CELL MULTIPLICATION AND MOVEMENT ANALYSIS OF SWIMMING EUGLENA CONFINED IN A FLOW-ISOLATED MICRO-AQUARIUM

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ABSTRACT

We developed a new microchip for cell culture, in which the space for cell culture was isolated from two bypass microfluidic channels for culture medium supply. The chemicals of culture medium permeate from the bypass channels into the flow-isolated micro-aquarium through porous poly-dimethylsiloxane (PDMS) walls. The multiplication of swimming microorganism (*Euglena gracilis*) from one single cell has been achieved with long-term cell culture more than one month. Unlike other conventional cell-culture microchips, where cells are located in the flow of culture medium, our device is advantageous to culture non-adherent cells including circulating tumor cells, with analyzing cell division, cell movement, or cell differentiation, under fresh culture medium supply.

KEYWORDS: *Euglena gracilis*, Micro-aquarium, PDMS, Cell multiplication

INTRODUCTION

Microorganisms with flagellum have high potentials for transporting micron-scale objects [1], sensing localized chemicals, and processing information with their survival strategies [2]. We have found that toxicity sensors can be made by confining microorganism cells in a microfluidic chip [3], where small molecules of test sample permeate through PDMS wall from microchannels into a separated and closed cell micro-chamber (micro-aquarium). The permeation of small molecules leads to an idea of flow-free cell culture micro-chamber for flagellated microorganisms, by separating the closed micro-chamber for cell culture from nutrition supply microchannels. Complete confinement of the cells in the closed micro-chamber enables the precise measurement of movements of microorganism cells for a long period of weeks or months, where the cell multiplication can be tracked with time together.

In this study, we examined a long-term culture of photosynthetic microorganic cells, *Euglena gracilis* [4], in a microfluidic chip with flow-free micro-aquarium. The cells were confined in an isolated micro-aquarium, and minerals/ions required to cell culture were supplied from two separate flow microchannels by permeation and diffusion through the porous PDMS walls. The quantitative measurement of cell movements in the micro-aquarium was achieved by counting pixels in trace images taken by a video camera [5]. The cell multiplication was observed at approximately 24h each, with synchronous manner. Two cells born from one cell by cell division behaved differently, suggesting that this deviation leads to diversity in cell characteristics.

MATERIALS AND METHODS

The key point of our study is to separate the cell culture area from the flow lines for supporting medium. As shown in Fig. 1, our cell culture microchip has one micro-aquarium, i.e., closed chamber for cell culture, and two bypass microchennels running aside of the micro-aquarium [3]. The micro-aquarium (2.5 mm in diameter) and bypass microchannels (200 µm in width) were separated by a 150-µm thick wall of porous PDMS. The minerals, ions, and gases molecules in culture medium flowing in the microchannels permeate through porous PDMS wall to the micro-aquarium, supporting the cell growth in the micro-aquarium. The volume of the micro-aquarium was only 0.7 µL.

One single cell (or a several single cells) cultured in CM medium was confined in the micro-aquarium on the day 0. CM culture medium (or pure water) was supplied for two bypass microchannels with a flow rate of 20 µL/h from two syringes on a syringe pump.

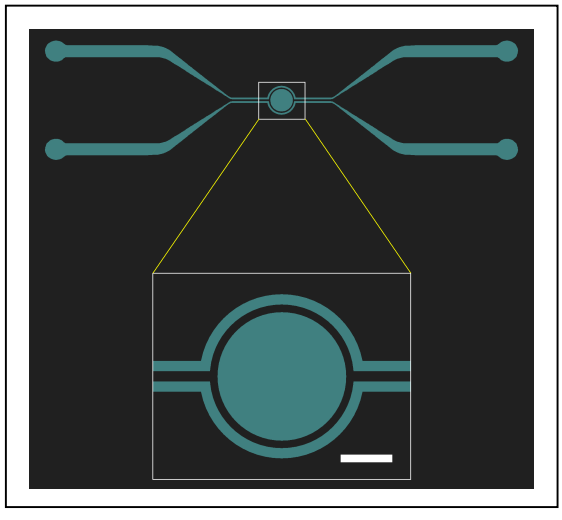

Figure 1: PDMS chip with a micro-aquarium and two bypass microchannels [3]. Bar = 1 mm.

