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Fabrication of Microfluidic Device for Quantitative Monitoring of Algal Cell Behavior using X-ray LIGA Technology

J. Ruenin, S. Sukprasong, R. Phatthanakun, N. Chomnawang, and P. Kuntanawat

***Abstract*—**In this paper, a simple microfluidic device for monitoring algal cell behavior is proposed. An array of algal microwells is fabricated by PDMS soft-lithography using X-ray LIGA mold, placed on a glass substrate. Two layers of replicated PDMS and substrate are attached by oxygen plasma bonding, creating a microchannel for the microfluidic system. Algal cell are loaded into the microfluidic device, which provides positive charge on the bottom surface of wells. Algal cells, which are negative charged, can be attracted to the bottom of the wells via electrostatic interaction. By varying the concentration of algal cells in the loading suspension, it is possible to obtain wells with a single cell. Liquid medium for cells monitoring are flown continuously over the wells, providing nutrient and waste exchange between the well and the main flow. This device could lead to the uncovering of the quantitative biology of the algae, which is a key to effective and extensive algal utilizations in the field of biotechnology, food industry and bioenergy research and developments.

***Keywords*—**Algal cells, microfluidic device, X-ray LIGA, X-ray lithography, metallic mold, synchrotron light, PDMS

I. Introduction

MICROALGAE play important role as primary producers in aquatic food webs and are major oxygen suppliers on the earth.

They can be found in forms of single cellular and multicellular. A great number of commercially valuable products such as fatty acids [1], proteins [2], bioactive compounds [3], natural pigments [4], and energy source [5] can be extracted from different species of algae. Although there are a large number of studies on several aspects of the algae within the past decades, the quantitative behavior, physiology and knowledge about their delicate architectures of the algal cells are still poorly understood. The major problem is the lifestyle of the algae, freely floating in a liquid body. Investigation of the cells under a few millimeter to submillimeter scale microscope's view of any particular cells or colonies, within a cloud of other cells under the constant natural water flow, is therefore next to impossible.

Recently, the micro-fabrication technology has been employed to resolve the problem. Pan *et al.* demonstrated that oil-suspended microdroplets of algal medium can be generated using micronozzle. With a well calibrated cell concentration, almost single cells are trapped inside their own compartment [6]. In another work [7], a hydrodynamic beam focusing allows a precise delivery of algal filament to a microcage structure.

J. Ruenin and P. Kuntanawat are with the King Mongkut's University of Technology Thonburi, Bangkok, Thailand (e-mail: bldejazaa@gmail.com).
S. Sukprasong and N. Chomnawang are with Suranaree University of Technology, Nakorn Ratchasima, Thailand (e-mail: sut.mems@gmail.com).
R. Phatthanakun is with the Synchrotron Light Research Institute (Public Organization), Nakorn Ratchasima, Thailand (e-mail: rungrueang@slri.or.th).

With this, the algal filament is captured by the structure within a continuous flow of the medium, where nutrient and waste exchange can take place. Although these works allow an extent of single cell monitoring, still there are some limitations. The first model is not flexible for complicated experiments, because manipulation of cells inside the droplets during the experiment is impossible. The second method may provide a good affinity capturing, but the microcage may not be suitable to trap several kinds of algae that come with a wide range of shape and size. To overcome these problems, we are proposing a rather simpler system that would be likely to be compatible with several species of algal cells. Based on X-ray LIGA technology, array of algal wells are created by PDMS soft-lithography and plasma bonding techniques. PDMS microfluidic device is attached on a microscope slide, which provides a positive charge on the bottom surface of the wells. After the algal cells are trapped inside the wells, they can be observed under different culture conditions. This device can be used to track the behavior of individual algal cells and improves the cultivation method for many days.

II. Microalgae Cultivation Design

Figure 1 shows a functional diagram of microfluidic device for quantitative cell tracking. It is a chamber of a microscope slide size, which contains an array of about 200 wells. Two layers of polymer are fixed together and bound to the glass substrate, to create an algal culturing system on a chip. The microwells' dimensions are about 1 mm (L) × 1 mm (W) × 0.5 mm (H), which is large enough to trap an algal cell at the bottom surface. In order to simplify the fabrication of the device, we employed poly-dimethysiloxane (PDMS) patterned using conventional soft lithography technique. PDMS mixture was casted onto metallic microwells mold, created by X-ray LIGA process. This is the first PDMS layer that functions as an array of algal cell wells. The second layer is a flow channel provided with an inlet and an outlet port of water.

