PARALLEL TRAPPING OF SINGLE MOTILE CELLS USING VIBRATIN-INDUCED FLOW ON MICROFLUIDIC CHIP

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ABSTRACT

We propose an on-chip cell manipulation method for a parallel trapping of single motile cells. The proposed method enables a parallel trapping of large (≳ 50 μm) motile cells, that is difficult to be achieved with conventional cell manipulation methods. We realize the trapping method by using a vibration-induced flow which is induced by applying a vibration to a microfluidic chip having microstructures on its surface. By applying a rectilinear vibration to a chip with a micropillar array, a localized flow pattern to trap single motile cells are generated around the micropillars. We succeeded in a parallel trapping of single *Euglena* (size is approximately 50-100 μm) by using the proposed method. Furthermore, we demonstrated one application of the method for an evaluation of motility of motile cells with a single cell level.

INTRODUCTION

Motile cells are known to have various motile characteristics, such as phototaxis, gravitaxis or chemotaxis [1][2]. Furthermore, some motile cells, such as *Euglena* or *Escherichia coli* have potential to be applied for biofuel, health food or bioactuators. To realize these applications, basic evaluations for motile characteristics of these cells with a single cell level are required.

In order to evaluate motile characteristics of single cells, trapping technology of a single motile cell is necessary. Previous researches proposed trapping method of motile cells based on an ultrasonic standing wave [3][4], surface acoustic wave (SAW) [5][6], optical or optoelectric trapping force [7][8]. However, it is difficult to trap a single cell with ultrasonic standing wave because of its large spatial resolution. While the SAW based trapping method is able to trap a single cell, it requires complex fabrication process using piezoelectric substrates. Optical or optoelectrical trappings are able to trap a single motile cell. And also, they evaluated a motility of *E. coli* by trapping a target cell with various laser power and observe if the cell escape from the trapping or not. Although the previous study succeeded in evaluating a motility of *E. coli* with size of ≈ 10 μm, it is difficult to apply this method to larger motile cells, such as *Euglena* with size of ≈ 50-100 μm due to its weak trapping force. Furthermore, it is difficult to realize parallel trapping and evaluation of motility with the optical force, that is to say throughput of this method is low. Therefore, parallel trapping method of single motile cell with large sizes (≳ 50 μm) is strongly demanded.

CONCEPT

In order to achieve the parallel trapping of large motile cells, we utilize vibration-induced flow. Vibration-induced flow is a kind of acoustic streaming caused by applying vibrations to microstructures on a chip, as shown in Fig. 1. Previously, we have proposed on-chip cell manipulation methods based on vibration-induced flow caused by applying a circular vibration to microfluidic chips having micropillar array with various configurations. We have succeeded in transportation [9], gathering [10] or rotation [11] of cells with vibration-induced flow.

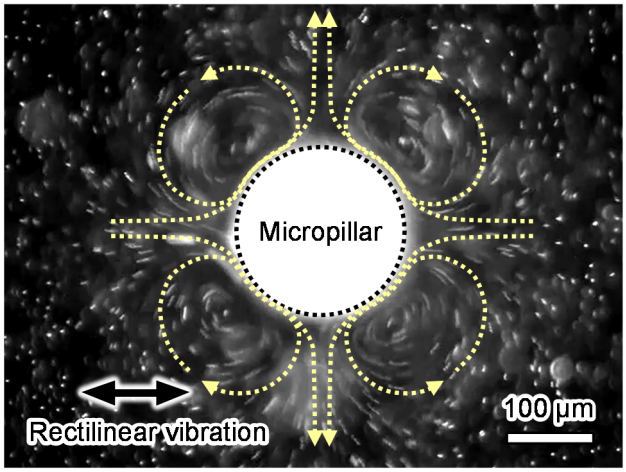

Figure 1: Vibration-induced flow around micropillar.

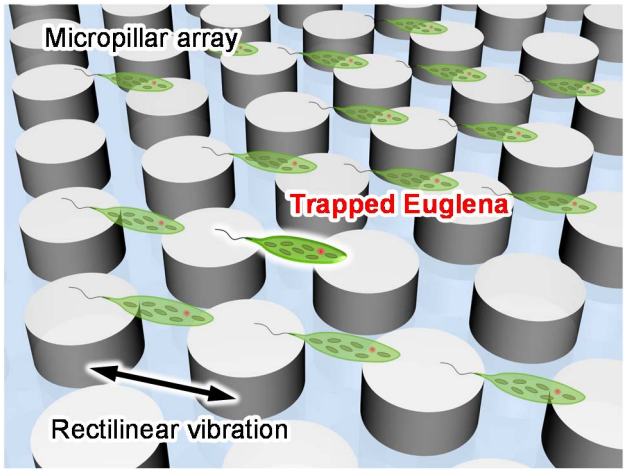

Figure 2: Concept of proposed trapping method based on a vibration-induced flow.

