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Culture and Motion Analysis of Diatom *Bacillaria paradoxa* on a Microfluidic Platform

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**Abstract**  We proved the feasibility of using a microfluidic chip to culture diatom *Bacillaria paradoxa*, and analyzed the gliding characteristics of its self-organized colony in detail. The optimal cultivation parameters of *B. paradoxa* for the designed chip made with polydimethylsiloxane are as follows: the preferable cells injecting rate for keeping the cells alive is 0.2 mL/h, the initial cell density for fast reproduction is $5.5 \times 10^4$ cells/mL, and the optimal replacement period of culture medium is 4 days. *B. paradoxa* tends to form a colony during their growth, and the colony can glide with a steady period of $29 \pm 3$ s along its axial direction in a constant stream, the amplitude of the colony will not decay (e.g., 24 μm of two-cell colony at 1.1 mm/s flow rate), and the colony rapidly adjusts its direction of gliding to the direction of water flow. The successful culture of diatoms on a microfluidic platform may be used for biosensing chips and the creation of gasoline-producing diatom solar panels.

**Introduction**

Diatoms are unicellular and photosynthetic algae widely distributed on the earth. Their rigid cell walls, called frustules, have delicate micro- and nanoporous structures that are composed of amorphous silica [1]. The frustules have well-arranged pore arrays [2], large specific surface area, good absorbability and biocompatibility [3], and special optical characteristics such as photoluminescence [2]. As an excellent hierarchical structure material, the frustules attract wide interests of research and show great potential for applications in micro/nano manufacturing, microfluidic devices, biosensors, energy devices, etc. [4–7].

Conventional methods of obtaining fresh frustules in laboratories often need to culture diatoms in flasks or bioreactors [8, 9] then remove the organic matter of diatom cells, and also might include filtration processes [10]. After that, some types of frustules with fragile structures are easily damaged due to the pressure and fluid impact, such as *Bacillaria* and *Ditylum* sp. [10], calling for more gentle culture and processing methods. Recently, microfluidic chips have been used to culture animal cells and tissues [11–14] due to their miniaturization, low power consumption, multi-functional integration, and portable characteristics. Microfluidic devices were also proved to be convenient and advantageous to culture, operate, and study microbes such as yeast, bacteria, and microalgae [15, 16]. However, there are few reports about culturing diatoms in microfluidic chips.

In this paper, we explore the possibility of culturing the diatom directly on a microfluidic chip. Considering diatom growth process needs not only nutrition in solution, but also photosynthesis and respiration, so we use polydimethylsiloxane (PDMS) to fabricate the microfluidic chips which is both optically transparent and permeable to gas. The culturing condition of diatom on the microfluidic chip is optimized, and the gliding motion of the diatom colony is analyzed in detail. Successful culture of diatoms in “microcosms” not only is meaningful for biosensing, but also may be essential to the creation of gasoline-producing diatom solar panels [17].
Materials and Methods

Materials and Devices

The diatom *B. paradoxa Gmelin* and culture medium formula [18] used in this experiment were obtained from the Diatom Laboratory of Xiamen University, China. Other devices and reagents include: lithography machine (model: URE-2000, the Institute of Optics and Electronics of the Chinese Academy of Science), heating plate (model: kw-4AH, Chemat Technology Inc), ultraviolet curing machine (model: kw-4AC, Chemat Technology Inc), vertical pressure steam sterilizer (model: BX-30R, Shanghai Boxun Industry & Commerce Co., Ltd), micropump (product model: JZB-1800, Jian Yuan Medical Technology Co. Ltd), square glass, PDMS pre-polymer (Sylgard 184) and crosslinker, CH$_3$CH$_2$OH (anhydrous ethanol), CH$_3$COCH$_3$ (acetone A.R.), CH$_3$(CH$_2$)$_4$CH$_3$ (n-hexane), etc.

