Flow Regulator Powered by the Stalk of Vorticella convarallia

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Abstract. We propose flow regulation by the stalk of Vorticella convarallia. The motion of stalk can be controlled by introducing Ca$^{2+}$ and chelator solutions alternatively. The fluid channel is narrowed by fully extended V. convarallia, which results in increasing fluidic resistance and reducing flow rate. The flow regulator consists of a microchannel and Vorticella. The microchannel has two layers. The top layer contains a fluid channel where Vorticella is stored to control the flow rate. The bottom layer includes a control channel which enables control of the fluid channel by an external compressed air supply. After cells were introduced to the device at $1.0 \times 10^3$ cells/µL, eight of 18 chambers contained more than a single cell. Four cells of Vorticella were cultured in one chamber at a maximum. The cells were alive for two days after the injection. We evaluated the change in mean flow rate through the fluidic channel. The flow rate without V. convarallia increased linearly from 5.5nL/min to 1.1µL/min with applied pressure from 4 to 18kPa. The switching time of solutions measured two seconds at 10 kPa. The response time for the device was estimated to be approximately four seconds after the partial removal of cell membrane.

1. Introduction

MEMS (Microelectromechanical system) devices have proven to be valuable in several subject areas. MEMS based physical sensors have contributed on improving the machinery performance. However MEMS based devices suffer from operation under water and dependency of an external driving circuit. They normally require a large power supply, which leads to poor portability. Biological actuators and chemical sensitive hydrogel have been developed to remove the power supply, because they obtain their driving energy from a solution. There is wide range of biological actuators available in micro scale such as cardiomyocytes (1)(2) or Mycoplasma (3). Those bio actuators consist of energy conversion circuits and sensing elements. Their energy conversion circuits convert chemical energy such as ATP to mechanical work (4). A flow regulator equipped with a hydrogel actuator successfully regulated a flow through a micro fluid channel. (5) The hydrogel actuator suffered from small displacement and slow response to start the actuation. The displacement and response time of the biological actuator are superior to the hydrogel. For example, Vorticella can contract from 60µm to 20µm in 2s after partial removal of cell membrane. (6) Therefore, integration of microorganism to a microdevice has great potential for many applications.

We propose the flow regulator powered by stalk of V. convallaria (Vorticella convallaria). The device is an example of integration of biological actuator and artificial structure. V. convallaria is classified as stalked ciliate protozoan which inhabits at fresh water area (Figure 1). It consists of a filament like motive organ called stalk, a bell shaped zooid and an array of cilia located around the oral region. V. convallaria is normally anchored onto a substrate. The contraction of stalk completes in 7–8 ms. This sudden contraction is occurred by rapid release of Ca$^{2+}$ produced within the zooid. The stalk returns to the original length in several seconds. The motion of stalk is controlled by partial removal of cell membrane with surfactant solution. After the treatment, it becomes permeable to external supply of Ca$^{2+}$. The motion of stalk can be controlled by varying concentration of Ca$^{2+}$. The flow rate regulation can be done by this particular movement of the stalk within the micro fluidic channel, which causes the change in fluidic resistance in the micro channel. The flow regulation is
critical for the micro fluidic device, e.g. micro total analysis system, since precise control of injecting reagents is essential to obtain desired chemical reaction. In this paper, we fabricated the multilayer fluidic device for flow regulation and investigated the flow speed and the flow rate through the channel. We evaluated the stalk growth and distribution of *V. convallaria* cells in the fluidic channel. On-chip permeabilize treatment was also carried out.

**Figure 1.** Schematic diagram of the motive organs of *V. convallaria*. a) Contraction and relaxation of stalk by injection and removal Ca\(^{2+}\) b) Image of *V. convallaria*.

**2. Operational principle**

The proposed micro flow regulator utilizes *V. convallaria* as biological actuator which changes its length by varying concentration of Ca\(^{2+}\). Figure 2 shows the operational principal of proposed flow regulator. When *V. convallaria* is introduced to the fluid channel, it swims freely and adheres on the wall of channel spontaneously. Then the cell membrane is partially removed by surfactant solution. The stalk is contracted and extended by introduction of Ca\(^{2+}\) and chelator solution alternatively, which results in two forms. The relationship between three variables, flow rate, \(Q\) (m\(^3\)/s), applied pressure, \(P\) (Pa), and fluidic resistance, \(R\) (Pa s/m\(^3\)), is given by the following expression.

\[
Q = \frac{P}{R} \tag{1}
\]

Under the assumption that the applied pressure is constant, we can select two different flow rates. (a) A fully extended stalk causes clogging in the channel. The total fluid resistance in a single element is the summation of the fluid resistance of element, \(R\), and the fluid resistance excreted by *V. convallaria*, \(\Delta R\). The flow rate is reduced to \(P/(R+\Delta R)\). (b) The injection of Ca\(^{2+}\) makes the stalk contract. The fluid

