MICROFLUIDIC GAS/LIQUID TOXICITY SENSING THROUGH THE CHEMOTAXIS OF EUGLENA CELLS CONFINED IN A MICRO-AQUARIUM

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ABSTRACT

We developed a new biosensor device for environmental gas/liquid toxicity sensing, which utilizes the chemotaxis of microbial cells confined in a micro-aquarium. The device has an isolated micro-aquarium to confine microbial cells separately from flowing gas/liquid in two bypass microchannels, and the molecules of the flowing gas/liquid permeate from the microchannels into the micro-aquarium through porous poly-dimethylsiloxane (PDMS) walls. We demonstrate that CO2-gas or ethanol liquid can be sensed by the device through the chemotaxis of *Euglena gracilis*. Since Euglena cells in the device can be maintained for more than two weeks, our chemotaxis device is suitable for long-term toxicity monitoring of environmental gases/liquids as well as for microbiological study on chemotactic reactions of motile microbes.

KEYWORDS: Chemotaxis, *Euglena gracilis*, Micro-aquarium, Gas diffusion, PDMS, Toxicity monitoring

INTRODUCTION

Flagellated microbes have high potentials as intelligent' micro-agents, which can be used as transporters of micron-scale objects [1], as localized sensors for chemical substances, and even as mediators of information processing [2]. By utilizing the chemotaxis of flagellated microbes and achieving the long-term culture of the cells, a small and convenient sensors monitoring environmental gas/liquid would be realized, which can replace the mine canary or fish-movement monitoring system used for the same purposes. Some devices of flow-mixing type or diffusion-separating type [3,4] have been developed to investigate the chemotaxis of *Escherichia coli*, however, even for the diffusion-separating type, the cells were injected in a small semi-closed chamber and not confined completely. To achieve the precise measurement of chemotactic movements of microbes for a long period of weeks, the cells should be confined completely in a micro-aquarium.

In this study, we developed a new type of chemotaxis device having an isolated micro-aquarium to confine the microbial cells and two separate flow microchannels to supply chemicals to the cells through the porous PDMS walls by permeation and diffusion. The chemotactic movements of *Euglena gracilis* confined in the micro-aquarium were measured *in situ* quantitatively by counting two-dimensional (2D) traces in a video image [5]. We tested CO_2 as a gas sample and diluted ethanol as a liquid sample. By flowing the gas/liquid in the bypass microchannels, *Euglena* cells exhibited clear negative (positive in some cases) chemotaxis.

MATERIALS AND METHODS

Figure 1 shows the design of the chemotaxis chips used in this study. The micro-aquarium in the chip had 16 walls to prevent the circumferential movements of the microbes along the outer edges, constructing 16 compartments around a center circle. The outer diameter of the compartments was 2.5 mm and the diameter of the center circle was 800 µm. Two microchannels of 200-µm wide, one for sample and one for reference, were isolated from the micro-aquarium with a 150-µm thick wall. The chip was fabricated with the conventional PDMS molding technique, and the height of the micro-aquarium and microchannels was approximately 150 µm. We confined 200-300 cells of *Euglena gracilis* as the chemotactic microbe in the micro-aquarium.

The chip was mounted on the stage of an optical microscope (Olympus, IX51) to observe the chemotactic movement of *Euglena* cells through a video camera (Trinity, IUC-200CK2) through 5x object lens. The chip was illuminated by red light from bottom side and observed from top side [5]. The *in situ* measurement of *Euglena* cells was carried out by counting the number of pixels in differential image (trace image) taken by the camera at each 1.3 s, which we call trace momen-

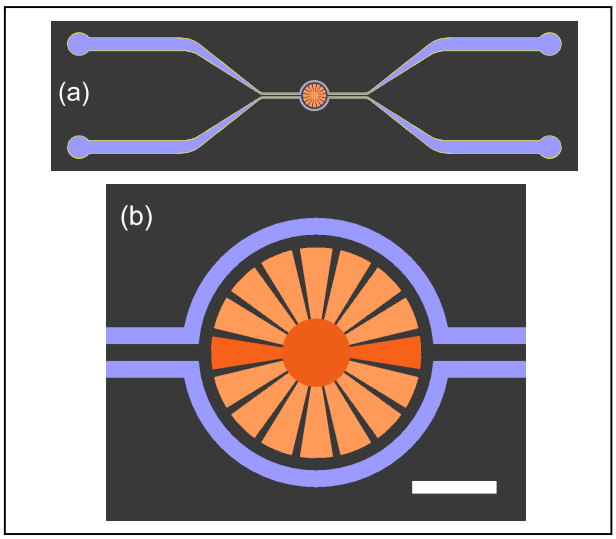

Figure 1: (a) PDMS chip with a micro-aquarium and two microchannels. (b) Enlarged drawing of the micro-aquarium with 16 compartments. Bar = 1 mm.

