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## On-chip microfluid induced by oscillation of microrobot for noncontact cell transportation

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The importance of cell manipulation and cultivation is increasing rapidly in various fields, such as drug discovery, regenerative medicine, and investigation of new energy sources. This paper presents a method to transport cells in a microfluidic chip without contact. A local vortex was generated when high-frequency oscillation of a microtool was induced in a microfluidic chip. The vortex was controlled by tuning the tool's oscillation parameters, such as the oscillation amplitude and frequency. The cells were then transported in the chip based on the direction of the tool's movement, and their position, posture, and trajectories were controlled. Bovine oocyte manipulations, that is, transportation and rotation, were conducted to demonstrate the capability of the proposed method, without any contact by the microrobot with high-frequency oscillation. © 2017 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1063/1.5009545

In biology, the study of cells attracts considerable attention because the cell is the basic structural, functional, and biological unit of all known living organisms. The importance of cell manipulation and cultivation has been increasing rapidly in various fields, such as drug discovery, regenerative medicine,<sup>1</sup> and investigation of new energy sources. Biomanipulations widely used in many applications show great commercial value, with over tens of billions of dollars invested into this field worldwide.<sup>2-6</sup> There are two types of methods for cell manipulation: contact<sup>7-9</sup> and noncontact manipulations. To achieve much more flexible actuation in a closed space, researchers have recently begun studying the noncontact manipulation of robots, especially for biological applications. Noncontact methods show great merits on non-contaminations and remote control. Therefore, many optional methods without contact, such as electric force,  $^{10,11}$  optical force,  $^{12,13}$  acoustic force,  $^{14,15}$  and magnetic force,  $^{16-18}$  have been well studied. For example, a rotating magnetic field was applied to drive Ni nanowires with a tumbling motion.<sup>19-21</sup> The local flows around the nanowire can be used to manipulate micro-objects without contact. However, the applied force on the object was small, and thus insufficient for the transportation of some large objects, such as mammalian oocytes.

In this paper, we propose a method for noncontact cell transportation by using permanent magnets to oscillate a microtool at a high frequency in a microfluidic chip. A local vortex could be generated when the tool is oscillated at a high frequency, and the fluid stream transports cells without tool contact. Figure 1 shows the concept of noncontact manipulation through the generated vortex. The vortex form varies depending on the tool oscillation form, and can thus be controlled by changing several parameters, such as the oscillation amplitude, frequency, and relative position between the tool and microchannel wall. Owing to their attraction to the vortex, the cells transport through the microtool, which oscillates at a high frequency in a microfluidic chip. The tuning of the oscillation parameters of the tool changes the viscous fluid dynamics, and the cells can be manipulated away from the tool without contact. As this is a noncontact operation, there are no concerns regarding cell adhesion to the tool, unlike for other methods in which there are occasional issues when the manipulator handles adherent cells. In addition, the tool can manipulate cells through contact as well because it has enough physical strength to handle cells.

A steady stream, which is a function of oscillation, is generated when an object is oscillated in the viscous fluid.<sup>22–24</sup> However, the Reynolds number is generally small at a microscale, and a high frequency is required to generate the vortex. Assuming that the steady stream occurs twodimensionally when a plate oscillates in a microfluidic chip, the local fluid velocity  $(U_x, U_y)$  can be calculated using the model proposed by Wang *et al.* and the modified Reynolds



FIG. 1. Concept of noncontact cell manipulation through high-frequency oscillation in a microfluidic chip.



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number  $Re = \omega a^2/\mu$ , where  $\omega$  is the frequency of the plate oscillation, *a* is the gap between the oscillating plate and channel wall, and  $\mu$  is the kinematic viscosity. Then,

$$U_x = xA\omega \cdot \frac{d}{dt}f(y,\lambda), \tag{1}$$

$$U_{y} = -xA\omega \cdot f(y,\lambda), \qquad (2)$$

$$\lambda = (1+i)\sqrt{Re/2},\tag{3}$$

where  $f(y, \lambda)$  is the function that considers the oscillation effects and *A* is oscillation amplitude. The imaginary number *i* is defined solely based on the property that its square is -1, and *Re* stands for Reynolds number.<sup>25</sup>

Figure 2 shows the simulation results when the microtool was oscillated using the permanent magnets with a  $\pm 0.5$  mm amplitude in the Y direction only, with a sine wave oscillating frequency of 30 Hz. Under this condition, a regular vortex was generated, and the speed of the vortex reached to tens of micrometers per second. The vortex direction regularly changed with the movement of the oscillating microtool. This result implies that a tool with high-frequency oscillation in a microfluidic chip can transport cells without contact when the cells are attracted by the vortex, and the vortex can be controlled by configuring the oscillation.