Important properties of the vibration-induced flow are followings; (i) strong manipulation force, (ii) simple fabrication process and (iii) large manipulation area. For (i), we can cause strong fluid force by inducing a flow with large flow velocity of the order of 100 μm/s. Thus, we can apply the flow to manipulate large cells [11]. For (ii), microfluidic chip for cell manipulation by using vibration-induced flow can be fabricated with only a single photolithography process. For (iii), vibration-induced flow can be induced around microstructures on whole area of a chip. Therefore, the flow is applicable to achieve parallel

cell manipulation on a chip.

In this study, we employed micropillar array with hexagonal configuration and applying a rectilinear vibration for parallel trapping of single motile cells, as shown in Fig. 2. When we apply a rectilinear vibration to the chip, local flow is induced between micropillars along a direction of the vibration axis, as shown in Fig. 3. Therefore, a single motile cell can be trapped between the micropillar. By using this trapping force between micropillars, we can achieve parallel trapping of single motile cells with micropillar array, as shown in Fig. 2. Furthermore, flow velocity of the vibration-induced flow can be controlled by changing an amplitude of applied vibration. Thus, we can change strength of trapping force by changing the amplitude of applied vibration and a motility of trapped cell could be evaluated by trapping the cell with various amplitude and confirm if the trapped cell escapes or not.

EXPERIMENTS

We designed micropillar array for parallel trapping of single *Euglena,* as shown in Fig. 4. According to the previous theoretical study in ref. [11], sizes of micropillar does not strongly affect the vibration-induced flow. Therefore, we determined diameter and height of micropillars as 100 μm and 50 μm, respectively. For pitch of micropillars, we determined as 150 μm, where the distance between micropillars is equivalent to 50 μm because typical size of *Euglena* is approximately 20 μm x 50 um, as shown in Fig. 4.

Experimental system setup is shown in Fig. 5. The system was constructed on a microscope (BX-71, Olympus Corporation, Tokyo, Japan) and microscopic images were recorded to the control PC by using CCD camera (Flea 3, Point Grey Research Corp., Tokyo, Japan). Micropillar array on the chip surface were fabricated from SU-8 photoresist (SU-8 3000, Nippon Kayaku Co., Ltd.) applied to a glass substrate by a standard photolithography process. The chip was attached to a mounting jig and a vibration was applied to the mounting jig by using XY piezoelectric stage (PK2H100-030U, Nano Control Co. Ltd., Tokyo, Japan). Source of a driving voltage of the XY piezoelectric stage was output from a function generator (WaveStation 2012, Teledyne LeCroy Japan Corp., Tokyo, Japan). This output signal was amplified by a high-voltage amplifier (9400, Gain x50, Toyo Corporation, Tokyo, Japan). We employed a sinusoidal wave to actuate the XY piezoelectric stage.

As a target cell, we used *Euglena gracilis* in this study.

RESULTS

First, we confirmed a flow pattern around micropillar array when a rectilinear vibration was applied to the chip. The expected trapping flow which is illustrated in Fig. 3 was observed, as shown in Fig. 6.

Next, we confirmed that the proposed method was able to realize parallel trapping of Euglena. When we applied rectilinear vibration with frequency of 1 kHz and amplitude of 20 μm, Euglena cells were successfully trapped, as shown in Fig. 7. According to the observation in Fig. 7, we succeeded in trapping of single Euglena at some trap points

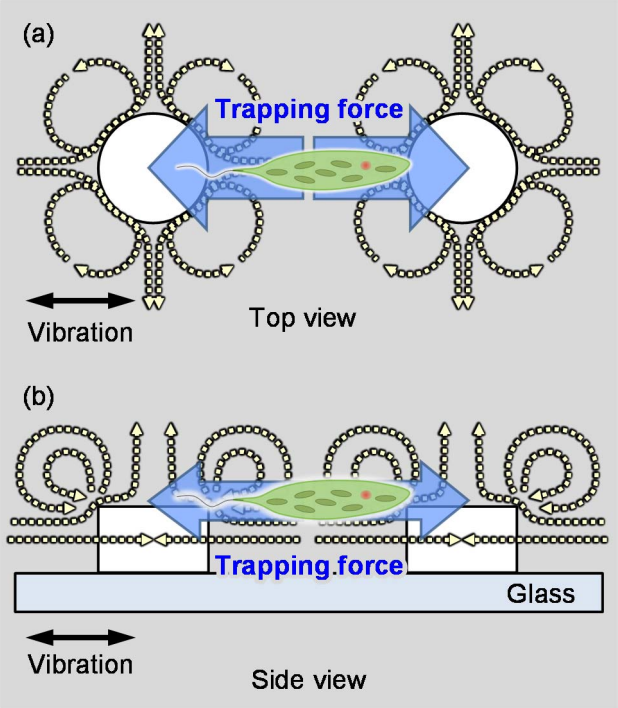

Figure 3: Schematic images of mechanism of a single cell trapping by using the proposed method. (a) Top view, (b) side view.

