ABSTRACT

Herein, a monolithic polycarbonate (PC) microdevice was developed to handle both DNA extraction and polymerase chain reaction (PCR) processes successively. Target cell – *Cochlodinium polykrikoides* microalgae, the causative species for harmful algal blooms in South Korea – was firstly captured on amine-functionalized microbeads via electrostatic interaction and then lysed to release DNA into subsequent PCR channel. Gram-negative *Escherichia coli* bacterium was used to illustrate the adhesion phenomenon based on its similar negatively-charged surface in comparison with algal cells. A 244-bp amplicon from the genome of *C. polykrikoides* algae was successfully amplified using this microdevice, demonstrating its potential application on further on-site analysis for early algal detection.

KEYWORDS: Amine-functionalized, *Cochlodinium polykrikoides*, Monolithic microdevice

INTRODUCTION

Harmful algal blooms (HABs) are aquatic phenomena caused by the excessive proliferation of microscopic algae in waterways that have threatened not only the fishery industry but also the public health in many regions all over the world for decades due to their highly potent toxins. In South Korea, recently, most of concerns have been placed on the annual occurrence of the unarmored microalgae *Cochlodinium polykrikoides* in the southern part of the South Sea [1]. Amongst bloom management solutions, early detection is one of the criteria because it helps reduce the amount of chemicals used and improve the treatment efficiency. Therefore, recently, many groups have investigated different approaches in order to realize precise, portable, and inexpensive systems replacing most of conventional methods such as satellite images or optical microscopic observation which require specific instruments, well-trained technicians as well as multistep operations.

In this study, we propose the fabrication of a monolithic thermoplastic microdevice which could be utilized to screen for the existence of *C. polykrikoides* in water samples by realizing both DNA extraction and PCR processes in one continuous flow. Cells were firstly captured on amine-functionalized microbeads and then lysed to release DNA into subsequent PCR channel. The underlying principle for cell capturing is based on the inherent negatively charged surface of the microalgal cells [2] which induces the electrostatic interaction between microalgal cells and the positively charged amine-coated surface. To illustrate such circumstance, the Gram-negative *Escherichia coli* bacterium with fluorescent property was used as a simulating model because *E. coli* cells also possess similar negative charges on their surfaces. As a result, a 244-bp DNA fragment from the genome of *C. polykrikoides* algae was successfully amplified from genome on a PC microdevice following the on-chip extraction which confirmed the effectiveness of integrated extraction and amplification steps.

THEORY

Like almost all other microalgal species, *C. polykrikoides* cells possess intrinsic negatively charged surface making its own electrophoretic property [3]. Therefore, to facilitate algal cells stabilization, insteads of applying external electric field as can be seen in microalgae harversting studies, we applied...
positively charged coating on microbeads surface to induce the electrostatic interaction between the coated surface and the cell surface (Figure 1).

Figure 1: Microbeads modification and interaction between the positively charged microbeads and negatively charged algal cells

**EXPERIMENTAL**

Figure 2 shows the design of integrated microdevice with large channel part served as separation and extraction site and the followed serpentine-type channels for DNA amplification purpose. Two pieces of PC substrates were adhered by simply thermal bonding method (130°C, 1 MPa, 30 min) and the microdevice was connected to inlet and outlet through silicon tubes (o.d. 2 mm, i.d. 1 mm).

Figure 2: Microdevice fabrication (a) Design of a microdevice with both DNA extraction and subsequent serpentine-type PCR channel and (b) Polycarbonate microdevice

Figure 3 shows the exact placement of PC microdevice on heater with two copper heating blocks and the result of temperature measurement performed on the interface of two PC substrate taken by infrared (IR) camera. The area ratio for each temperature zone was decided based on the desired residence time for denaturation and annealing/extension steps in which the temperatures were controlled at approximately 95 °C and 65 °C, respectively.

Figure 3: Temperature control of microdevice for realizing PCR reaction (a) Photo of the polycarbonate microdevice on heaters and (b) IR camera images taken from the top of the device

**RESULTS AND DISCUSSION**

Figure 4a shows the optical microscopic image of the channel part for DNA extraction with microbeads infixed inside and the results of experiment for illustrating cell capturing model (Figure 4b, 4c, 4d). As can be seen in Figure 4b and 4c, the background fluorescent intensities of the bare as well as amine-functionalized microbeads were relatively weak. Meanwhile, as shown in Figure 4d, the amine-functionalized coating significantly improved the binding of *E. coli* cells on microbeads surface indicated by the increase in the fluorescent intensity after flushing bacterial suspension (10⁴ CFU/mL) through the bead-embedded channel and washing with the copious amount of water 3 times.

Figure 5 shows the results of agarose gel electrophoresis for the continuous-flow PCR performed on the integrated PC microdevice. A 244-bp target amplicon was successfully amplified from the nuclear-
encoded large subunit (LSU) ribosomal DNA (rDNA) region after running 40 thermal cycles. The total analysis time was 2 hours including 15 minute for on-chip purification and extraction step. Although the average intensity of the amplified product obtained using the microdevice was approximately 20% of that obtained using a commercial thermal cycler, the target amplicons were clearly distinguishable.

Figure 4: Cell adsorption modeling (a) Optical microscopic image of microbeads captured inside a microchannel; Fluorescent image of (b) bare microbeads (c) amine-functionalized microbeads (d) amine-functionalized microbeads with bacterial adhesion

Figure 5. On-chip continuous-flow PCR for algal DNA amplification. (a) Results of agarose gel electrophoresis and (b) graphs showing relative intensity scales of the target amplicons obtained in (a)

CONCLUSION
In this research, a monolithic polycarbonate microdevice was fabricated for the fast detection of the toxic *C. polykrikoides* responsible for the majority of harmful algal blooms in South Korea. The amplification results confirmed the effectiveness of integrated extraction and amplification steps, demonstrating the potential application of this microdevice for further on-site analysis.

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CONTACT
* N.Y. Lee; phone: +82-31-750-8556; nylee@gachon.ac.kr