A biological sensor platform using a pneumatic-valve controlled microfluidic device containing *Tetrahymena pyriformis*†

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In this study, we introduce a microfluidic device equipped with pneumatically actuated valves, generating a linear gradient of chemoeffectors to quantify the chemotactic response of *Tetrahymena pyriformis*, a freshwater ciliate. The microfluidic device was fabricated from an elastomer, poly(dimethylsiloxane) (PDMS), using multi-layer soft lithography. The components of the device include electronically controlled pneumatic microvalves, microchannels and microchambers. The linear gradient of the chemoeffectors was established by releasing a chemical from a ciliate-free microchamber into a microchamber containing the ciliate. The ciliate showed chemotactic behaviours by either swimming toward or avoiding the gradient. By counting the number of ciliates residing in each microchamber, we obtained a precise time–response curve. The ciliates in the microfluidic device were sensitive enough to be attracted to 10 pmol glycine-proline, which indicates a 10^5 increase in the ciliate's known sensitivity. With the use of blockers, such as DL-2-amino-5-phosphonopentanoic acid (APPA) or lanthanum chloride ($LaCl_3$), we have demonstrated that the NMDA (*N*-methyl-D-aspartate) receptor plays a critical role in the perception of chemoeffectors, whereas the Ca^{2+} channel is related to the motility of the ciliate. These results demonstrate that our microfluidic chemotaxis assay system is useful not only for the study of ciliate chemotaxis but also for a better understanding of the signal transduction mechanism on their receptors.

1 Introduction

Tetrahymena pyriformis is a 50-μm long, unicellular, ciliated freshwater protozoan. Because of their high motility, ciliates can respond more quickly to outside stimuli than any other organism. Their superb chemotaxis property has been used to develop biological sensors for assaying environmental pollutants and chemicals of medical interest.[1,2]

The traditional method used in the study of chemotactic motility is the two-chamber capillary assay.[3] In previous experiments with this assay, 10^{-6} M glycine weakly repelled *Tetrahymena*, whereas glycine-proline was a strong attractant.[3,4] Precise control of the chemical gradient of compounds being tested is not possible with this assay.

In order to generate a repeatable chemical gradient, many microfluidic devices have been developed.[5–8] However, many of the devices were designed to use pressure-driven microflows to generate chemical gradients, and thus the devices are not suitable for ciliates that are inclined to follow the flow direction.[5,6] Herein, we report a microvalve-controlled chemotaxis assay system in which the gradient of chemicals can be generated by diffusion in one chamber while opening the valve located at the other chamber containing the chemicals of interest. For the precise control of flow in microfluidic channels, multilayer soft lithography[9] was used to construct microvalves, and the microvalves were electronically actuated. The microfluidic device was placed on the stage of a conventional stereomicroscope to obtain time-lapse micrographs of ciliates responding to a linear gradient of a chemoeffector.

2 Experimental

Fabrication and device control

The microfluidic device consists of two PDMS (poly(dimethylsiloxane), Dow-Corning, Cortland, NY, USA) layers, as shown in Fig. 1(e). The diameter of the chambers is 1 mm, and a connecting channel between the chambers is 100 μm wide, 900 μm long and 12.5 μm deep. The top layer contains three microchannels (100 μm wide) ended by a square (300 μm × 300 μm) and an inlet hole (600 μm diameter). The overall size of the microfluidic device is 15.5 mm long and 4.1 mm wide.

The microfluidic device was fabricated using multi-layer soft lithography.[9] The top, thick layer was prepared with a ratio of 1 : 10 (curing agent : PDMS), whereas the bottom thin layer is 1 : 30. The surfaces of PDMS and a microscope slide were simultaneously treated in an O_2 plasma cleaner (Harrick Scientific, Ithaca, NY, USA) at 50 Watts for 30 s, and the two surfaces were then brought together to form an irreversible bond. Pneumatic valves were connected through the Tygon tubing (Fisher Scientific International Inc., Hampton, NH, USA) to solenoid valves (Lee Company, Westbrook, CT, USA), which can vent or pressurize the PDMS valves. The

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† Electronic supplementary information (ESI) available: Video clips showing action on ciliates of repellent and attractant chemoeffectors: video 1: 100 μM Gly; video 2: 100 pM Gly-Pro. See DOI: 10.1039/b617357h

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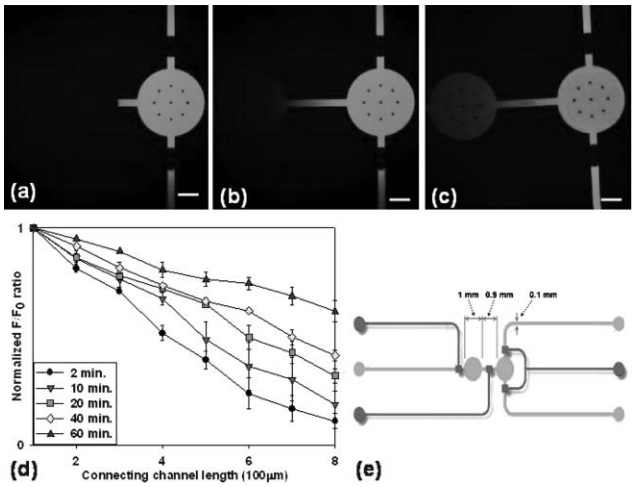

