A Microfluidic Flow-switching Device Powered by *Vorticella* Stalk

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**Abstract.** Bioactuators are an attractive alternative for mechanical components of MEMS devices. We propose a flow-switching device active to calcium ion based on bioactuator of *Vorticella*. We develop a fundamental procedure for immobilization of *Vorticella* in a microfluidic chamber and control of contraction and extension of stalks. Cells were trapped in microfluidic chambers and allowed to adhere. After treatment of cells, stalks were contracted and extended by injecting solutions. Flow speed changed during the motion. Our developed method presents a strategy for application of bioactuator.

**1. Introduction**

Microfluidic device is widely used for chemical and bioanalysis applications [1]. Whereas application of active elements is preferred for controllable systems [2], traditional active elements enlarge the systems because a driving circuit is required. Use of cellular actuators (bioactuators) can eliminates an external circuit and enables us to achieve miniaturization and functionalization of the systems, since energy conversion cycle is contained in a cell body. Bioactutors are an attractive alternative of active elements. We have employed a contractile stalk from *Vorticella* [3]. Stalk of *Vorticella* is several tens of µm in length and composed of filamentous spasmoneme. After permeabilization of stalk, it contracts and extends in Ca²⁺ solution at the concentration range from 10⁻⁸ to 10⁻⁵ M [4]. In this paper, we propose a flow-switching device active to Ca²⁺ based on *Vorticella* stalk. Figure 1 shows a flow-switching mechanism powered by the motion of *Vorticella*. We develop a method to immobilize this highly motile organism in a targeted microchamber. We demonstrate control of its reversible linear actuation by changing concentration of Ca²⁺ and measure change in flow speed.
2. Experimental Methods

2.1. Design and fabrication of a fluidic device for cell immobilization and motion control

We design and fabricate a flow channel device (Figure 1a) for immobilization and motion control of *Vorticella*. We use the approach developed by W. Tan et al. [5] and modify the design to trap a highly motile cell in a microchamber. Figure 2 shows hydrodynamic trapping of *Vorticella* into a microchamber with two narrow segments. A cell is pressed into the entry narrow segment, trapped in a target microfluidic chamber (Figure 2a, b). Ratio of flow through trapping channel ($Q_1$ through path1) to by-pass channel ($Q_2$ through path2) was designed to satisfy $Q_1/Q_2 > 1$ to trap *Vorticella* in a chamber. $Q_1/Q_2$ is expressed as follows,

$$ \frac{Q_1}{Q_2} = \left( \frac{C_2(\alpha_2)}{C_1(\alpha_1)} \right) \left( \frac{L_2}{L_1} \right) \left( \frac{W_2 + H}{W_1 + H} \right)^2 \left( \frac{W_1}{W_2} \right)^3 $$

(1)

where $L$ is channel length, $W$ is channel width, $H$ is channel height, and $\alpha$ is aspect ratio. $C(\alpha)$ denotes a
constant that is a function of $\alpha$. $Q_1/Q_2$ is designed to be 5.23. A channel was fabricated in PDMS (Sylgard 184) by standard soft-lithography [6]. We used SU-8 3050 as a mold of flow channel. SU-8 was spin-coated on silicon wafer at 3,000 rpm, baked, and exposed. The dimensions of the channel are listed in Table 1.

### Table 1. Dimensions of microfluidic channels to trap a cell.

| Design          | Path 1 | Path 2 |
|-----------------|--------|--------|
|                 | Width, $W_1 \mu$m | Length, $L_1 \mu$m | Width, $W_2 \mu$m | Length, $L_2 \mu$m | Height, $H \mu$m |
| Fabricated      | 16     | 40     | 100     | 5600     | 30       |
| $(n=5)$         | 18.3±1.3 | 40.9±1.0 | 104.0±0.6 | 5517±3   | 34.9±0.6 |

#### 2.2. Cell preparation

*Vorticella* for injection was prepared according to the method reported by Matsumoto [7]. We stored the prepared cells at a concentration of $4 \times 10^3$ cells/µL in a reservoir and injected them into a flow channel by gravitational force. Flow speed was adjusted to 50 µm/sec at which *Vorticella* was trapped.

*Vorticella* was treated and controlled with three solutions shown in Table 2. Cells adhered to the surface was permeabilized by treating with surfactant solution containing saponin to control the stalk length by $Ca^{2+}$. EGTA solution collects $Ca^{2+}$ from a stalk and extends the stalk. Concentration of free $Ca^{2+}$ was adjusted to 1 µM under equilibrium condition with EGTA (ethylene glycol tetraacetic acid) and $Ca^{2+}$.