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tum (TM). It should be noted that fluorescence-dying technique is not required for the observation, since the size of *Euglena* cell was large enough for conventional transmission observation and only the mobile cells were detected in the differential images.

The sample/reference gas/liquid was put into the microchannels by syringe pumps with a speed of 3.0 mL/h for gases and 0.2 mL/h for liquids. The reference substance, air for CO_2 and pure water for ethanol, was injected to the counter microchannel to eliminate unintentional artifacts. Since a capillary tube of 1-m length was used to connect the syringes to the input ports of the chip, a delay time of several ten seconds was expected. The measure TM was obtained for the seven compartments close to the sample (reference) microchannel, and named *Msam* (*Mref*), resplectively.

RESULTS AND DISCUSSION

Figure 2a shows the temporal change of *Euglena* movements in the seven compartments of sample side and of reference side, observed when 100%-CO_2 gas (air) was introduced in the sample (reference) channel for 20 min (3.0 mL/h). The *Euglena* movements in sample (reference) side were decreased (increased) 2-min after starting CO_2 injection. After 10-min of CO_2 introduction, the *Euglena* movements in sample side was reduced to less than 10% of the original level, whereas that in reference side increased to 2.3 times higher than the original level, indicating that *Euglena* showed negative chemotaxis against CO_2. The result also revealed that CO_2 molecules flowing in bypass microchannel permeate through PDMS wall and dissolve into water in the micro-aquarium. The escape movements of *Euglena* were more quick than the recovery as shown in Fig. 2a, probably because the ventilation of CO_2 in the water in the micro-aquarium took much time compared to CO_2 dissolution into the water.

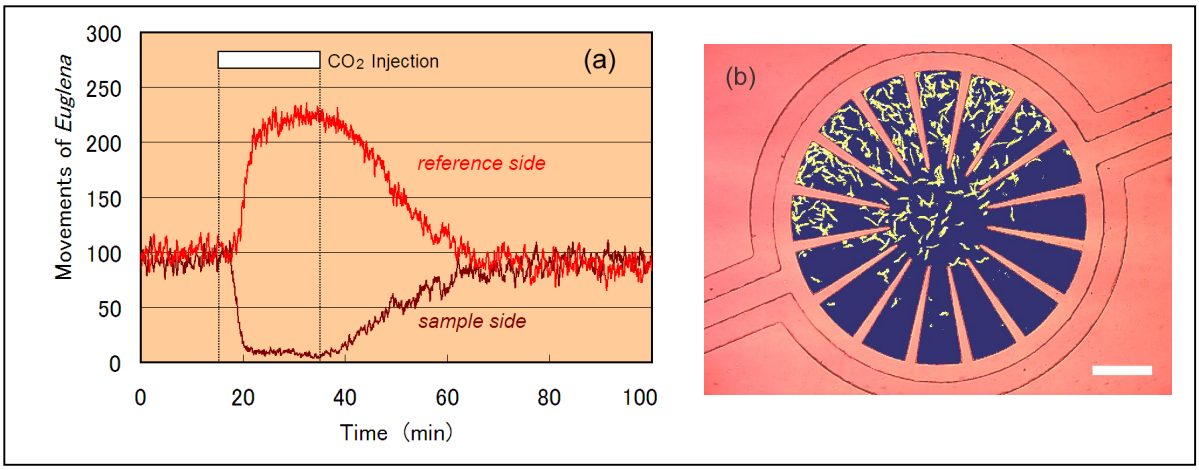

Figure 2: (a) Temporal change of Euglena movements obtained by 100%-CO_2 injection. (b) Trace image of Euglena cells for 1.3 s taken 10 min after CO_2 injection started, with CO_2 (air) gas flowing in the lower (upper) microchannel. Scale bar indicates 0.5 mm.

The *Euglena* movements observed at 10-min after the CO_2 injection is given in Fig. 2b, where the most cells were swimming in the reference side. The increase/decrease unbalance in Fig. 2a was due to the evacuation of *Euglena* cells from the center circle to reference side (Fig. 2b). The strength of chemotactic reaction can be measured by chemotactic reaction ratio defined as (*Mref* - *Msam*)/(*Mref* + *Msam*), where *Mref* and *Msam* were *Euglena* movements for reference- and sample-side, respectively. The chemotactic reaction ratio in Fig. 2a reached approximately 0.94.