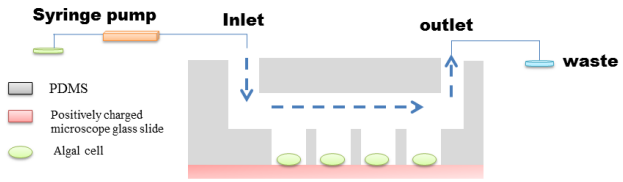


Fig. 1 Functional diagram of microfluidic device of algal cell culture

III. Fabrication Process

A. Deep X-ray LIGA for Metallic Mold

X-ray lithography is an advanced technique, which uses synchrotron light in micromachining fabrication.

High aspect ratio microstructures can be achieved after lithography process. This technique is usually used for high accuracy fabrication of micromolds and microparts for many applications. The process of X-ray LIGA fabrication of the metallic mold and polymer replication is illustrated in Fig. 2. The key material of this technique is SU-8 negative photoresist, which is hardened by X-ray radiation and remained after the development. The process was started by deposition of 1000 µm-thick SU-8 photoresist film on a stainless steel substrate, as shown in Fig. 2(a). Then an X-ray mask, which comprised a graphite substrate and gold absorber patterns of algal cell wells (as shown in Fig. 3), was placed over the photoresist. By using X-ray radiation, the pattern was then transferred to the photoresist layer, as shown in Fig. 2(b). After rinsing the sample with SU-8 developer, unexposed areas were dissolved, resulting in a micromold of SU-8 microwells, which was later used for metal electroplating, as shown in Fig. 2(c). Wood's Strike technique was applied to the substrate as shown in Fig. 2(d). The micromold was then immersed in electrolytic bath, where the SU-8 microwells were filled with nickel by electroplating process, as shown in Fig. 2(e). The electroplated metal was then polished to remove the excess, in order to obtain a thickness of about 900 µm, as shown in Fig. 2(f). Finally, the SU-8 photoresist mold was removed by PG remover (MicroChem®) and O_2/CF_4 plasma etching, as shown in Fig. 2(g).

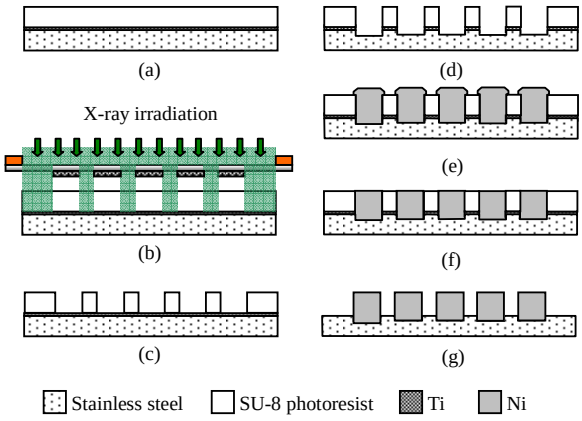


Fig. 2 Fabrication sequence of the metallic mold for X-ray LIGA

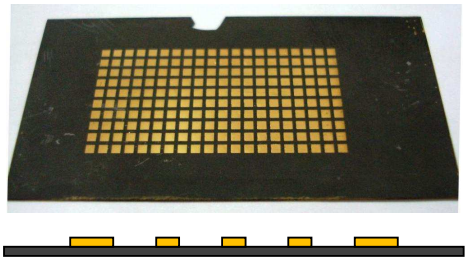


Fig. 3 X-ray mask of graphite sheet with gold absorber

Figure 4 shows a complete metallic mold for algal cell microwells.

There are 200 nickel bricks separated by a distance of about 500 µm from each other. Because of the high energy and low divergence of X-ray radiation of synchrotron light, the metal structures with vertical smooth side walls were easily obtained. These features are very useful for PDMS replication, because they reduce the resistance during mold peeling.

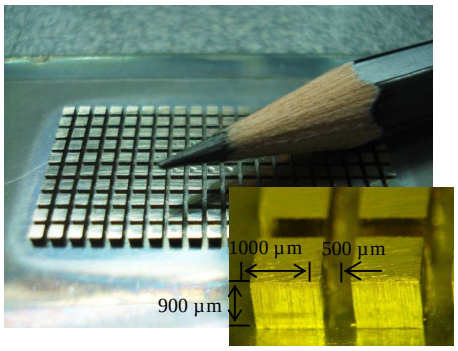
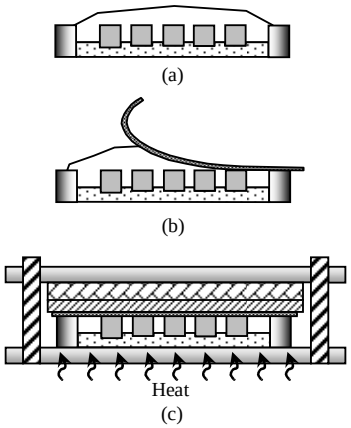