Fig. 1 Characteristics of a valve-controlled microfluidic device for a ciliate sensor. (a) At the initial stage, the connecting valve is closed. (b) The concentration gradient forms 10 min after the valve is opened. (c) The gradient after 20 min. Scale bars are 300 μm. (d) The gradient profile is measured at 8 different positions in the connecting channel at various times (2, 10, 20, 40 and 60 min) after the connecting channel was opened. F is a fluorescence intensity at a defined position and F_0 is a mean fluorescence intensity of the microchannel at a defined time. (e) The microfluidic device consists of two PDMS layers. The bottom layer is the fluidic channel while the top layer is a pneumatic microvalve layer.

solenoid valves were controlled by transistor switches (Comfile Technology, Seoul, Korea) which in turn were switched by a 5 V TTL signal from a digital IO card (National Instruments Co., Austin, TX , USA). The pressure was set at 30 psi by a mini pressure regulator, which was connected to a bottle of N_2 gas. The switching of the valves was controlled by LabVIEW (National Instruments Co.).

Device operation

The microvalves were pneumatically operated by controlling the flow of N_2 gas into the valves. In detail, each microvalve was first filled with water through an inlet hole and tubing connecting the inlet hole with a solenoid valve that is electronically controlled by the Labview® program. A pressure of 30 psi applied by flowing N_2 to the microvalve enabled water in the microvalve to lift the PDMS layer. The expanded PDMS layer on the top pressed down the microchannel on the bottom, resulting in the blocking of solution flow. The volume of each chamber on closing the valves was about 0.01 μL.

To prepare chemotaxis analysis about 30 ciliates in medium were injected into the chamber on the left while opening all the valves. The valves surrounding the left chamber were closed after the ciliates were introduced. To prevent any pressure increase in the chamber caused by closing the valves, the valve on the connecting microchannel between the two chambers was turned on and off for a few seconds. Then, the right chamber was filled with chemoeffectors through the remaining valves surrounding the chamber while the valve on the connecting microchannel was closed. The chamber was closed only when the flow of solution containing chemoeffectors completely stopped. After turning off the valve on the microchannel connecting the two microchambers, the mixing between the microchambers started with chemical diffusion and ciliate motility.

The microfluidic chip was placed under a stereo-microscope (Nikon Co., Tokyo, Japan) equipped with a Peltier cooled CCD camera (SPOT INSIGHT™, Diagnostic instruments, Sterling Heights, MI, USA). Chemotactic responses of the ciliates were analyzed by counting cells residing in chambers over time. All experiments were repeated at least three times, unless otherwise mentioned. Fluorescence intensity and motility of the ciliates were measured by the Image J program (NIH, Bethesda, MD, USA). Data were evaluated by the SigmaPlot 2000 program (Systat Software Inc., San Jose, CA, USA). For a new chemotaxis assay the fluidic channels and microchambers were flushed out with distilled water while all the valves were opened. The microfluidic device was reused with the flushing step at least five times without affecting the results.

Ciliate and reagents

T. pyriformis was obtained from ATCC (Manassas, VA, USA). Cells were cultured in ATCC medium 357 containing 5% protease peptone (Difco 0120), tryptone and 0.2% K_2HPO_4. After incubation at 25 °C for 12 h, cells were grown to early growth phase to a concentration of 1.1–1.4 × 10^5 cells mL^{-1}. Cells were centrifuged at 500 g for 2 min to collect a high density of cells in a volume of 100 μL and resuspended in the medium. Amino acids, dipeptide, DL-2-amino-5-phosphonopentanoic acid (APPA) and lanthanum chloride ($LaCl_3$) were purchased from Sigma Chemicals (St. Louis, MO, USA). For the control experiment of diffusion, a synthesized diethyl iminodiacetate fluorescein (691.42 MW, λ_{ex} = 505 nm/λ_{em} = 510 nm) was used.[10]

3 Results and discussion

To characterize a linear gradient formed by diffusion in the device, the gradient of a fluorescein derivative (691.42 MW) was monitored in real time. After opening the connecting channel between the microchambers, the dye from the right chamber diffused into the left chamber, as shown in Fig. 1. Images under UV were taken at 2 min intervals. The dye reached equilibrium after 140 min. The gradient was measured over the connecting channel in a 100 μm × 100 μm area in eight different positions (Fig. 1(d)). To compare the gradient profile at different time points, the intensities were normalized. We observed that over time the change in concentration decreased. This change in concentration reflects the change in the linear gradient over the connecting channel, with the standard deviation being 6.5%. The diffusion time was estimated from Stokes–Einstein equation.[11] According to Einstein's theory on Brownian motion, the velocity of the molecule is proportional to the square root of its mass. Thus, we calculated that the diffusion time to reach the equilibrium concentration of each chemoeffector is 70 min for glycine-proline and 47 min for glycine. These diffusion times are sufficient enough to measure the chemotactic response of the ciliate, which is usually completed within several minutes.