When 2.0%-ethanol diluted in water was injected into the sample channel with pure water in the reference channel, a similar negative chemotaxis of *Euglena* was observed. Figure 3 shows the distribution of *Euglena* cells obtained by integrating the traces of cells for circumferential direction for the trace images taken at 0 and 10 min after the injection of 2.0% ethanol. The distribution was unbalanced to the reference side 10 min after the injection, revealing that the negative chemotaxis of Euglena to ethanol. The occurrence of chemotaxis means that ethanol molecules permeated into the micro-aquarium through the PDMS wall, indicating that our chemotaxis device can be used for liquid samples with small molecules as well as gases samples.

The dependence of the *Euglena* movements on gas/liquid concentration can be evaluated as the chemotactic reaction ratio, defined as the difference ratio of *Euglena* movements between sample and reference side. The dependence obtained for CO_2 and ethanol is given in Fig.4, which shows that chemotactic threshold of *Euglena* in our device was less than 20% for CO_2 and less than 0.5% for ethanol.

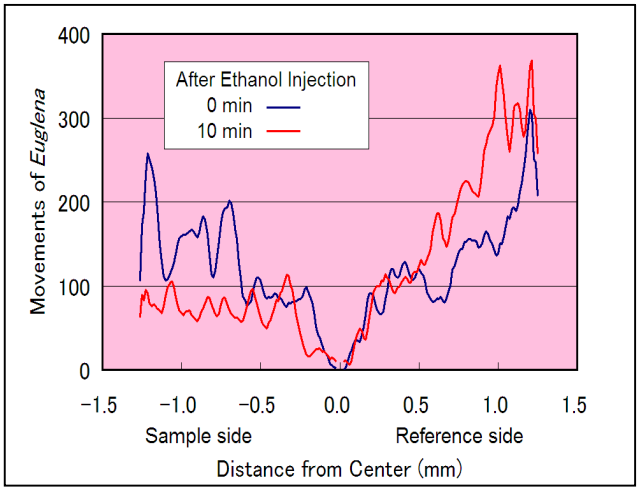

Figure 3: (a) Distribution of Euglena movements integrated for circumferential direction plotted for 0 and 10 min after the injection of 2.0% ethanol.

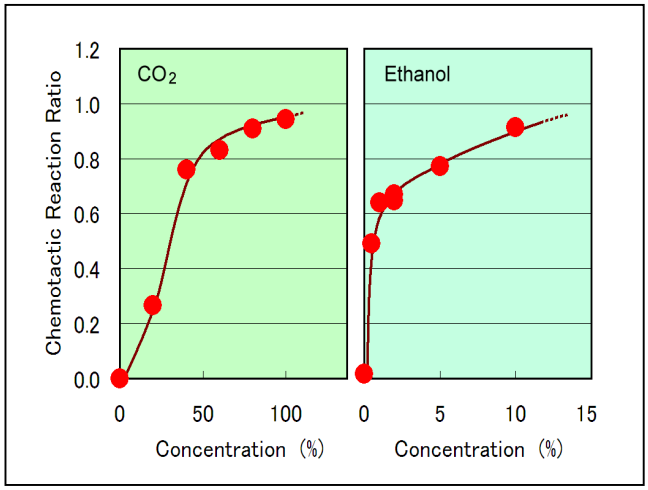

Figure 4: Chemotactic reaction ratio measured in our device for CO_2 and ethanol, both representing the negative chemotaxis of Euglena.

Separately, we succeeded in maintaining *Euglena* cells alive in the micro-aquarium for more than two weeks, which shows that a long-term toxicity monitoring will be realized through the chemotaxis of *Euglena* cells confined in our chemotaxis device. The results obtained in this study clearly show that our chemotaxis device is suitable for long-term toxicity monitoring of environmental gases/liquids as well as for microbiological study on chemotactic reactions of motile microbes.

CONCLUSION

Gas/liquid toxicity sensing was demonstrated by using the chemotactic movements of *Euglena* cells confined in a small space of micro-aquarium. Sample gas/liquid was supplied in one of two microchannels running aside of the micro-aquarium, and gas/liquid molecules permeate through PDMS wall into the micro-aquarium. Because the cells are confined in the micro-aquarium completely, our device requires only a small amount of microbial cells for a long-term sensing. We observed a large repulsive chemotaxis of *Euglena* to CO_2 and ethanol, and measured the dependence of chemotactic response on CO_2 and ethanol concentration. Our device with *Euglena gracilis* cells is promising for cytotoxicity sensing of environmental gases/liquids including volatile agrichemicals.

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