Fabrication of the Microfluidic Chip

Soft lithography [19] was used to fabricate the PDMS substrates with microchannels. A layer of SU-8 negative photoresist was spin-coated on a square glass slide (the thickness of photoresist layer is 35 μm), and then exposed and developed to form molding patterns of microscale channels. Prepolymer mixtures of PDMS were cast and cured to obtain microchannel substrates and coverslips. Both substrates and coverslips were made with PDMS in this experiment to make chips possess better air permeability. The thickness of them was 2.8 ± 0.3 mm. The refractive index of PDMS is 1.410 [20]. The coverslip was punched with a sharpened glass tube to fabricate one inlet and three outlets with diameter of 1.1 mm. After ultrasonic cleaning by hexane, acetone, and ethanol for 15 min in order, the microchannel substrates and coverslips were ultraviolet irradiated in a UV curing machine for 3 h, and then fit together rapidly and laid aside for 48 h to seal the microchannel.

Injecting Diatom Cells into Microfluidic Chips

The injection speed is one of the key parameters which affects the number of living cells in chips. In this study, we use the proportion of intact cells (diatom cells that have cytoplasm and unbroken frustules) to assess the culture condition. *B. paradoxa* with mean size of 100 μm in length and 6 μm in width were cultured in an incubator first, then they were moved into a disinfected beaker when they were in logarithmic phase, and the beaker was gently shaken to make diatom colonies separate into single or double cells. Since some diatoms tend to settle at the bottom even with slight shaking, to avoid the influence of different diatom concentrations at different depths, only the supernatant culture media were used, in which the density of the diatom was around 5.6 × 10$^4$ cells/mL. Then the sample was injected into 7 chips at different speeds (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0 mL/h) for 2 min through an automatic micropump and a syringe. After that, the number and proportion of intact cells in 7 chips were measured separately under a microscope.

On-chip Diatom Culture

Culture medium was sterilized at 121 °C for 20 min at 15 psi. Three chips were fabricated to determine the optimal replacement period of medium. After the diatom cells were injected into the 3 chips (0.2 mL/h, 0.2 min), the chips were placed in big beakers filled with culture medium to avoid the water in the chip from evaporating away and cultured in a light incubator. The culture temperature was maintained at 21 ± 1 °C, and the illumination intensity was 2,000 lux with a 12:12 h light/dark period. The number of cells in each chip was measured every day under a microscope, and colony formation situation was also observed simultaneously.

In order to study the effect of the initial density of diatom cells on their reproduction rate, the diatom cells were injected into 24 chips at a speed of 0.2 mL/h for different injecting times (0.5, 0.67, 1, 2, 3, 4, 5, 6 min) to make the initial cell density inside the chips different (3 × 10$^4$ cells/mL). After being cultured for 4 days, the cell numbers inside the 24 chips were measured under a microscope.

Motion Analysis of the Colony of Diatom *Bacillaria paradoxa*

A previous study has proved that the movement of the *Bacillaria* colony is impacted by the water flow [21, 22]. Here, we further study its motion characteristics under different speeds of water flow in the microfluidic chip. The cell colony motility rhythms in still water and flowing water were measured by shooting the videos (VSI USB2.0 Digital Camera, #pixel: 5.2 × 5.2 μm, frame rate: 30 f/s) of colony movement under a microscope (XDS-1B, objective: 25×, field of view: 1.98 × 10$^4$ μm$^2$, the depth of focus is more than 10 μm), and the microstructure of *Bacillaria* frustule was observed with SEM to analyze its motion mechanism.