By using the previous calculation results, the drag force from the generated vortex acting on a single cell can be estimated by

$$F_d = \frac{1}{2}\rho v^2 C_d A,\tag{4}$$

where  $F_d$  is the drag force,  $\rho$  is the fluid density, v is the speed of the object relative to the fluid,  $C_d$  is the drag coefficient, and A is the reference area. From the equation, we can easily find that the drag force is relative to the fluid velocity. In our experimental condition, we used the culture medium, the kinematic viscosity of which approached that of water so that  $\rho = 1 \text{ g/cm}^3$ , and the frequency is 30 Hz. When the microrobot moved in the microchip, the average gap between

the oscillating plate and channel wall was 35 mm. Therefore, the Reynolds number was approximately equal to  $2 \times 10^5$ . The drag coefficient can be approximated as follows:<sup>26</sup>

$$C_d \approx \frac{24}{Re} + \frac{6}{1 + \sqrt{Re}} + 0.4 = 0.4135.$$
 (5)

The reference area is estimated as  $A = \pi \times R^2 \approx 3.14 \times (75 \times 10^{-6})^2 = 1.77 \times 10^{-8} \text{ m}^2$  (R is the cross-sectional radius). Thus,

$$F_d = \frac{1}{2}\rho v^2 C_d A = 3.66 \times 10^{-6} \times v^2.$$
 (6)

According to Eqs. (1), (2), and (6), the drag force is proportional to the oscillated frequency, thus when the frequency increases the force also increases.

The fabrication process of microrobot is described as follows.<sup>27</sup> The silicon-based microrobots can be made into diverse shapes through deep reactive-ion etching (DRIE). First, the photoresist OFPR (Tokyo Ohka Kogyo, Co.) was coated on the substrate. After exposure on the OFPR side, an OFPR pattern was developed. Next, DRIE was conducted from the OFPR side, and the silicon was etched to a depth of 125  $\mu$ m. By exposing both sides, we coated the opposite side of the silicon with OFPR and performed the patterning and exposure after the DRIE process. Then, we finished creating the silicon-based microrobot. The images obtained using the scanning electron microscope show the two-time DRIE-fabricated microrobot. Last, four neodymium (Nd<sub>2</sub>Fe<sub>14</sub>B) magnets (Magfine, Co., Japan), that is,  $\varphi$ 0.5 mm × 0.5 mm permanent magnets, were assembled to shape the microrobot.

To control the microrobot oscillation in a closed microfluidic chip, the magnetic control had many obvious advantages, such as non-contact, high-speed actuation<sup>28</sup> (up to 90 Hz), and precise positioning accuracy<sup>29</sup> (micrometer order) in the X-Y plane. Therefore, a magnetically driven microtool<sup>18</sup> (MMT) was the optimal option for the oscillating tool. The experiments showed the velocity vector field on the microfluidic chip when the microrobot was moving



FIG. 2. Simulation results of velocity vector field in a microfluidic chip (arrow represents the velocity vector, and the contour is the magnitude). The microrobot oscillated in the (a) positive direction of the Y-axis and (b) negative direction of the Y-axis.

with high-frequency oscillation. Fluorescent beads of  $2 \mu m$  diameter were added to the fluid to visualize the actual distribution of the vortex. The shape of the microrobot made of silicon and nickel is shown in Fig. 1, in which four permanent magnets are fixed at four corners. The size of the oscillation tool was 0.3 mm (width)  $\times$  0.2 mm (height), and the width and height of the microfluidic chip were 3 cm and 0.5 mm, respectively.

