Electrolysis of selectively patterned *Vorticella* with pneumatic microchambers and electrodes

Moeto NAGAI*, Ryoga KOHARA*, Tuhin Subhra SANTRA** and Takayuki SHIBATA*

*Department of Mechanical Engineering, Toyohashi University of Technology

1-1 Hibarigaoka, Tenpaku-cho, Toyohashi, Aichi 444-8580, Japan

E-mail: nagai@me.tut.ac.jp

**Department of Engineering Design, Indian Institute of Technology Madras,

Chennai, Tamil Nadu 600036, India

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Abstract

Living machines are expected to expand the limits of silicon microtechnology. *Vorticella convallaria* is a ciliated protozoa and a promising linear actuator. The use of *Vorticella* as a Ca^{2+} -responsive actuator, in a small system, requires a reduction in the size of the membrane treatment of selectively patterned *Vorticella*. However, selective cell patterning and the electrolysis of cell membranes have not yet been combined; therefore, the development of a combined method is essential. The objective of this study is to develop a method for combining selective cell patterning and the electrolysis of cell membranes. A two-layer pneumatic device was fabricated in polydimethylsiloxane (PDMS), and the device and electrodes were clamped with acrylic plates. The deformation of the PDMS membrane was controlled by compressed air. The electrochemical reactions were visualized with fluorescein, and the temporal pH change was evaluated. *Vorticella* cells were introduced into a microfluidic device and were captured in chambers at a pressure of 21 kPa. They were immobilized in the chambers after incubation for 6 h. Further, OH^- was generated from the cathode by the electrolysis of water, and the bell-shaped bodies of *Vorticella* became spherical. This permeabilization treatment stopped both spontaneous contraction and cilia movement. The application of DC 3 V was sufficient for the electrolysis of *Vorticella*. Selective patterning and electrochemical membrane treatments are beneficial for facilitating the use of *Vorticella* as a Ca^{2+} responsive actuator in a small system.

Keywords: Selective patterning, Electrochemical treatment, Bioactuator, Pneumatic channel, *Vorticella*, Permeabilization

1. Introduction

In the future, applications such as drug delivery and autonomous parallel computing are predicted to provide improved medical treatments and to solve mathematical problems. Living machines have been used as autonomous microrobots (Carlsen and Sitti, 2014) and are expected to expand the limits of silicon (Si) microtechnology. Biomolecular motors have been applied for parallel computation (Nicolau, et al., 2016), and bacteria have been used for drug delivery (Din, et al., 2016). The cilia motion of *Vorticella* (Nagai, et al., 2013a) has mixed laminar flow, and *Vorticella* has rotated a microstructure and functioned as a linear actuator (Nagai, et al., 2019a). *Volvox* has been observed to stop a flow and function as a spherical microvalve (Nagai, et al., 2015), while a sperm flagella-driven micro-biorobot has also been developed (Magdanz, et al., 2013).

Among living machines, *Vorticella convallaria* is a ciliated protozoa and a promising linear actuator. It is composed of a bell-shaped body (zooid) with a diameter of about 30–40 μm . Its stalk is 100 μm in length and 2–3 μm in diameter, and it anchors itself to a surface (Ryu, et al., 2017; Upadhyaya, et al., 2008). A permeabilized stalk was contracted with Ca^{2+} and extended with a chelating agent of glycol ether diamine tetra acetic acid (EGTA) (Katoh and Naitoh, 1994; Ochiai, et al., 1979). The contraction/extension cycle of the *Vorticella* stalk was repeated over 100 times (Asai, et al., 1978), and the actuation cycle was demonstrated in a microfluidic channel (Nagai, et al., 2010; Nagai, et al., 2013b).