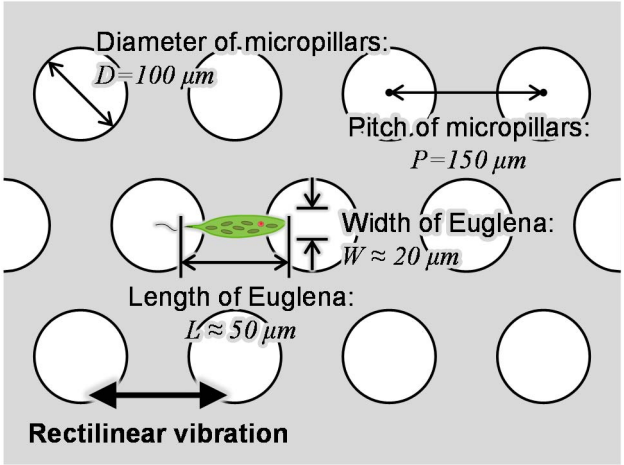

Figure 4: Design of micropillar array for trapping of Euglena

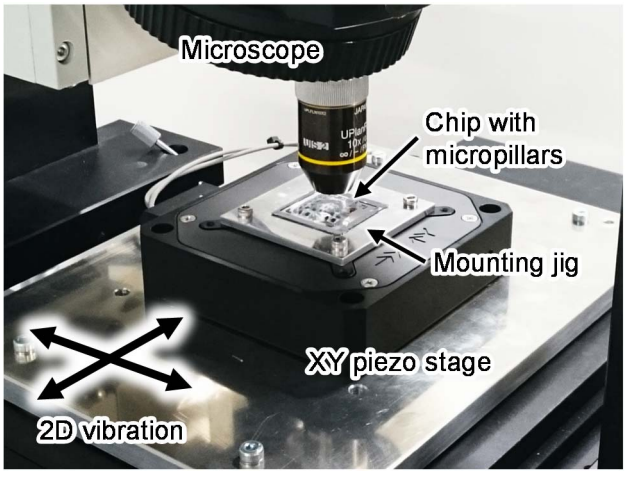

Figure 5: Experimental system setup

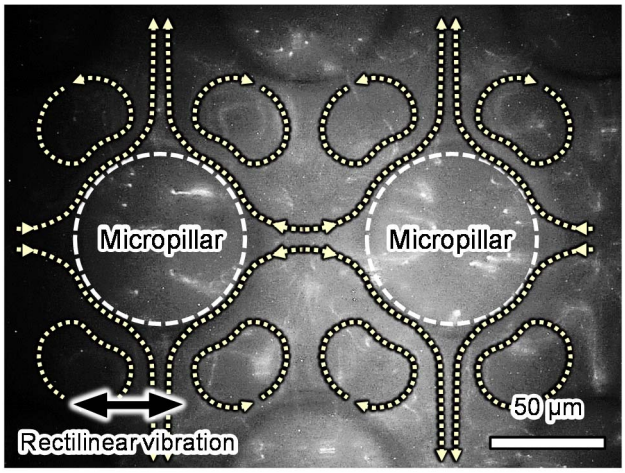

Figure 6: Streamline around micropillar array for trapping of Euglena visualized by using fluorescent microparticles. Frequency and amplitude of applied vibration was 400 Hz and 10 μm, respectively.

(shown in blue dotted circle in Fig. 7), but double Euglena were also trapped (shown in red dashed dotted circle). For our original purpose, parallel trapping of single motile cells, this double trap was not favorable. Thus, we evaluated trap rate which is defined as

$$Trap\ rate = \frac{number\ of\ trapped\ cells}{number\ of\ trap\ point} \quad (1)$$

with various amplitude of applied vibrations, as shown in Fig. 8. By changing the amplitude of applied vibration, we succeeded in parallel trapping of single Euglena with 52 % trap rate. Furthermore, viabilities of all trapped Euglena were confirmed by stopping applying the vibration and observing that they moved same as before the trapping.