When the microrobot oscillated at the Y-axis by permanent magnets, objects were pulled to the edge of the microrobot. Then, only the object was transported by moving the microrobot on the X-Y plane. Therefore, by moving the microrobot, the fluorescent beads can easily be arranged in the plane and the microfluid distribution can be visible, as shown in Fig. 3 (Ref. 30) (Multimedia view). Figure 3(a) shows the high-frequency oscillating microrobot. Figure 3(b) shows the movement of the microrobot from left to right, whereas Fig. 3(c) shows the movement in the opposite direction. To move the microrobot from the rear side to the front side, the fluorescent beads can be arranged as shown in Fig. 3(d). Figure 3 also shows the measured velocity vector of the fluorescent beads under a set condition: amplitude of  $\pm 0.5$  mm and frequency of 30 Hz. The object velocities were estimated using a method based on image processing. In this study, we used the block matching theory, which estimates motion between two images or two video frames using "blocks" of pixels. Although the velocity vector field was slightly different from the simulation results because of the limitations of the robotic shape, the vortex around the edge was based on the simulation analysis. According to velocity vector analysis, the highest velocity of the fluid was at the edge of the tool. Therefore, the cell transportation and rotation could be achieved by these vortexes. In addition, in the process of moving the microrobot with high-frequency oscillation, the vector field slightly changed, and the vortex changed with change in the oscillating conditions. Of course, these are only some simple experiments to prove that the microrobot with high-frequency oscillation can produce a certain flow field distribution. However, more flow fields can also be achieved by adjusting the fluid factors, such as oscillation amplitude and frequency.

Figure 4 shows the results of the hydrodynamic force before and during oscillation. The oscillating conditions included amplitude of  $\pm 0.5$  mm, oscillation frequency of 30 Hz. After the velocity of the fluid was analyzed, the hydrodynamic force distribution was calculated using the equation of drag force [Eq. (6)]. The simulation shows that the hydrodynamic force dragging a single cell could reach the order of micro-Newton. According to Fig. 4, before the oscillation, only a slight force was generated away from the tool because of the external interference. Moreover, when the microrobot was oscillating, the drag force around the tool edge was significantly higher than that at other areas, while the hydrodynamic force was the highest at the tip area of the microrobot. Thus, this hydrodynamic force can drag cells when the microrobot with high-frequency oscillation moves.

The basic experiments and simulations of the velocity vector field showed that with microrobot with high-frequency oscillation was able to transport cells without contact. Therefore, by using the bovine oocytes as the manipulating object, we demonstrated its actual transport state. Except for the diameter of the oocyte, which was  $150 \,\mu$ m, the other conditions were the same as those in the experiments of fluorescent beads, that is, the oscillating amplitude was 0.5 mm and frequency was 30 Hz. Figure 5 shows that by using a microrobot, the cell can be easily transported to the designated spot



FIG. 3. Experimental result using fluorescent beads ( $\Phi 2 \mu m$ ): (a) microrobot oscillation; (b) microrobot moving from left to right; (c) microrobot moving from right to left; and (d) microrobot moving from rear to front (the red arrow represents the microrobot movement direction, and the blue arrow represents the oscillation direction. Multimedia view: https://doi.org/10.1063/1.5009545.1



FIG. 4. Hydrodynamic force distribution in a chip: (a) before the microrobot oscillated and (b) during microrobot oscillation.



FIG. 5. Bovine oocyte manipulation using the microrobot: (a) oocyte kept stationary; (b) oocyte was transported to the rear side; (c) oocyte was transported from left to right; and (d) oocyte was transported from right to left (the red arrow represents the microrobot movement direction, and the blue arrow represents the oocyte transporting direction. Multimedia view: https://doi.org/ 10.1063/1.5009545.2

(Multimedia view). Figure 5(a) shows that the cell was stationary and away from the microrobot before its oscillation. The oocyte can be transported from the front side to the rear side as shown in Fig. 5(b). Figure 5(c) shows the transportation of the oocyte from left to right, while Fig. 5(d) shows the transportation in the opposite direction. If the relative position of the cell and microrobot was very close, cells would be occasionally adsorbed into the tool. However, this had no significant effect on the process of transporting other cells without contact. If necessary, the microrobot can also perform cell manipulation, such as cutting and injection, through contact, as presented in our previous works.<sup>31,32</sup>

In summary, the movement of the microrobot with high-frequency oscillation in the microfluidic chip generated a velocity vector field, the distribution of which depends on the conditions of the oscillation and movement trajectory. Cells could be attracted by the vortex flowing around the edge of the oscillating microrobot and follow the tool without contact when the microrobot moved in the microfluid chip. This noncontact manipulation has many advantages, including larger control of the area and the movement of the cell in a predetermined trajectory. Although sometimes cells accidentally adhered to the tool, other noncontact manipulations were not disturbed, and the tool could conduct contact operation on the cell.<sup>31,32</sup>

In this paper, we demonstrated the noncontact transportation of a single cell. It is also possible to simultaneously manipulate several cells by using the experimental results of fluorescent beads. In addition, the tuned movement trajectory or oscillation parameters of the microrobot can change the transporting route of the cell, thus achieving the manipulation requirements. Our future work would involve the achievement of high-precision movement of noncontact manipulations in a three-dimensional microfluidic chip.

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