The movements of *Euglena* cells were taken by a video camera through 5X objective lens with a microscope. The captured images were process with differentiation to extract the moving cell traces, and superimposition to show the trace clearly. The movement of the whole area was evaluated by counting up the pixels of all the traces in the micro-aquarium [5].

RESULTS AND DISCUSSION

Figure 2a shows the temporal evolution of swimming movement of *Euglena* cells observed for more than one month, starting with a few cells (Fig. 2b) at day 0. Red light of 18 mW/cm^2 was irradiated to the micro-aquarium. Pure water was supplied in the bypass microchannels for 0–16 days and CM medium for the 17th day and later. The cells grew with photosynthesis using minerals and ions in the initial CM medium in the micro-aquarium. The cell number once increased at the initial stage decreased after 10 days due to the shortage of culture chemicals in the micro-aquarium. When CM medium was supplied in the microchannels instead of the pure water at day 17th, the cells started growing rapidly in number again. This indicates that chemicals in the CM medium, minerals and ions, were supplied to the flow-isolated micro-aquarium by permeation through PDMS. The number of cells increased continuously, and the traces of the cells became overlapped as shown in Fig. 2c, which resulted in the saturation in measured cell activity in Fig. 2a after day 27th. The growth of cell number continued for more than two months [3], as the optical transmission through the micro-aquarium was continuously decreased by multiplied cells.

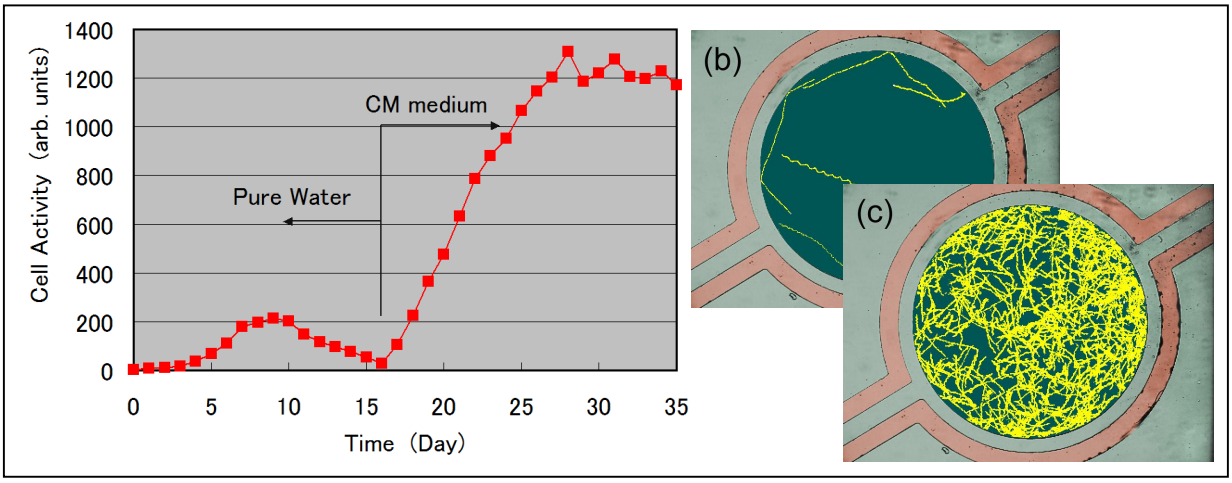

Figure 2: (a, light) Temporal change of cell activity, with pure water (0–16 days) or CM medium (17th day and after) supplied in the bypass microchannels [3]. The number of Euglena cells increased only temporally when pure water was supplied, whereas it grew with CM medium, revealing that chemicals in CM medium permeate through PDMS. (b,c) Swimming traces of cells observed on 1st day (b) and 18th day (c).