The use of *Vorticella* as a Ca^{2+} -responsive actuator in a small system requires a reduction in the size of the membrane treatment of selectively patterned *Vorticella*. To control the actuation of a *Vorticella* stalk via the surrounding concentration of Ca^{2+} , the cell membrane of *Vorticella* is partially removed using the surfactant, saponin. Chemical permeabilization is simple (Jamur and Oliver, 2010; Shehadul Islam, et al., 2017); however, one disadvantage is that a liquid tank, valve, and pump are required for liquid exchange (Nagai, et al., 2010; Nagai, et al., 2013b), which is problematic when reducing the system's size. In previous studies, hydroxyl ions and detergent were used to permeabilize the cell membrane; hydroxide ions were generated by a low DC voltage, and the cell membrane was lysed in a microfluidic chip (Di Carlo, et al., 2005; Lee, et al., 2010; Nevill, et al., 2007). A low DC voltage has the potential to reduce the size of a system for cell lysis. Single cells were patterned at specific locations with pneumatic microchambers (Hümmer, et al., 2016), and a pneumatic valve (Nagai, et al., 2013a) and magnetic force (Nagai, et al., 2019b) were utilized to position *Vorticella* in a microfluidic channel. To date, cell patterning and the electrical lysis of cell membranes have only been studied separately; therefore, it is necessary to develop a combined method.

The current research developed a technique for combining selective cell patterning and the electrolysis of cell membranes. For selective cell patterning, pneumatic chambers were selected because they are available for a wide variety of cells. *Vorticella* was selectively patterned in pneumatic chambers, and its cell membranes were lysed by the electrolysis of water. A device for the patterning and electrochemical permeabilization of *Vorticella* was fabricated. The authors designed a fluidic channel and an electrode for immobilizing *Vorticella* to target specific areas. A DC voltage was applied to a pair of electrodes, and the pH change was observed using fluorescent dyes. Multiple cells of *Vorticella* were placed in a microfluidic channel using valves in a two-layer flow channel. *Vorticella* was electrochemically treated, and the changes in its shape and motion were observed.

2. Experimental methods

2.1 Design of pneumatic microchambers integrated with electrodes

This study developed a mechanism to place *V. convallaria* cells between electrodes, and then permeabilize them through electrochemical treatment (Fig. 1). The location of *Vorticella* was controlled to enable its use as a bioactuator. The bottom polydimethylsiloxane (PDMS) layer was used as a flow channel into which *Vorticella* was introduced (Fig. 1a). The top PDMS layer functioned as a pneumatic channel for applying pressure to the thin membrane. The thin PDMS membrane functioned as a valve; it was depressed by pneumatic air pumped through the pneumatic channel, and a chamber was formed (Fig. 1b). *Vorticella* cells were maintained in the chamber, where they adhered to the surfaces and produced stalks (Fig. 1c). After the valve was opened, electrochemical treatment was conducted to permeabilize the *Vorticella* cell membranes (Fig. 1d).

The authors prepared a device for the electrochemical treatment of selectively patterned *Vorticella* (Fig. 2). Table 1 presents the major dimensions of the PDMS chambers and electrodes. To trap *Vorticella* cells, the height of the flow channel was set at 30 μm , and a total of nine chambers were arranged along a straight flow channel (Fig. 2c). A ϕ 240- μm chamber was designed to accommodate approximately 10 cells, while a few *Vorticella* cells were kept in a ϕ 120- μm chamber. Four electrodes were designed in the straight flow channel (Fig. 2a); the two left electrodes were the cathode,

Fig. 1 Schematic of a device for the electrochemical treatment of selectively placed *Vorticella* cells. (a) Introduction of *Vorticella* to a channel. (b) Chamber formation by the deformation of the pneumatic valve. (c) Spontaneous adhesion of *Vorticella* to the channel's surface. (d) Electrochemical treatment of the *Vorticella* cells.

Fig. 2 Schematic of a PDMS chip and electrode substrate clamped with a pair of acrylic plates. (a) Top view of the entire chip. (b) Entire view of flow channels and a partial view of four electrodes. (c) Enlarged view of four electrodes and nine chambers along a channel. (d) Cross-sectional view of a clamp, PDMS chip, and glass slide.

Table 1 Dimensions of PDMS chambers and electrodes.