Results and Discussion

Microfluidic Culture Platform

The culture platform consists of a microfluidic chip, a micropump, a syringe, an injection tube, and an inverted microscope (Fig. 1a). The microfluidic chip made with
PDMS contains one inlet in the center of the chip, twelve culture chambers, and three outlets which were all uniformly circularly distributed (Fig. 1b). The diameter of the inlet chamber is 6 mm, which is big enough to make the fluids and diatom cells flow into 12 culture chambers. Twelve uniform-sized small culture chambers with diameter of 2 mm and height of 35 μm can facilitate the observation of the cells' growth situation. Three outlets are designed to let the fluid distribute uniformly in the chip. The automatic micropump was used to accurately control the injection speed, and the culture media with diatom cells were injected equally into each culture chamber. The flow channels (Fig. 1b) have a width of 120 μm and height of 35 μm, which could allow the separate Bacillaria cells (90–110 μm in length, 6 μm in width) to pass through freely. Arrayed barriers were designed at the far end of culture chambers to keep cells in culture chambers (Fig. 1c, d). Since the microfluidic chip is made of PDMS, which is not only optically transparent but also permeable to gases, the diatoms cells in the culture chamber can grow and reproduce by absorbing nutrient of medium in the chip.

Optimal Injecting Speed for Keeping Diatom Cells Alive

The cell density of the Bacillaria sample was $5.6 \times 10^4$ cells/mL. After injecting the sample into chips at different speeds (0.1–2 mL/h) for 2 min, the cell number and proportion of intact cells in different chips differed. As shown in Fig. 2a, the number of intact cells increased first along with the increase of injecting speed, and reached up to 273 cells at 0.3 mL/h, and then decreased to 138 cells at 2 mL/h. However, the proportion of intact cells decreased along with the increase of injecting speed (Fig. 2b), dropping down to 75 % at 0.3 mL/h and even lower at higher speeds.

The B. paradoxa cell has a rod-like structure, which is more fragile than other species of diatoms: in a previous study we found that a force of 25 μN could break the cells [10]. Figure 2 indicates that the cells could be moved to culture chambers with the medium flow rate we applied, yet they would accumulate in the central chamber and even break in a strong current. By comparison, an injecting speed of 0.1–0.2 mL/h is more suitable for moving cells to

![Fig. 1](image-url) The microfluidic chip-based culture platform. a Overview of the platform, b microscopic image of the microfluidic chip. The arrows show the flow direction, c SEM image of a microchannel, d SEM image of the barriers in the microchannel marked in e
culture chambers while keeping a cell survival rate of more than 90%. Considering the low efficiency of 0.1 mL/h, we choose 0.2 mL/h as the injection speed for the following experiments.

Culture of Diatoms in Microchambers

According to our previous study, the optimal replacement period of culture medium for diatom flask culture is seven days, but in the microfluidic chip culture it is different (Fig. 3a). The initial number of cells in the 3 chips was 225 ± 10 cells. The living cells increased to 281 ± 11 cells at 1st day, reached the top growth of 456 ± 12 at the 4th day, and then decreased to 112 ± 8 cells at 7th day. The decrease of cell count for 5th, 6th, and 7th day is probably due to insufficient nutrients. After culturing for 4 days, over half of the cells formed colonies and the longest colonies contained 8–9 cells. From the experimental data, replacing medium in the microfluidic chip every four days seems to meet the nutrient requirement of diatom cells.

To analyze the relationship between the initial cell density and the cells’ incremental increase in number, the diatom cells were injected into 24 chips with different injecting times at the same speed to make the initial number of living cells range from 56 to 650. After being cultured for 4 days, the cell number in different chambers was measured and compared (Fig. 3b). From the figure we can see that at the lower density, the cell number increased faster when initial cells are denser, and it reached the fastest point at injecting time of 2 min, with which the initial cell number is 209, the corresponding cell density is calculated to be about 5.5 × 10^5 cells/mL, and the cell growth rate could reach 115% in 4 days. But when the initial cell density is even higher, the reproduction rate of the cells decreased: for example, when the cell density was above 1.03 × 10^5 cells/mL, the cell growth rate decreased to less than 20%. The results follow the trend of flask culture: the diatom cells reproduce slowly when the cell density is too low or too high. Through culture in microfluidic chips, we can conveniently get the relationship between the initial cell density and the growth rate, which can be used to guide the culture of diatom in a flask and natural waters.