**Figure 2.** Schematic diagram of operational principle
resistance is reduced to $R$. The flow rate increases to $R/P$. The fluid resistance exerted in each element is enhanced and averaged by arranging 18 bottle neck shaped elements (the volume of single element: $300\times100\times28\ \mu\text{m} = 0.84\ \text{nL}$) in series for the fluid regulation effect to be greater. The flow regulator is equipped with two separate regulator sections. This allows us to carry out two different conditions for flow regulation in a single device by setting different concentration of $V.\ \text{convallaria}$ on each section. The device consists of two layers of fluidic channel, fluid layer and control layer. There are three main elements in the device: 1) Regulator section on fluid channel to contain cells and control flow rate. 2) Pneumatic valves on control channels to select solutions. 3) Fluid channel with four inlets and one outlet. All reagents including Ca$^{2+}$ and chelator solutions are delivered to the fluid channel with pneumatic valves by compressed air. The target solution of the current device is limited to water in order not to damage $V.\ \text{convallaria}$. A Wide variety of fluids can be used by making a partition and separating working fluid and analytic fluid.

3. Experimental method

3.1. Fabrication of multi-layer fluidic channel
We fabricated the multilayer fluidic channel to achieve fluid flow regulation. The device was fabricated by multilayer soft lithography technique (7). Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) is used to cast the two channel layers. The upper and lower layers are fluid and control channels, respectively. The push-up valve structure is employed in our device (8). The fluid channel is pushed upwards by the control channel via the valve membrane using compressed air.

Figure 3 shows a schematic diagram of the fabrication process. The fabrication is broken down in three main processes, (a) mould fabrication for fluid channel layer, (b) mould fabrication for control channel layer and (c) casting and bonding of channel layer. (a) We spin-coated a thick positive photoresist (AZ P4903) on silicon wafer with the thickness of 30 µm. The photoresist was patterned by standard photolithography. Upon development, patterned photoresist was reflowed by heating over the glass transition temperature, 110 °C for 15min to obtain semi elliptic cross sectional shape. Epoxy resin mould (Devcon ET 300, Illinois Tool Works Inc) was prepared by replica molding technique to enhance the durability of the mould. (b) The mould of control channel was made of thick negative photoresist, SU-8 3050. The photoresist was spun on silicon wafer to obtain the thickness of 30 µm. The mould was patterned by photolithography. (c) Two PDMS layers of control and fluid channels were fabricated individually. The base polymer and catalyst of PDMS, we use in this paper, were all mixed with the ratio on 10:1. Both moulds were treated with the vapor of Trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich) at 60 °C for 4min to improve the release of PDMS. Uncured PDMS was poured onto the epoxy resin mould and obtained about 1 cm thickness of fluid channel layer. For the control channel layer, the PDMS was spun at 1800 rpm for 60s. Both layers were cured at 80 °C for 1h. The fluid channel layer was peeled off from the mould. The access holes were punched using a biopsy punch (0.5mm diameter, Harris, Uni-Core, Ted Pella Inc, Redding, CA) and trimmed to shape. This layer was cleaned with isopropyl alcohol and dried with nitrogen gas. Both control and fluid layers were bonded after the corona discharge treatment with the corona treater, BD-20AC (Electro-Technic Products, Inc.) for 20 s. We aligned both layers under the microscope and baked the assembled device at 80 °C for 40 min. The assembled layer was peeled off from the mould and the access holes for the control channel were punched. The final device was made by bonding cover slip and the assembled layer by the same bonding technique.
3.2. Device calibration

The manipulation of reagents (solution input and replacement) was calibrated using blue and red food dye solutions for flow visualization. The concentration of the dye solutions was adjusted to 10 mg/mL with deionized water. We monitored a flow through the fluid channel with the inverted microscope, Eclipse TE2000-U (Nikon Instruments Inc.) with objective lens of 10× and 20×. The flow rate was determined by measuring the mean flow velocity through a vinyl chloride tube with inner diameter of 3.2×10² µm connected to the exit. The mean velocity was obtained by travelling time of dye solution in unit length. The cross-sectional area of the tube is 8.6×10⁴ µm². The mean flow rate was obtained by the multiplication of the cross-sectional area of the tube and the mean velocity.

3.3. Preparation and injection of V. convallaria

We prepared floating cells for injection. V. convallaria was cultured in the 1L flask containing the extraction of wheatgrass powder (Pines International Inc.) in deionised water. Then V. convallaria was detached from the flask surface by shaking the flask at 100 rpm for 24 h. The detached V. convallaria was transferred to a large Petri dish and incubated at 20 °C for 6 h to allow adhering to the bottom of dish surface. The adhered cells were rinsed with spring water twice and were scraped with a cell scraper. They were centrifuged at 3,000 g for 10 min to obtain concentrated suspension. The concentration of suspension was adjusted to be approximately 1,000 cells/µL. The suspension of V. convallaria cells was introduced to the fluid channel using a syringe. The cells were incubated at 20 °C for 48 h to allow adhering and stalk growth.