Channel width	Electrode width	Chamber diameter
500 μm	400 μm	240 μm
300 μm	200 μm	120 μm

and the two right electrodes were the anode (Fig. 2c). For the ease of connection of wires, square electrode pads of size $8 \times 8\text{-mm}$ were used. The gaps between the up and down electrodes were 200 μm for the 300- μm wide channel and 500 μm for the 400- μm wide channel.

2.2 Fabrication of pneumatic microchambers integrated with electrodes

2.2.1 Preparation of SU-8 mold

The bottom and top microchannels were molded from SU-8 molds, which were prepared in SU-8 3050 photoresist (Nippon Kayaku Co., Ltd.) by using photolithography (Fig. 3a, c). First, a 3-inch Si wafer was cleaned with acetone and isopropyl alcohol (IPA). The Si substrate was spin coated with SU-8 at 3,000 rpm for 60 s using a spin coater (1H-DX2, Mikasa Corp.). For the introduction of *Vorticella*, the target thickness was set to 30 μm . The wafer was pre-baked at 65°C for 2.5 min, at 95°C for 20 min, and at 65°C for 2.5 min. A two-stage heating and cooling was employed to reduce the difference in thermal expansion between the SU-8 and the wafer. The SU-8 film was exposed in contact mode for 15 s via a film mask with a double-sided mask aligner (PEM-800, Union Optical Co., Ltd.). The amount of integrated light was 200 mJ/cm^2 . The SU-8 was post-baked at 65°C for 9 min, at 95°C for 5 min, and at 65°C for 2.5 min. It was developed

Fig. 3 Fabrication process of a two-layer microfluidic device using thin PDMS film and the integration process of the device and electrodes. (a) SU-8 mold fabrication and (b) PDMS molding for flow channel. (c) SU-8 mold fabrication and (d) PDMS molding for pneumatic channel. (e) Bonding of the bottom and top layers. (f) Patterning of platinum (Pt)/titanium (Ti) electrodes. (g) Integration of the PDMS on the electrodes.

in 2-methoxy-1-methylethyl acetate (Wako Pure Chemical Industries, Ltd.) and rinsed with acetone for 10 min. The surface of the flow channel mold was treated in a vapor of trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma Aldrich), which was vaporized at 80°C for 2 min to enhance the releasability of PDMS from the SU-8 mold.

2.2.2 Fabrication of top and bottom polydimethylsiloxane layers

The top and bottom layers were replicated in PDMS (Sylgard 184, Dow Corning) from the SU-8 molds by soft lithography (Fig. 3b, d). The main PDMS agent and a curing agent were mixed at a ratio of 10:1, and air bubbles in the PDMS were removed with an aspirator (Dry Aspirator DAS-01, As One Corporation). Degassed PDMS was poured over the flow channel mold with a height of 30 μm and was spin coated at 1,500 rpm for 60 s. The wafer was maintained at room temperature for 10 min to flatten the PDMS. It was then cured at 50°C for 1 h on a hotplate (RSH-1 DR, As One Corporation) and again at 80°C for 1 h (Fig. 3b). Degassed PDMS was also poured over the top SU-8 mold, and air bubbles in the PDMS were removed with the aspirator. The PDMS was cured at 80°C for 40 min on the hotplate (Fig. 3d). The cured PDMS was released using a scalpel and tweezers and was shaped with a razor. Inlets and outlets of 0.5 mm were punched in the PDMS chip under a stereomicroscope (SZ61, Olympus Corporation).

The PDMS chip was then ultrasonically cleaned (VS-100III, As One Corporation) at 45 kHz in deionized (DI) water and was dehydrated on the hot plate at 80°C. Both PDMS surfaces were treated with the corona treater (BD20AC, Electro-Technic Products, Inc.) for 40 s (Haubert, et al., 2006). The top PDMS chip was then aligned with the bottom layer, and the assembled chip was subjected to light finger pressure (Fig. 3e). After baking at 80°C for 1 h, the bonded PDMS chip was released from the SU-8 mold.

