MICROALGAL CULTURE, LIPID PRODUCTION AND EXTRACTION USING AN INTEGRATED MICROFLUIDIC SYSTEM
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ABSTRACT
We present a novel method for simple and efficient lipid analysis by integrating multi-process including microalgal culture, lipid production and lipid extraction on a single microfluidic device. We devised a double-layered PDMS device, composed of cell culture chamber and connecting channel filled with micropillar array between culture chamber and output reservoir. Lipid accumulation was induced by supply of nitrogen deficient media into microchannel and lipid extraction was performed by alcohol solvents such as 70% ethanol, isopropanol. Cell debris was removed by micropillar filter and lipid extract from output reservoir was analyzed by thin layer chromatography and gas chromatography.

KEYWORDS: Microfluidics, Microalgae, Culture, Lipid production, Lipid extraction, Micropillar filter

INTRODUCTION
Microalgal lipid has gained increasing interest as a promising alternative to fossil fuels due to its sustainability. Simple and efficient lipid analysis is required for selection of desirable strains for biofuel production. However, conventional method for lipid accumulation and extraction requires multiple centrifugation steps and long processing time for cell harvesting, media exchange, lipid extraction using toxic organic solvents. Microfluidic approaches enable multiple processes in a single integrated device. Although there have been numerous reports on extraction and detection of biological molecules such as DNA [1], protein [2], RNA [3] in microfluidic systems, few reports on lipid extraction in microfluidics [4]. This might be caused by toxic organic solvent used for lipid extraction, which require solvent-resistant material such as glass [4]. In the present work, we also demonstrated PDMS-based microfluidic device can be applied to lipid extraction by using alcohol solvents such as ethanol and isopropanol.

EXPERIMENTAL
A double-layered PDMS device was fabricated by soft lithography. The lower layer containing a cell culture chamber with a diameter of 8 mm and output reservoir was bonded to glass slide after oxygen plasma treatment. The upper layer was designed to have connecting microchannel filled with micropillar array with 2 µm gaps to filter microalgal cell and bonded to lower layer after plasma treatment (Fig. 1).

Chlamydomonas reinhardtii (CC-503) was grown in microfluidic culture chamber including TAP (Tris-Acetate-Phosphate) medium under continuous light at 50 mol photon/m²/s, 23°C. Cell growth was monitored by measuring optical density (at 800 nm) using plate reader (Tecan). After 4 days, lipid accumulation was induced by supply of nitrogen deficient TAP medium into culture chamber. Lipid accumulation was monitored by measuring fluorescent intensity at the excitation wavelength of 530 nm and emission wavelength of 580 nm after staining cells using a fluorescent dye, Nile red.

After lipid accumulation, cell lysis and lipid extraction were performed by injecting various alcohol solvents such as 70% ethanol, isopropanol and methanol into microchannel at 60°C using PHD syringe pump (Harvard Apparatus). Neutral lipid content and fatty acid content in lipid extract obtained from output reservoir was analyzed by thin layer chromatography (TLC) and gas chromatography.
RESULTS AND DISCUSSION

As shown in Figure 2, we fabricated double-layered PDMS device composed of cell culture chamber and connecting channel filled with micropillar array between culture chamber and output reservoir. We evaluated the performance of this integrated microfluidic system. For the growth of microalgal cells, seed culture was injected into culture chamber through the inlet. We observed culture chamber is filled with TAP medium including microalgal cells (Fig. 3A) and micropillar array filter completely block the movement of cells (Fig. 3B).

![Integrated microfluidic device fabricated as double-layered PDMS](image)

Figure 2: Integrated microfluidic device fabricated as double-layered PDMS

![Image of (A) microalgal cells in culture chamber and (B) connected microchannel with micropillar filter](image)

Figure 3: Image of (A) microalgal cells in culture chamber and (B) connected microchannel with micropillar filter

We monitored cell growth in culture chamber by measuring optical density using microplate reader and optical density ($A_{800}$) increased from 0.2 to 1 for 4 days. After growth for 4 days, culture medium was exchanged with nitrogen deficient TAP medium to induce lipid accumulation by supply nitrogen deficient medium into culture chamber through the microchannel. Lipid content was monitored by measuring fluorescence intensity of microalgal cells stained with Nile red using microplate reader.

When lipid accumulation reached maximum value, alcohol solvents such as 70% ethanol, isopropanol were injected into culture chamber through microchannel for cell lysis and lipid extraction. In this study, we utilized alcohol solvents to minimize swelling effect on PDMS. Before extraction in microfluidic device, we analyzed lipid extraction ability of each alcohol solvent compared to conventional solvent such as chloroform/methanol (2:1, v/v). Although overall fatty acid content in lipid extract by alcohol solvent was reduced compared to that extracted by chloroform/methanol, the difference was not significant (Fig. 4A). We found that double-layered PDMS device maintains its structure during extraction process using alcohol solvent such as 70% ethanol and isopropanol. In addition, cell debris occurred by cell lysis was successfully removed by micropillar array filter and we obtained transparent lipid extract from integrated device using alcohol solvents (Fig. 4B). When we analyzed the lipid extract from integrated device by TLC, we observed neutral lipid (TAG) was obtained using alcohol solvent such as 70% ethanol in PDMS-based microfluidic device (Fig. 4C). Despite simple process, neutral lipid (TAG) or total fatty acid content obtained from integrated microfluidic system was comparable to that obtained from conventional Bligh-Dyer method.
CONCLUSION

Microalgal lipid analysis requires multi-step processes for cell culture, lipid production, and lipid extraction including multiple centrifugation steps, long processing time and usage of organic solvents. Microfluidic approaches allow performing multiple processes in a single integrated device by simply changing flow of medium and solvents. In conclusion, we report that integrated microfluidic device developed in this study can provide effective means to develop simple and efficient process required for lipid analysis.

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