Fig. 4 Metallic mold for algal cell wells

B. Microfluidic Device by Soft Lithography Technique

The replication sequence of algal cell microwells using soft lithography is demonstrated in Fig. 5. The process started by encompassing a polymer frame around the metallic mold. PDMS mixture was carefully poured onto the mold as shown in Fig. 5(a). To create smooth surfaces on both sides of the PDMS layer, a transparent plastic sheet was gradually placed on the mold as shown in Fig. 5(b). Another glass slide was also placed on top of the transparent sheet to reinforce, followed by a rubber sheet, preventing glass slide breaking. All of them were pressed together by aluminum clamping and then heated on a hotplate at 60°C for 60 minutes to solidify the PDMS, as shown in Fig. 5(c). After removing from the metallic mold, as shown in Fig. 5(d), the PDMS was oxygen plasma treated and bonded on a glass slide substrate, as shown in Fig. 5(e).

The flow channel layer was replicated with the same technique as the microwells. The mold for the flow channel was created by stacking several layers of PET tape, as shown in Fig. 6. A 1000 µm-thick flow channel was casted on the PET tape mold, which was surrounded by a 1500 µm-thick rectangular PET tape frame.



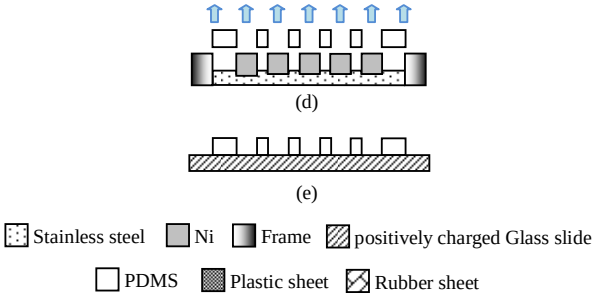


Fig. 5 Replication sequence of the algal cell microwells by soft lithography

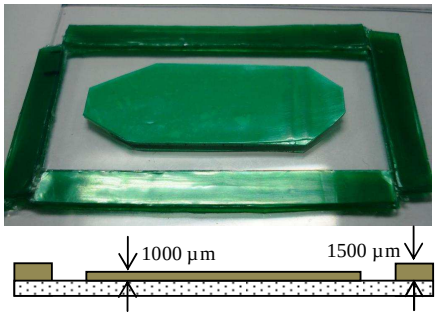


Fig. 6 Simple PET mold for flow channel layer

To provide the inlet and outlet, polymer tubes, with inner diameter of 1 mm and 3 mm outer diameter, were fixed at the end of the PET mold as shown in Fig. 7(a). Then PDMS mixture was poured onto the mold and heated on the hotplate at 80 °C for 30 minutes as shown in Fig. 7(b). The hardened PDMS was peeled out from the mold. Some PDMS residues stuck in the tubes were punched out as shown in Fig. 7(c). Finally, the flow channel was bonded on the algal cell microwells using PDMS mixture as glue, as shown in Fig. 7(d). Both layers were heated on the hotplate at 60°C for 60 minutes. Figure 8 shows the complete microfluidic device.

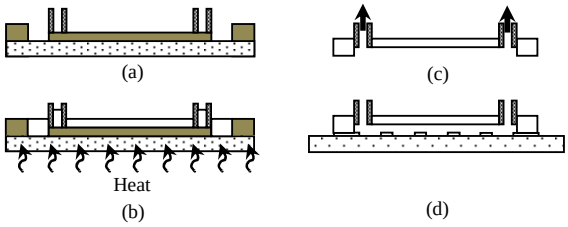


Fig. 7 Fabrication process of the microfluidic device

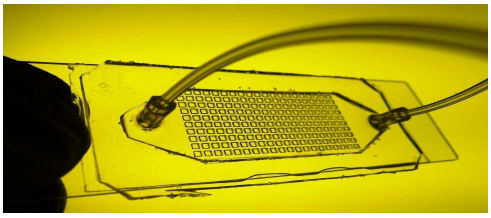


Fig. 8 The complete microfluidic device

IV. Experimental Setup

Figure 9 illustrates the experimental set up to monitor the behavior of algal cells. This setup comprises the microfluidic device, a reservoir, a valve, a beaker for waste, a syringe and a microscope. To avoid gas bubbles trapped inside the wells during algal suspension injection into the device, ethyl alcohol, which has a density lower than water, was first introduced into the channel first, followed by DI water for wells cleaning. After rinsing the wells, liquid medium in the reservoir was then loaded into the microfluidic device. By adjusting the concentration of algal cells in the loading suspension, it is possible to obtain wells with a single cell. Seeding the cells into the device was done by injecting the algal suspension into the mainstream flow via the T-connector at the inlet port. Figure 10 demonstrates the experimental setup under the environmental control for algal cell culturing.