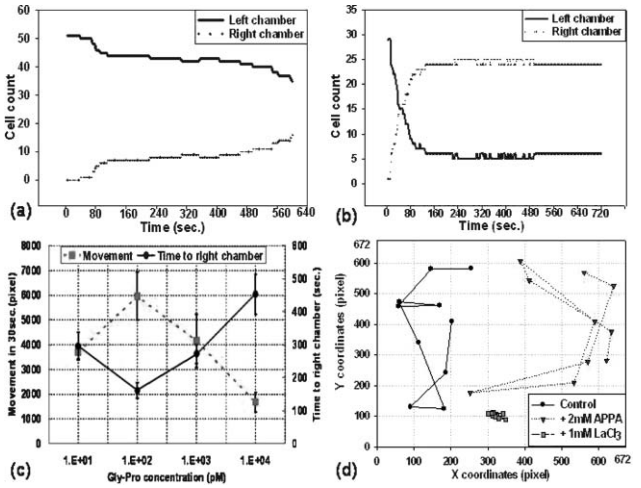

Fig. 2 Chemotaxis analysis of *T. pyriformis*. (a) Response of ciliates to distilled water with no chemoeffector over 10 min. (b) Chemotactic response of ciliates to 10 pM Gly-Pro. (c) Chemotactic behaviour of ciliates to various concentrations of Gly-Pro. (d) Effect of inhibitors on chemotactic response of a ciliate to 10 pM Gly-Pro. Representative trajectories of three different ciliate populations previously exposed to the inhibitors (APPA or $LaCl_3$) at the previously described conditions or with no exposure to any of these inhibitors.

To verify the performance of our microfluidic platform, the chemotactic response of *T. pyriformis* to glycine (Gly) as a repellent and glycine-proline (Gly-Pro) as a attractant were analyzed.[3,4] Also, as a control experiment, the chemotactic response of the ciliate was performed with distilled water containing no chemoeffector (Fig. 2(a)). Images were taken every 4 s for 10 min. This control experiment was repeated 10 times under identical conditions. The average number of ciliates moving from the left to the right chamber was 36% (σ = 0.16) for the first 10 min. After about an hour, the equilibrium of cell number over the chambers was reached (data not shown). This is roughly twice as fast as the diffusion time of the fluorescein derivative.

An analysis of ciliate response to each chemoeffector was performed in the same manner as just described. After the opening the connection channel, a chemoeffector diffused from the right to the left chamber, and the ciliates in the left chamber responded to the chemoeffector by moving toward or away from the right chamber at various speeds, depending on the concentration and nature of the chemoeffectors. For example, 100 μM Gly acted as a repellent (video 1 in ESI†), while 1 μM Gly was a weak attractant. On the other hand, 1 pM Gly-Pro had no effect (data not shown), whereas Gly-Pro was a strong attractant at 10 pM (Fig. 2(b)), which is five orders lower than a previously reported minimum concentration that resulted in attraction.[4] According to the behavioural analysis of ciliates (Fig. 2(c)), it is estimated that the strongest and weakest attractions to Gly-Pro were observed at 100 pM (video 2 in ESI†) and 10 nM, respectively.

To demonstrate the capability to perform more elaborate experiments in our microfluidic platform, the effects of chemotaxis inhibitors on chemotactic response to an attractant were investigated. For this specific purpose, an NMDA (*N*-methyl-D-aspartate) receptor[12] and Ca^{2+}-channel blocker, APPA (DL-2-amino-5-phosphonopentanoic acid) and $LaCl_3$ (lanthanum chloride)[13] were used separately.

Before the exposure to the gradient of 10 pM Gly-Pro, the ciliates were incubated in 2 mM APPA or 1 mM $LaCl_3$ for 5 min at 25 °C in the left chamber. The cells did not show a response to the attractant and remained in the left chamber. To differentiate the inhibitory effects of the blockers, the trajectory of ciliate motion was analyzed. Images were taken every 1 s. The representative trajectories of a ciliate from three different populations either experiencing exposure to the inhibitors or with no exposure to any of these inhibitors was presented as a position of the cell on the x, y coordinates (Fig. 2(d)). APPA did not impede the motility of the ciliate (0.34 mm s^{-1}), which is faster than the motility (0.22 mm s^{-1}) of cells with no previous exposure to any of the blockers. On the other hand, the ciliates previously exposed to $LaCl_3$ showed poor motility (0.018 mm s^{-1}). These results suggest that APPA inhibits the chemotactic response of ciliates by interfering with the function of the NMDA receptor without impairing motility, whereas $LaCl_3$ blocks Ca^{2+} influx and thus decreases motility.

Acknowledgements

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