3.4. The treatment of V. convallaria in microchannel

The introduced cells were permeabilized with a surfactant solution, 0.1%(w/w) saponin in a chelator solution (0.1M KCl, 4 mM EGTA [Ethylene Glycol bis(2-aminoethylether) Tetraacetic Acid], 50 mM Tris [Tris(hydroxymethyl)aminomethane]-HCl buffer, pH 7.0) for 5 min. The surfactant solution was washed away with the chelator solution. The surfactant and chelator solutions were stained for visualization with red and blue food dyes, respectively. We observed the cell at 10 fps using the inverted microscope equipped with 10× and 20× lenses and a CCD camera.
4. Result and discussion

4.1. Flow velocity and flow rate evaluation
We measured flow velocity and rate through the fluid channel of the fabricated device (Figure 4). The mean flow speed was obtained by measuring the traveling time and channel lengths from A to C and B to C. The lengths from the inlet 3 point A, the first chamber of flow regulator, point C is 7.7 mm (see the inset of figure 5). The length from the inlet 4, point B to point C is 9.8 mm. Figure 5 shows the result of fluid velocity measurement. The mean velocity increased steadily from 0.1 to $5.8 \times 10^3 \mu m/s$ with the applied pressure from 2 to 18 kPa. We measured the flow speed five times. Error distribution in figure 5 represents standard deviation. The slope of the flow speed was $3.2 \times 10^2 \mu m/kPa \cdot s$. This agrees well with the trend in Poiseuille flow. From the velocity measurement, we also evaluated the possible device response time. As the travelling time of solution to the regulator was found to be about 2 s and the time taken for the contraction of peameablized stalk, the device response time could be roughly 4 s.

Figure 6 shows the result of flow rate measurement at the exit of the fluid channel as the function of applied pressure ranging from 2 to 18 kPa. We measured the flow rate five times. Error distribution in figure 6 represents standard deviation. At the inlet pressure of 6kPa and above, the flow rate increased linearly from 5.5nL/min to 1.1µL/min with the applied pressure from 4 to 18kPa. The slope of flow rate was $0.5 \times 10^2 nL/kPa \cdot min$. However, the for inlet pressure below 6kPa, the result did not follow the trend. This shows the existence of threshold pressure for flow at the exit of fluid channel. This phenomenon is currently under investigation.

![Figure 5: Flow speed from inlet 3 and 4 to regulator element as function of applied inlet pressure](image1)

![Figure 6: Flow rate at fluid channel exit as function of applied inlet pressure](image2)
4.2. The distribution of adhered V. convallaria and stalk growth within regulator section

We confirmed that 12 cells adhered to the surface two hours after cell introduction. Figure 7 shows the distribution of *V. convallaria* cells. Four cells adhered in a single chamber at maximum. The cells grew their stalks to 39±11 µm (n=12) at 48 hours of incubation (Figure 8). There was no detachment of the cells after seven days of incubation. However, the result of cell introduction was below the target values, 2–3 cells on each regulator chamber and the stalk length of 100 µm. Poor distribution of cell adhesion was caused by the property that the cells swim freely and adhere to their desired location. The cells adhered more to the inlet port than the chamber. The diameter of the inlet is approximately 0.5 mm, which is larger than the chamber. The number of cells adhered to the regulator chamber can be improved by confining them with additional control valves. The lack of nutrition in the suspension of mineral water may result in short length of stalk. The stalk growth can be improved by addition of the culture medium in the suspension.

![Figure 7. Distribution of cells within a regulator chamber 48 hour after the injection](image1)

![Figure 8. Image of *V. convallaria* after 48 hours of incubation](image2)

4.3. Partial cell membrane removal of adhered cell at regulator chamber

We observed the reduction of mechanical strength of the treated cell. This suggests the partial removal of cell membrane. Figure 9 shows the time progress images of chelator introduction to the regulator chamber. The zooid of the adhered cell was detached by the introduction of the chelator solution. As shown in Figure 9(a), the cell remained to regulator chamber at the introduction of the chelator. One second after the introduction, the zooid was detached and only the stalk remained attached (figure 9(b)). The treated cell could not withstand the fluid velocity at regulator chamber. When applied at 10 kPa, we estimate the flow velocity at around 3.3×10³ µm/s. The inlet pressure should be reduced to prevent the zooid detachment after the permeabilize treatment. The inlet pressure of 6–8 kPa should be reasonable.

![Figure 9. Time series images of the chelator solution injection at the inlet pressure of 10 kPa.](image3)

(a) At the instant of chelator solution introduction. (b) Detachment of the zooid was observed at 1s after the injection of solution.
5. Conclusion
We fabricated a multilayer fluidic channel, which is a platform of the proposed micro flow regulator. Introduction, stalk growth, adhesion and permeabilize treatment of V. convallaria were achieved on a chip. The stalk grew to 39±11 µm (n=12) after 48 hours of incubation. There were 12 cells in the entire regulator elements with the maximum of four cells in the single element. From the on-chip permeabilize treatment, the partial removal of cell membrane was successfully achieved by observing the detachment of zooid during the chelator solution injection. As for the future work, we will conduct the measurement of change in stalk length under varying concentration of Ca\textsuperscript{2+} and flow rate variation during stalk actuation. The stalk length will be improved by replacing mineral water to culture fluid. The number of adhered cell is planned to increase by actuating the control channel for regulator section in order to limit the movement of cells. The mechanism of flow regulation will also be investigated by visualizing the fluid flow with fluorescent particles.

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