2.2.3 Preparation of platinum/titanium electrodes

A glass slide (76 $\times$ 26 $\times$ 1 mm, white, No. 1, Matsunami Glass Industry Co., Ltd.) was cut into three equal parts (each 26 $\times$ 26 mm). It was cleaned with a mixture of hydrogen peroxide and sulfuric acid at a ratio of 1:3. The glass slide was dehydrated at 140°C for 5 min. Hexamethyldisilazane was spin coated on the glass surface at 3,000 rpm for 60 s, and it was baked at 110°C for 90 s. A positive resist (OFPR-8600, 52 cp) was spin coated onto the glass slide at 3,000 rpm for 60 s and was pre-baked at 110°C for 90 s. The resist was exposed in contact mode for 20 s (integral amount of light: 250 mJ/cm²). The photoresist was then developed in NMD-3 (Wako Pure Chemical Industries, Ltd.) for 10 min. The wafer was rinsed with DI water and was post-baked at 140°C for 5 min. Thin Ti and Pt films were deposited on the patterned glass via a sputtering apparatus (L-250S-FH, Anelva Co., Ltd.). Target film thicknesses of 10 nm for Ti and 100 nm for Pt were applied. The Pt/Ti films were removed in acetone for 10 min by ultrasonic cleaning at 100 kHz (Fig. 3f). The surface was cleaned with IPA and was blown with nitrogen.

2.2.4 Integration of the polydimethylsiloxane pneumatic chamber and electrodes with an acrylic clamping jig

The PDMS flow channel and electrode substrate were clamped with an acrylic jig and M3 bolts (Fig. 2d and Fig. 3g). To enable the reuse of the electrode substrate, permanent bonding was not performed. Transparent hard-coated acrylic resin plates (Acrysunday Co., Ltd., acrylic resin cast, pencil hardness: 6H, MR2-001, thickness: 2 $\pm$ 0.2 mm) and an inverted microscope were used to observe the inside of the PDMS chip. The plates were scratch resistant and highly permeable (total light transmittance: 93%). The plate was milled and drilled to match the size of the microfluidic device (Fig. 2a).

2.3 Observation of fluorescence for pH measurement

OH^- was generated from the cathode and was measured using fluorescein (Acid yellow 73, Tokyo Chemical Industry Co., Ltd.), which is a pH-dependent fluorescent dye. Fluorescein powder was dissolved in acetone and diluted to 50 μM with DI water. The state of the electrochemical reaction between the electrodes was observed using an inverted fluorescence microscope, and the intensity was measured from the image. The fluorescence intensity over the distance between the pair of electrodes was measured to evaluate the pH change. Based on the obtained intensity distribution, the

electrochemical reaction between the electrodes was investigated.

For fluorescence observation, a fluorescence microscope (Ti-U, Nikon Instruments Inc.) equipped with a cooled charge-coupled device (CCD) camera (DS-Qi1Mc, Nikon Instruments Inc.) was used. Blue light was produced from a fiber light source (Intensilight C-HGFI, Nikon Instruments Inc.) via a fluorescence cube (B-2A, EX 450–490 nm, DM 505 nm, BA 520 nm, Nikon Instruments Inc.).

2.4 Injection and trapping of *Vorticella*

A cell suspension of *V. convallaria* was prepared according to a previously described method (Nagai, et al., 2013b; Vacchiano, et al., 1991). A culture medium was extracted from 1-g/L wheatgrass powder (Pines wheatgrass, Pines International, Inc.) by first, autoclaving at 121°C and second, removing the solid content by filtration. The cells were incubated in a 1-L flask containing a medium at 20°C while agitating for 24 h to obtain floating *V. convallaria* cells.

A PDMS flow channel was filled with mineral water (Morinomizu, natural spring water, Japan Coca-Cola Co.) in a vacuum chamber. A suspension of *Vorticella* was introduced, and pneumatic pressure was applied from a compressor (Mini air compressor AC-500, Tow Marker Products CO., LTD.). The valve was gradually deformed to guide cells to the center of the chamber.