Finally, we demonstrated parallel evaluation of motility of single Euglena by using the proposed trapping method. After we trapped Euglena with the amplitude of 20 μm, we decreased the amplitude step-by-step and observed number of Euglena escaped from the trapping. We performed this evaluation for 45 Euglena with one trial and evaluated escape rate which is defined as following equation.

$$Escape\ rate = \frac{number\ of\ escaped\ cells}{number\ of\ trapped\ cells} \quad (2)$$

As shown in Fig. 9, most of Euglena escaped under the condition of amplitude was 6 μm. However, small number of Euglena, approximately 2 % of total, escaped under the higher amplitude such as 16 μm. This result indicate that the Euglena sample escaped at 16 μm could have higher motility than usual samples. Thus, we confirmed possibility of our proposed method for high-throughput evaluation of motility of motile cells with single cell level.

CONCLUSIONS

In this study, we propose an on-chip cell manipulation method for a parallel trapping of single motile cells. The parallel trapping of single motile cells is achieved based on a vibration-induced flow. The vibration-induced flow for trapping of a single motile cell is induced around micropillar array on a chip by applying a rectilinear

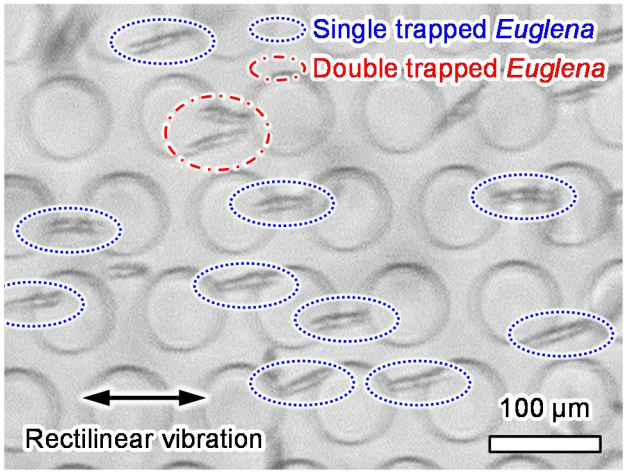

Figure 7: Microscopic image of trapped Euglena with proposed method. Frequency and amplitude of applied vibration was 1 kHz and 20 μm, respectively.

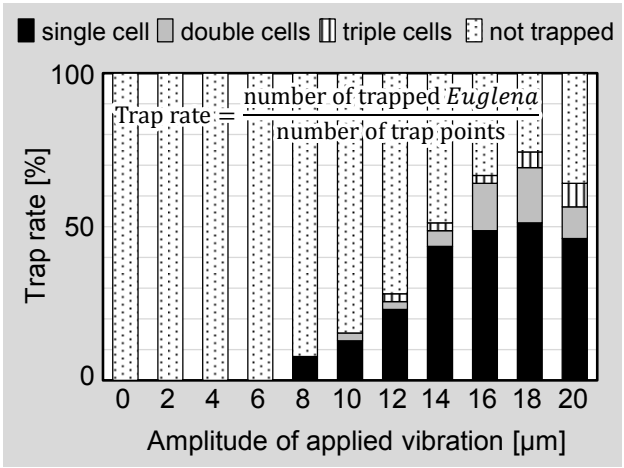

Figure 8: Trap rate of Euglena with various amplitude of applied amplitude.

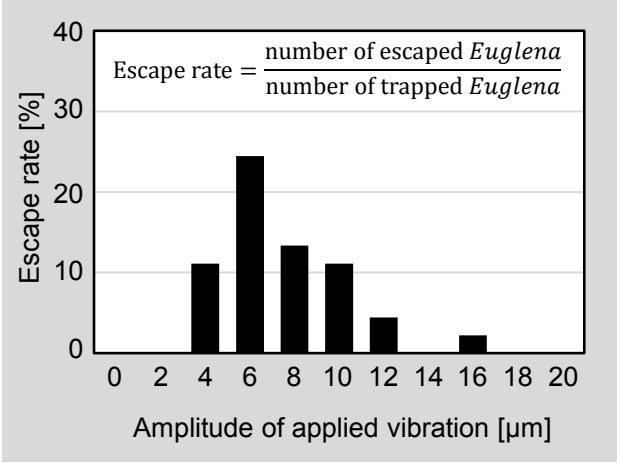

Figure 9: Escape rate of trapped Euglena with various amplitude of applied vibration

vibration to the chip. The proposed method enables a parallel trapping of large (≳ 50 μm) motile cells, that is difficult to be achieved with conventional cell manipulation methods based on ultrasonic standing wave, SAW, optical or optoelectric forces.

We succeeded in a parallel trapping of single Euglena (size is approximately 50-100 μm) by using the proposed method with 52 % trap rate. Furthermore, we demonstrated

evaluation of motility of Euglena with single cell level.

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