SIZE SELECTIVITY AND TRAPPING EFFICIENCY OF SINGLE-CELLS WITH A HYDRODYNAMIC WELL IN A MICROFLUIDIC DEVICE
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ABSTRACT
This paper reports size selectivity of single cells and trapping performance of a hydrodynamic well (HW) in a microfluidic device. HW can trap single cells non-invasively with little dependence on cell and/or medium properties. Computational results reveal the pressure gradient and a background flow can enhance cell trapping. Cell size selection was characterized and shown a typical range of single cell size can be trapped with a high success trapping performance of 98%. The maximum number of trapped cell is depended on the length of plate’s edge and cell’s dimension. This noninvasive trapping platform using the hydrodynamic well should be useful for variety of cellular microfluidic applications.

KEYWORDS: Oscillatory plate, Lorentz force, Hydrodynamic well, Single-cell trapping

INTRODUCTION
Single-cell analysis has been increased to individual cell response and was a cell-cell interaction. Analyzing individual cells provides a more precise understanding of different cells’ behavior than bulk analysis. Noncontact method of cell trapping should be of interesting for studying non-adherent cells [1-2]. We have measured the trapping force of a single particle [3], as well as real-time monitoring in an immunofluorescent assay [4]. Here, we demonstrate that HW is able to trap a specific range of cell sizes with high trapping efficiency.

THEORY
Figure 1(a) shows the main structure of the microdevice. The main structure of the microdevice is a suspend microplate. The geometry of the suspended structure is 1.3μm thick, 20μm wide, and 750μm long, with a square microplate (100μm by 100μm) in the middle. The gold-plated structure is driven to resonate laterally (at 140 kHz) by an alternating current (AC) under an external magnetic field due to Lorenz force. At natural frequency of 140 kHz, the oscillatory microplate can generate a sharp pressure gradient close to plate’s edge. The sharp pressure gradient coupled with background flow is leveraged upon as hydrodynamics well to trap a single cell. The microdevice was bonded with a PDMS microchannel, see Fig. 1(b).

Simulation (Comsol Mutilphysics) was commenced to illuminate the physics of the phenomenon. Figure 2 showed the computational results of the time-averaged pressure distribution above the mid-plate plane. Results show definitive pressure variation near the edge: the pressure signature maximizes on the plate’s surface, decreases sharply towards the edge, and returns to ambient beyond the edge. Hence, the plate’s edge shows a distinct pressure valley forming a hydrodynamic well.

EXPERIMENTAL
Fabrication methods utilized conventional lithographic micro-fabrication. First, silicon nitride (∼1 μm) was deposited on the silicon substrate with a low-pressure chemical-vapor deposition system (LPCVD). Then, a metallic layer (0.3 μm thickness of Au/Cr) was sputtered on the silicon nitride surface. A photoresist (S1813, Shipley) layer defined the microelectrode structure, then metal-etchant. Next, photoresist on the metal was removed by acetone.
Repeating the procedure of coating photoresist and development, the cavity underneath the suspended structure was formed. Using reactive-ion-etching (RIE) to remove the non-covered. Then, a bulk micromachining process was used to remove unwanted silicon below the plate/beam structure using potassium hydroxide, thus suspending the structure.

Only two masks are needed in the whole fabrication procedure. First, the microchannel was fabricated with polydimethylsilane (PDMS, Sylgard 184, Dow Corning) via soft-lithographic technique. The ratio of elastomer/curing agent used was 10:1 by weight, mixed uniformly and degassed at low pressure for 20 minutes. Next, the pre-mixed PDMS was poured on a master and baked at 85°C for 1 hr. After cutting and peeling off from the master, the replica PDMS channel is formed.

Jurkat, Clone E6-1, an immortalized line of T lymphocyte cells, are used to study acute T cell leukemia. Jurkat was grown in RPMI-1640 (Gibco/Invitrogen) medium containing 10% fetal bovine serum, 1% penicillin-streptomycin and 1.5 g/L sodium bicarbonate. Then, cells were incubated in a 75-T culture flask at 37°C under an atmosphere of 5% CO₂. Jurkat cells were stained by Calcein AM in advance for a high contrast observation. Acetomethoxy derivate of calcein (Calcein AM, C3100MP, Invitrogen) was prepared in stock solution (50 μg Calcein AM in 9.6 μl DMSO) and diluted with PBS in a volume ratio of 250. Calcein AM can transport through the cellular membrane into cells, then hydrolyzed by an intracellular enzyme resulting in strong green fluorescence. As enzymes in the dead cells are degraded, only live cells are marked. All fluorescent images were taken via a CCD camera (DP-70, Olympus) on the microscope (IX-71, Olympus).

RESULTS AND DISCUSSION

Jurkat cells were utilized to interrogate the efficacy of the HW in trapping cells of different size. In Fig. 3, the blue histogram shows size variation within a population of Jurkat cells, indicating a normally distributed population (black dotted line), where the pink histogram reveals the capability of the HW in trapping a distinct range of cell size between 9-17 μm in diameter. The device was operating under driving voltage of 3Vpp and flow rate of 2 μl/min.

Probability for single cell trapping was tested by randomly choosing interested cells (cell sizes from 10 μm to 15 μm). The device was actuated while the targeted cell approaching to the trapping zone. There are 90 runs of single cell trapping/release in the test, and only one cell missed. Successful trapping ratio (r) is 98.9% for one single cell. Multiple cells trapping, collecting more than one cell, was tested — successful two-cells trapping is for 97.8% (~r²) and threecells 96.4% (~r³), see FIG. 5 and FIG. 6(a)-(c). We discovered the Successful trapping ratio for those cell numbers could be estimated by r^n, where r is the successful trapping ratio of a single cell (98.9%) and n is the number of trapped cells. From the results, each test is like an independent even. That shows the trapped cells would not apparently affect interior of flow field of the HW as well as the trapping ability. It presents the HW has good reliability for different cell numbers trapping, at least 1-3 cells. The maximum number of cell trapped after one manipulation was seven which depended on cell’s diameter and the length of plate’s edge, see Fig. 5(d).

Figure 3: Size spectrum of original sample (blue) and trapped (pink) cells. The cell diameter of sample ranges from 4 μm to 24 μm. The result demonstrated the hydrodynamic well can trap a specific range of cell sizes.

Figure 4 : Trapping efficiency of a single cell and multiple cells after one trapping/release manipulation. We chose interested cell sizes which are from 10μm to 15μm during the trapping and release operation. Trapping efficiency of single cell is 98.9%, and two single cells 97.8%, three single cells 96.4%.
CONCLUSION
Our work demonstrated the hydrodynamic well is capable of trapping a specific range of cell sizes with high successful trapping ratio. These features should render the noncontact cell trapping microdevice useful for cellular analysis in microfluidic systems.

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REFERENCES

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Figure 5. Images of trapped single cells. Corresponding to figure Fig. 4, images (a)~(c) show Jurkat cells trapping from one single cell to three multiple cells. (d) Image shown the maximum number of trapped cells is seven, and cells were trapped and lined up along with the microplate’s edge (length: 100 μm).