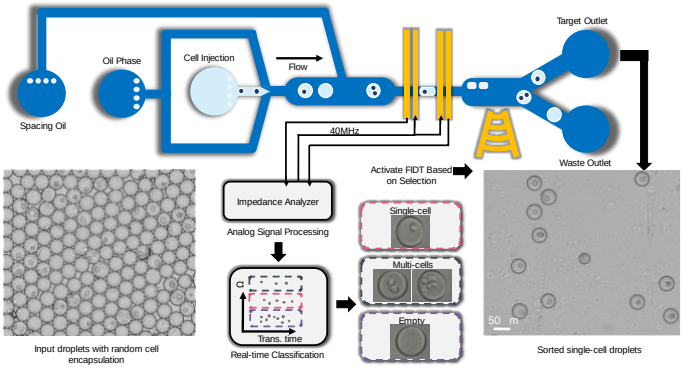

Fig. 3 Electrical impedance activated sorting of single-cell droplets from droplets with random cell encapsulation. Droplets containing no cells, single-cell and multiple cells can be identified based on a real-time impedance sensing that instantaneously activates the acoustic sorting of single-cell droplets.

3. Conclusions

We present a microfluidic biophysical cytometry integrated with high-precision acoustic manipulation that enables biophysical phenotyping activated sorting of single cells and droplets in a high-throughput manner. The biophysical cytometry allows concurrent electrical and mechanical phenotyping through single-particle electrical impedance characterization in a continuous flow. The high-precision sorting at single-cell or single-droplet level is implemented by a highly focused acoustic beam upon the activation of biophysical phenotyping. We have demonstrated this new microfluidic sorting platform for different sorting applications, for example sorting of live cells from cryopreserved cell samples and purification of droplets containing one cell and one gel-bead for single-cell sequencing.

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