2.5 Voltage power supply and observation setup for electrolysis of *Vorticella*

The electrodes were connected to a DC power supply (ePS80WL, Fujitsu Access Co., LTD.), and DC voltage was applied to the pair of electrodes. The fluorescence of the dyes and the shape changes of *Vorticella* were observed under a microscope. Trapped *Vorticella* cells were electrochemically treated to permeabilize the cellular membrane with the hydroxide ion (OH⁻) generated from the cathode. *Vorticella* cells were observed with an inverted microscope (Eclipse TE2000-U, Nikon Instruments Inc.) equipped with a CCD camera (Digital Sight DS-2Mv, Nikon Instruments Inc.).

3. Results and discussion

3.1 Fabrication of pneumatic microfluidic chambers

Each PDMS layer was replicated from an SU-8 3050 mold, and the first flow layer and the second pneumatic layer were permanently bonded following plasma treatment (Fig. 4a). A PDMS membrane was formed between the first and second layer to create pneumatic valves and was used to capture and immobilize *Vorticella* cells in the chambers. A pneumatic channel was then properly aligned with a flow channel, and circles were placed in the center of the flow channel. Four circles (φ 240 μm) were placed in a 500-μm wide channel and five circles (φ 120 μm) were placed in a 300-μm wide channel. A thin PDMS membrane was not formed on these circles, and the membrane enabled the formation of chambers for capturing *Vorticella* cells. The diameter of the φ 240-μm chambers for placing multiple *Vorticella* cells was $228.5 \pm 4.2 \mu\text{m}$ (N = 4) (Fig. 4b), and the diameter of the φ 120-μm chambers was $97.6 \pm 6.7 \mu\text{m}$ (N = 5) (Fig. 4c).

A cross section of the PDMS pneumatic channel was measured (Fig. 4d, e); the height of each channel was $28.9 \mu\text{m} \pm 1.6 \mu\text{m}$ (N = 6). The height of the circular parts was sufficient to bond the upper pneumatic layer to the lower PDMS flow channel layer. Using the channel height and the thickness (around 50 μm) of the PDMS that was spin coated at 1,500 rpm for 60 s, the thickness of the PDMS membrane was estimated at 20 μm (Zhang, et al., 2004). The widths of the typical PDMS membranes were approximately 80, 130, and 280 μm, and these widths affected the deformation of the membrane.

3.2 pH measurement with fluorescein

The fluorescence of fluorescein was measured, while H⁺ was generated by the electrolysis of water in a flow channel (Fig. 5). A pair of electrodes with a 400-μm gap was used for simplicity. The upper electrode was the cathode, and the lower electrode was the anode. The pH of a solution was changed by the following electrochemical reactions.

Anode reaction:

Fig. 4 Microscope images of a fabricated device. (a) A pneumatic valve device, which was composed of a two-layer flow channel. Top view of a pneumatic channel on the (b) left and (c) right sides. Cross-sectional views of the (d) 500- μm and (e) 300- μm wide PDMS pneumatic channels. $\phi 240\ \mu\text{m}$ and $\phi 120\ \mu\text{m}$ circles were placed in the middle of 500- μm and 300- μm wide channels.

Fig. 5 Visualized hydroxide ions with fluorescein in a region between a pair of electrodes with a gap of 400 μm . The applied voltage was DC 2 V. (a)–(d) Time-series microscope images of fluorescein around the electrode pair. (e) Fluorescence intensity along a line connecting the electrodes.

Cathode reaction:

The fluorescence decreased from the end of the anode immediately after voltage application (Fig. 5a–d). The fluorescence intensity near the cathode remained or slightly increased, while the intensity near the anode remained almost

constant (Fig. 5e). After applying voltage for 40 s, the intensity change decelerated. The increase in the value of the applied voltage increased the amount of electrochemical reaction. A similar pH profile to this result was observed using pH sensitive dyes, sodium alginate, and calcium carbonate (Cheng, et al., 2011a; Cheng, et al., 2011b). This intensity change suggests that H^+ and OH^- were generated around the anode and cathode, respectively. Hydroxide ions generated around the cathode were applied to the electrochemical treatment of *Vorticella*.