Gliding of the Bacillaria paradoxa Colony

The cells of B. paradoxa Gmelin usually remain connected with each other after they divide, and form a chain-like cluster. The motility rhythms of Bacillaria colonies has been reported: cultures of Bacillaria grown under an alternating light/dark regime were found to exhibit rhythms in cell motility with the majority of colonies being motile during the light periods and non-motile and aligned during the dark periods [22, 23]. But here we mainly focus on the colony movement in water flow since the flow rate can be precisely controlled in the microfluidic chip, and neglect the influence of the photokinetic response. Though the cells can form long colonies (as shown in Fig. 4a), to simplify the study on their basic motion characteristic, we selected several colonies with only two cells for this study.

The different statuses of typical colony movement in still and flowing water are shown in Fig. 4b–h, and detailed statistical information such as vibration amplitude, period and trends of the colony movement is shown in Fig. 5. In still water, the stretch amplitude (A, as shown in Fig. 4f) gradually decreases to nearly zero (0.5 μm) in 0.2 h, and the vibration period is 29 ± 3 s, meaning that the variation is within ±10.3% (Fig. 5a, b). When the flow speed is 1.1 mm/s, the stretch amplitude is kept at 24 ± 2 μm and the period is also 29 ± 3 s, meaning that the variation is within ±12.5% and ±10.3%, respectively (Fig. 5a, b). The colony tends to turn its stretching direction to the fluid flow direction. As shown in Fig. 4g, we use the angle α to express the included angle of the colony vibration direction and the flow direction. For most colonies, α tend to be about 0° within 10 min (as shown in Fig. 4h), indicating that the diatom colony tends to vibrate along the direction of the least flow resistance.
To further investigate the gliding mechanism of the diatom colony, SEM was used to observe the detailed structure of the *Bacillaria* frustules, as shown in Fig. 4i–k. An irregular flange (rf) structure is found in the middle of the valve, which may function as gliding rails to make the adjacent cells glide relative to one another. One flange is bigger than the other (Fig. 4l), so that cells can mesh with adjacent cells to form a regular chain-like colony [21]. Besides, the adjacent valves of two cells are connected with elastic fibrils (EF) to prevent them from departing away, especially when the colony is impacted by water flow. The fibulae structure (F) marked in Fig. 4k could fix EF and strengthen the frustule [21, 23]. Through experiments, we found that the motion of the colony is not uniform, though the vibration period is relatively steady, yet the colony not always stretch to its maximum amplitude, as shown in Fig. 5d. The EF may act as the weak spring, which determines the motion period and frequency of the colony, and limit the maximum stretch amplitude, yet can’t assure its stretch amplitude uniform within different periods. Though the other researcher has reported that the colony vibration was caused by the photokinetic response [22, 23], yet in our study, not all the colonies in the chip vibrate at the beginning, only slightly shaking or flushing.
with a water flow with speed $>0.6$ mm/s can cause the large-amplitude vibration of the two-cell colonies.

Conclusion

We proved the feasibility of using a microfluidic chip to culture diatom cells. The optimal cultivate parameters of diatom *B. paradoxa Gmelin* for the designed chip are as follows: the optimal cells injecting rate for keeping the cells alive is 0.2 mL/h ($5.6 \times 10^4$ cells/mL), the optimal initial cell density for the fastest reproduction is $5.5 \times 10^4$ cells/mL, and the optimal replacement period of culture medium is 4 days. In addition, we studied the motion of a two-cell *Bacillaria* colony using the chip. With slightly shaking or being motivated by a water flow $>0.6$ mm/s, most two-cell colonies can do large-amplitude vibration. In a constant stream, the colony will rapidly adjust its direction of vibration to the direction of water flow and keep the amplitude of vibration (e.g., 24 $\mu$m of two-cell colony at 1.1 mm/s average flow rate), and the vibration period of the two-cell colony is $29 \pm 3$ s. If the chamber was set still, then the vibration amplitude of the colony would decrease gradually and reach less than 0.5 $\mu$m in about 10 min. The culture of diatom on a microfluidic chip provide a miniaturized, convenient, and easy tracking tool for the biological study of diatoms, such as the morphology, physical, growth and motion characteristics, and culture parameters of diatoms.

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