### Table 2. Composition of solutions for treatment of *Vorticella*.

| Solution                  | Composition                                                                 |
|---------------------------|-----------------------------------------------------------------------------|
| Surfactant solution       | 0.1%(w/w) Saponin, 0.1 M KCl, 4 mM EGTA, 50 mM Tris-maleate buffer pH 7.0   |
| Extension solution        | 0.1 M KCl, 4 mM EGTA, 50 mM Tris-maleate buffer pH 7.0                      |
| Contraction solution      | 0.1 M KCl, 3 mM CaCl$_2$, 4 mM EGTA, 50 mM Tris-maleate buffer pH 7.0        |

#### 2.3. Observation

Cells were observed under the inverted microscope (Eclipse TE2000-U, Nikon Instruments Inc.) equipped with objective lens $\times 10$ or $\times 20$. Movies were recorded with a CCD camera at 5 fps. Stalk length was analyzed with image analysis software (Image J). Flow speed was measured by tracking fluorescent particles ($\phi 0.5 \mu$m).

#### 3. Results and discussion

##### 3.1. Trapping and immobilizing Vorticella

Figure 3 shows a single cell trap in a chamber. When *Vorticella* reached an entry site, the cell was introduced into a chamber via Path1 and immobilized on a trapping site (Figure 3a). After the cell was trapped, the next coming cell reaching the entry site flowed via Path2 (Figure 3b-d).
After Vorticella was trapped, flow speed was reduced. Although Vorticella freely swum in the chamber (Figure 4a-c), the cell was unable to move outside the chamber for itself. This is because the width of an entry site ($W_1$, 18.3 µm) is smaller than Vorticella with a diameter of 30 µm. 16h after introduction, most of the cells adhered in the chamber and grew their stalks as shown in Figure 4d.

Figure 5 is cell number distribution of immobilized Vorticella in microchambers. 46 cells were trapped in 39 of 68 chambers. 16 h after the trap, 26 cells adhered in the chambers. Stalks were treated with surfactant solution before motion control. Following surfactant treatment, total 12 cells remained in the chambers. Cells trapped in the chambers decreased from 34 to four. The number of chambers with two to three cells decreased from five to three.

**Figure 3.** Trapping of a single Vorticella in a chamber.

**Figure 4.** (a)-(c) Motion of pressed-in Vorticella in a microchamber. (d) Vorticella adhered in a chamber.
3.2. Motion control of stalk

Figure 6 and 7 shows control of stalk contraction and extension in a microchamber by changing the concentration of Ca$^{2+}$. By alternately injecting chelator solution (containing EGTA for removing Ca$^{2+}$) and contraction solution, we controlled stalk length reversibly. The lengths of stretched and contracted stalk were 35.4 ± 9.13 µm and 9.37 ± 3.29 µm (n=6), respectively. Transition time from extension to contraction and from contraction to extension was 9.11 ± 0.63 s (n=3) and 14.9 ± 1.28 s (n=3), respectively. Change in flow speed was measured by tracking fluorescent particles. Flow speed was changed accompanied by the motion of *Vorticella*. A flow speed through path1 was 345 ± 99 µm/s (n=4) in contracted state, while the speed was 138 ± 36 µm/s (n=4) in extended state. This decrease may be attributed to the increase of fluidic resistance. Change of flow speeds shows the possibility of flow switching.

![Figure 5](image1.png)

**Figure 5.** Histogram of number of *Vorticella* in a single chamber during trap and cell treatment.

![Figure 6](image2.png)

**Figure 6.** Micrographs of three cells in a single chamber. Time series extension of the three stalks.
Figure 7. Reversible control of three stalks by changing the concentration of Ca$^{2+}$. (a) Contraction of the stalks by injection of Ca$^{2+}$ solution. (b) Extension of the stalks by injection of chelator solution.

4. Conclusions

In this research, we proposed a flow-switching device active to Ca$^{2+}$ based on Vorticella. We demonstrated immobilization of Vorticella in a microchamber. Reversible actuation of Vorticella was achieved in a microchamber by exchanging solutions. Change of flow speeds shows the possibility of flow switching. Our developed method has the potential to accelerate the application of bioactuators.

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