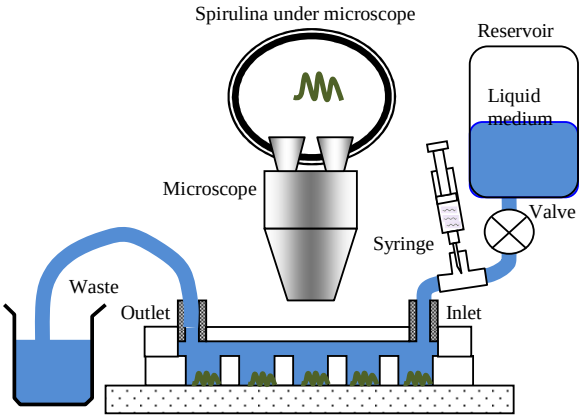


Fig. 9 Diagram of algal cell experimental setup

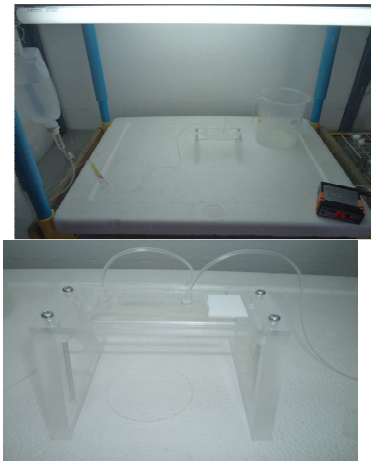


Fig. 10 Experimental setup under the environmental control

V. Experimental Results and Discussion

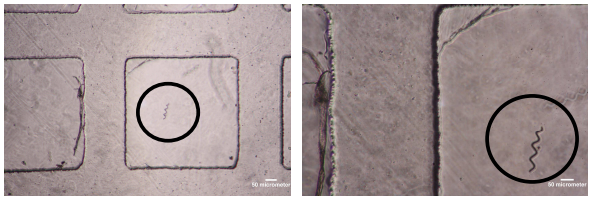
After algal suspension, with concentration of about 1-4 cells/ul, was fed into the microfluidic device, we observed some algal cells entrapped in the microwells. We believed, as we expected, that this is because the stream forced some algal cells to approach the bottom of the wells.

Then the positive charges found on the surface attracted the cells. Electrostatic force was strong enough to hold the cells in place. Therefore the cells remained in the wells, although they were still capable of moving around on the bottom. The vertical walls constrained the movement of the algae to a certain area, therefore the cells were always found in the field of observation.

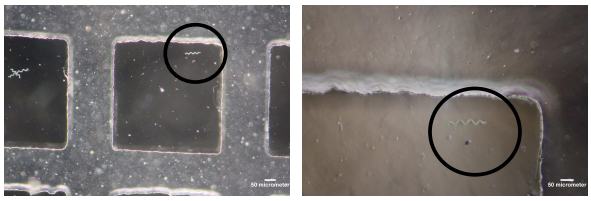
Figure 11 shows spirulina trapped inside the wells. By varying the concentration of algal cells in the loading suspension, we found it is possible to improve the number of wells with a single cell.

In addition, the flow rate in the microchannel was easily controlled by adjusting the reservoir's valve, so it is possible to generate experimental condition specified by the user.

With our system, algal cell could be monitored under controlled conditions, which could be either static or dynamic such as nutrient decline and starvation.



(a)



(b)

Fig. 11 Spirulina cells trapped inside the well after feeding with laminar flow of liquid medium

VI. Conclusion

Microfluidic device fabricated by X-ray LIGA and soft lithography techniques are proposed to monitor the behavior of individual algal cell. The device consisting of an array of wells with vertical side walls was designed. A metallic mold of 200 wells was constructed on the stainless steel substrate for multiple replications of the microwells PDMS. The algal cells can be trapped on the bottom surface by electrostatic force, due to the positive charge found on it. The complete device allows observation of cell behavior under controlled conditions and more complicated experimental requirements compared to other previously mentioned devices. This could lead to a better understanding of algal biology and utilization.

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