3.3 Trapping *Vorticella* with pneumatic chambers

A suspension of *Vorticella* cells (185 cells/ μ L) in a wheatgrass medium was introduced into a flow channel by hydrostatic pressure. *V. convallaria* was transported into the vicinity of a chamber by fine adjustment of the hydrostatic pressure. Pneumatic microchambers were formed by applying pneumatic pressure, which was gradually increased from 1 kPa to prevent the cells from being caught by the PDMS membrane. *Vorticella* escaped through small gaps between the valves near a 240- μ m diameter chamber at 16 kPa, possibly due to insufficient deformation. To retain *Vorticella* in the chamber, the pressure was increased to 21 kPa; 9 of 10 cells were guided into a chamber with a diameter of 240 μ m and captured in it (Fig. 6). 1 of 10 cell was not guided into the chamber and was sandwiched between the valve membrane and the substrate. The valve functioned without being peeled off, even at a pressure of 21 kPa.

Pneumatic pressure was applied to form a 120- μ m chamber. *Vorticella* passed through the gaps between a PDMS membrane and the bottom glass substrate and cells were not captured. Since the width of the thin film around a 120- μ m chamber was narrower than a 240- μ m diameter chamber, the PDMS membrane was not sufficiently deformed at 21 kPa. A higher pressure is needed to form a 120- μ m chamber.

To ensure the adherence of *Vorticella*, pneumatic pressure was maintained at 21 kPa for 6 h. The valve was returned to the initial position by releasing the pneumatic pressure. *Vorticella* cells were selectively immobilized in the right three of the four chambers, where they grew stalks (Fig. 7). Cells were caught between the membrane around the left chamber and were pushed away from the valve (Fig. 7a). The width of the PDMS membrane around the left chamber was 80 μ m, which was smaller than the 280 μ m of one of the right three chambers; hence, the valve deformation was insufficient for the selective patterning of *Vorticella*. Left unwanted cells shown in Fig. 7(a) did not interfere with the next experiment and they were ignored. The selective patterning method with a pneumatic valve (Hümmer, et al., 2016; Nagai, et al.,

Fig. 6 Time-lapse images of nine *Vorticella* cells captured in a pneumatic microchamber. Some cells swam in this chamber but did not escape. One *Vorticella* cell was not guided to the chamber and was captured with the PDMS valve.

Fig. 7 Microscope images showing *Vorticella* cells around (a) the two left chambers and (b) the two right chambers 6 h after the closure of the pneumatic valves.

Fig. 8 Time-lapse microscopy images showing 18 *Vorticella* cells before and after 60 s of 2-V electrolysis.

Fig. 9 Time-lapse microscopy images showing 18 *Vorticella* cells during 20 s of 3-V electrolysis.

2013a) was expanded for the electrochemical treatment of *Vorticella* positioned at the central part of the channel.

3.4 Electrochemical treatment of patterned *Vorticella*

Hydroxide ions were generated by the electrolysis of water from the cathode, and the permeabilization treatment of the *Vorticella* cells between the electrodes was conducted. After the 6-h capture and immobilization of *Vorticella*, the valve was opened, and the culture solution in the channel was replaced with spring water. *Vorticella* cells were observed with an inverted microscope in two chambers in the flow channel; the chambers had a width of 500 μm and an inter-electrode distance of 400 μm . DC voltages of 2 and 3 V were applied between left and right electrodes.

After the application of DC 2 V to the electrodes, 18 *Vorticella* cells were observed. Fig. 8 contains the microscopy images of *Vorticella* at 0 s and 60 s after voltage application. The shape of *Vorticella* did not change, and the *Vorticella* cells were observed to spontaneously move, even after 4 min of voltage application. If permeabilization is successful, *Vorticella* stops all movement; however, there was no evidence that the cellular membranes were permeable, and it was concluded that DC 2V was insufficient for the electrolysis of *Vorticella*.