We examined the detailed analysis of cell movements in real-time for a separated experiment with starting cell culture from one single cell at day 0. CM medium was supplied in the bypass microchannels for the entire duration of the experiment, and white LED light was irradiated to the micro-aquarium with high/low (2.0/0.2 mW/cm^2) intensity for 12 h each. Figure 3 shows the temporal change of swimming movement observed for 10 days. As clearly seen in Fig. 3, the cell division took place synchronously, as a manner of multiplication as 2, 4, 8, 16. Since the cells stopped swimming during the cell division period (Fig. 4), swimming movement dropped to zero at the timing of each cell division. After 5th day, the timing of cell division shifted to the dark phase in illumination, which revealed that photo-synchronization was achieved by shortening the cell multiplication cycle.

We also succeeded in tracking the cell division process in the micro-aquarium with a higher magnification of 50X, as shown in Fig. 4. The cell division took 3-4 hours in our experiments. Interestingly, the divided cells started swimming with a time interval of approximately 10-40 min, i.e., one of the two cells departed the site first while the other cell still immobile at the site for 10-40 min. This reveals that the divided cells are not equal in the sense of growth phase or metabolic status, leading to the diversity in cell characteristics after the cell division. We confirmed that the cells grown from one single cell behave differently when they encounter a light gradient. Some cells have higher resistance for a stronger light than the others showing that metabolic status in their cell bodies differ from others even for those multiplied from a single cell.

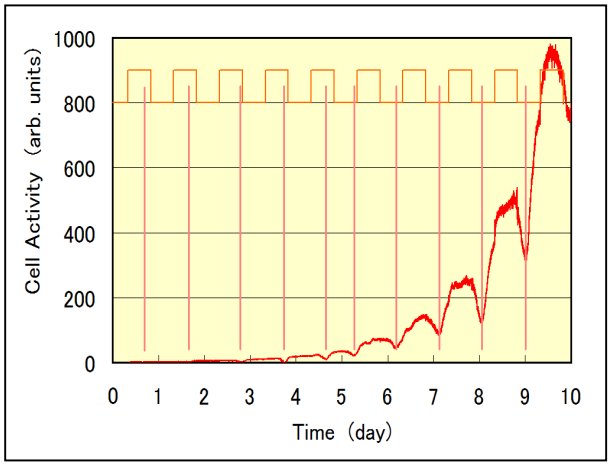

Figure 3: Temporal evolution of cell activity at the early stage of cell culture, starting with one single cell. The cell division (vertical line) occurred with light-on/off synchronization after 5th day.

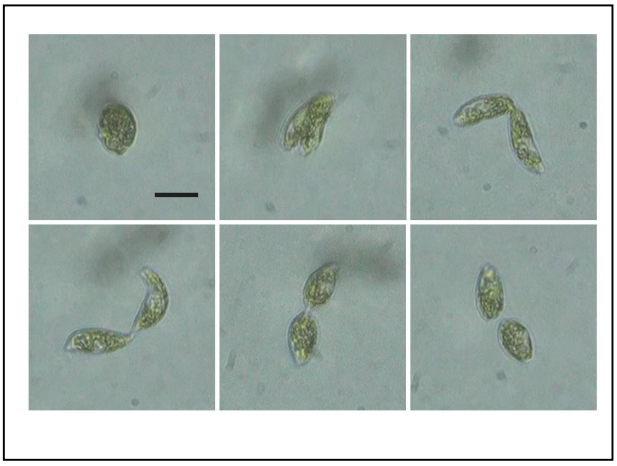

Figure 4: Sequence of cell division observed in the micro-aquarium, from top left to bottom right (duration 1.9 h). Scale bar = 20 µm. The cell divided into two with rotating the body at the site.

CONCLUSION

We achieved the month-long cell culture of photosynthetic microorganism *Euglena gracilis* in the flow-free closed-space of micro-aquarium isolated from bypass microchannels. Minerals and ions in cell culture medium in bypass microchannels were supplied to the isolated micro-aquarium via the permeation of chemicals through porous PDMS wall. The cell multiplication was tracked by evaluating swimming traces in the micro-aquarium, revealing the synchronized cell division took place according to illumination light on/off. Our study shows that the flow-separated micro-aquarium design employing bypass microchannels to supply chemicals through porous PDMS wall is promising for the investigation on cell growth, multiplication, and differentiation, under chemical stimulations.

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