To increase the effect of electrolysis, the voltage was increased from DC 2 to 3 V. The 18 cells started to contract with higher frequency, and their bodies became spherical (Fig. 9). Most of the cells suddenly became spherical after spontaneous contraction. The change ratio of *Vorticella* bodies over time is plotted in Fig. 10(a). All *Vorticella* changed from a bell shape to a spherical shape at 20 s and did not exhibit spontaneous contraction and cilia movement. The shape changed faster on the right side of the chambers than on the left side. Fig. 10(b) shows visualized hydroxide ions in a region between left and right electrodes. The fluorescence was stronger on the side close to the anode. This fluorescence

distribution is consistent with the faster treatment of *Vorticella* on the right side of the chambers. The shape change was similar to that observed with a solution of saponin, which is a detergent used for chemical permeabilization. This shape change and the cessation of spontaneous movement suggest that the cellular membrane was permeable. For further confirmation of membrane permeabilization, *Vorticella* was kept for 12 h in a channel and permeabilized at 3 V. After the permeabilization, a stalk of *Vorticella* contracted in spring water containing calcium ion. Spring water was replaced with a chelating solution (EGTA 4mM, KCl 100mM, Tris maleate 50 mM, pH7.0) and the stalk was extended. Although electroporation potentially cause permeabilization, the electric field strength is assumed to be too low to cause electroporation. The gap between the cathode and anode was 400 μm , and the maximum average electric field strength was 50 V/cm at 2 V and 75 V/cm at 3 V. These electric field strengths were relatively low to induce electroporation.

A microcomponent was rotated by the contraction of *Vorticella* in a microchannel (Nagai, et al., 2019b). However, a *Vorticella* cell contracted and elongated spontaneously and it was not responsive to Ca^{2+} . This cell permeabilization technology can provide Ca^{2+} -responsiveness to this actuation system. The permeabilization of *Vorticella* has been chemically performed in previous studies by exchanging solutions for which a solution-exchange mechanism was required (Nagai, et al., 2010; Nagai, et al., 2013b). In the current study, a DC power supply was applied for permeabilization, and a solution-exchange mechanism was omitted. This evaluation is useful for reducing the size of the permeabilization system for *Vorticella*.

Fig. 10 (a) Ratio of *V. convallaria* with shape change over time at DC 3 V. The shapes did not change at DC 2 V. (b) Visualized hydroxide ions with fluorescein in a region between left and right electrodes in flow. The left two electrodes were cathode and the applied voltage was DC 6 V.

4. Conclusions

This study successfully achieved the combined selective patterning of *Vorticella* and its electrochemical permeabilization. A pneumatic valve with electrodes was designed and developed. A two-layer pneumatic device was fabricated, and the deformation of a PDMS membrane was controlled to capture *Vorticella* in chambers. *Vorticella* was selectively patterned with pneumatic valves, and water electrolysis was used to permeabilize the *Vorticella* membranes. *Vorticella* was captured in a chamber at a pressure of 21 kPa and immobilized in a culture medium after 6 h of incubation. Further, OH^- was generated from the cathode by DC 3-V electrochemical reactions, and the bodies of *Vorticella* changed from a bell shape to a spherical shape within 20 s. The electrochemical reaction was also visualized with fluorescein, and the temporal change of the pH was evaluated to confirm the generation of hydroxyl ions. The permeability treatment stopped all spontaneous contraction and cilia movement. It was demonstrated that the electrical approach was able to remove the mechanical parts required for solution exchange.

The authors' future work will involve reducing the size of the entire system and developing microdevices incorporating microorganisms while removing the fluidic exchange system. A 9-V battery has previously been used to reduce the size of a microfluidic system (Addae-Mensah, et al., 2010). This concept can be applied to the objective of downsizing an entire system and could lead to the practical application of *Vorticella*-derived Ca^{2+} -responsive linear

actuators.

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