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A Microfluidic Device for Single and Small Population Cell Trapping and Lysis of *Pseudo-nitzschia*

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Abstract—**We present a microfluidic device that is capable of trapping single and small populations of *Pseduo-nitzschia* for subsequent cell lysis and domolic acid (DA) extraction. Microcages are integrated within a microchannel to capture the cells and hydrodynamic focusing is utilized to guide the cells into the cages. The microchip is coupled with an ultrasonication device, which enables for rapid cell lysis. Our results demonstrate effective trapping of single and multiple cells of various sizes, which can be lysed 10x faster than conventional methods. This device provides a simple and efficient tool for studying the biological mechanisms of DA production by *Pseudo-nitzschia*, which is crucial for minimizing the threat of amnesic shellfish poisoning. Moreover, the portability of this device allows for the integration of on-chip components and sensors enabling for in field DA detection.**

Keywords-microfluidics; domoic acid (DA); single-cell trapping; Pseudo-nitzschia

I. INTRODUCTION

Recently, phytoplankton blooms containing toxic species of *Pseudo-nitzschia* have become more common, especially along the western U.S. coast, posing a serious threat to the health of sea mammals, sea birds and humans [1]. Several members of this species produce domoic acid, a deadly neurological biotoxin, which is the causative agent for amnesic shellfish poisoning (ASP) in humans through the ingestion of contaminated shellfish [2]. At present, the production mechanism of DA by *Pseudo-nitzschia* is unclear due to the high variability in DA production by individual cells and different species [3]. To completely understand the biological mechanisms of DA, it is necessary to investigate DA production at the single and multi-cell level, which can avoid the loss of information associated with ensemble averaging. Recent advancements in biosensors have allowed for highly sensitive, single-cell analysis through the detection of various intracellular molecules (i.e., DNA, RNA, proteins) [4]. However, the rigid crystallized silica cell membrane of *Pseudo-nitzschia* and the lack of efficient tools for single-cell trapping and lysis have made DA extraction at the single-cell level a difficult task by conventional cell lysis methods.

To address this issue, this study is the first to extract DA from single cells and small populations of *Pseudo-nitzschia* through the utilization of a microfluidic device and ultrasonication, enabling for subsequent DA detection and analysis. Recently, microfluidic platforms fabricated from poly(dimethylsiloxane) (PDMS) have been developed for various single-cell studies [5, 6], which have the advantages of lower reagent consumption, faster analysis times and higher temporal resolution compared with benchtop laboratory methods. Such platforms provide novel methods for handling and analyzing single cells from bulk cell suspensions.

Previous work has been done utilizing ultrasonic waves for the disruption of cancer cells [7] and bacterial spores [8], which demonstrates the versatility of this approach for cell lysis. In addition, ultrasonic cell lysis avoids the common problems of conventional methods including chemical reagent contamination and excessive heating, which can denature and damage the intracellular components. Therefore, in this study, we have coupled ultrasonication with a microfluidic chip for the trapping and lysis of single and small populations of *Pseudo-nitzschia*. Compared with current methods, this system offers high precision for single and multi-cell analysis, which can greatly facilitate the understanding of DA production by *Pseudo-nitzschia* and diminish the risk of shellfish poisoning.

II. MATERIALS AND METHODS

A. Device Fabrication

PDMS microchannels were fabricated using conventional soft lithography techniques [8]. A thin layer of SU-8 photoresist (MicroChem, Newton, MA), was spin-coated onto a silicon wafer (Techgophers, Los Angeles, CA). UV lithography was performed to pattern the resist, followed by development and baking at 150°C for 5 min. Deep reactive ion etching (DRIE) was utilized to form microstructures (~55 μm in depth) in the wafer, serving as the PDMS mold. PDMS pre-polymer and crosslinker (Sylgard 184, Dow Corning, Midland, MI) was mixed at a weight ratio of 10:1, degassed for 30 min, poured onto the surface of the wafer and cured at 80°C for 2 hours. The cured PDMS was peeled off and inlets and outlets were punched using 1 mm diameter blunt-tip needles (Corning, Lowell, MA). PDMS chips and glass slides were exposed to an O_2 plasma and immediately placed together to form an irreversible bond. After assembly, blue dye was injected into the device to verify that the bonding process resulted in leak-proof sealing.

A photograph of the microfluidic chip is presented in Fig. 1. The device is composed of three inlets: the center inlet is loaded with the cells and the two outer inlets are loaded with

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buffer solution for hydrodynamic/cell focusing. Microcages are positioned further downstream of the inlets and are used to trap the cells. The cages, 50 µm long and 20 µm wide, were designed according to the size of *Pseudo-nitzschia* cells and are incorporated into the microchannels.

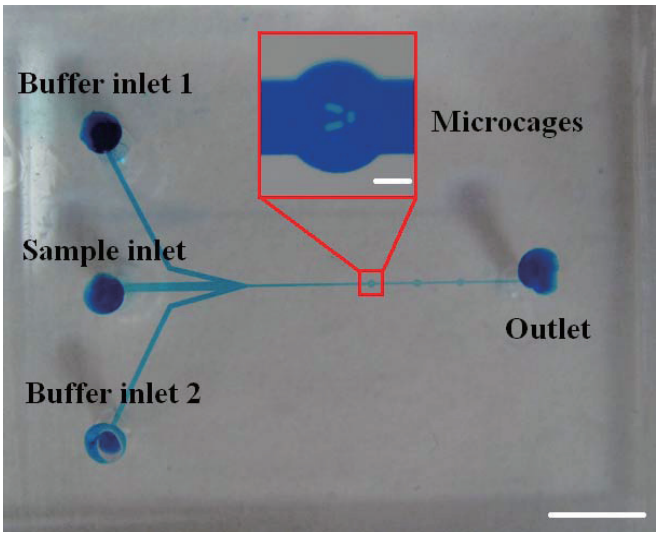

Figure 1. Photograph of the microfluidic chip. The inset shows an enlarged view of the microcages for cell trapping. Blue dye is flowed into the device for enhanced visualization. (Scale bar = 1 mm; inset scale bar = 100 µm)

B. *Cell Culture and Reagents*

Pseudo-nitzschia and sterile seawater was kindly provided by Dr. Caron's laboratory at University of Southern California. The growth media, 50x Guillard's (F/2) marine water enrichment solution, was purchased from Sigma-Aldrich (St. Louis, MO). Cell culture flasks with vented cap were purchased from Fisher Scientific (Tustin, CA). Cell culture media was made up by diluting the growth media with sterile seawater at the ratio of 1:50. In addition to cell culturing, the culture media was used as the buffer solution for hydrodynamic focusing. The cells were incubated in a mini-refrigerator and maintained at a temperature between 17-20°C. A lamp was fixed inside the refrigerator to generate sufficient light for cell growth. The lamp was controlled by a programmable timer that was set to generate light/dark cycles every 12 hours. The cell culture media was refreshed every two weeks. Cell concentrations were determined using a hemocytometer (Gibco, UK) and adjusted to 10^5 cells/mL before being loaded into the device.

C. *Experimental Setup*

A schematic illustration of the experimental setup is presented in Fig. 2. A Branson 2510 ultrasonicator (Danbury, CT) was utilized for cell lysis. Silicon rubber tubing was glued to the inlets and outlets of the microfluidic chip and connected to 2 mL glass syringes (Popper & Sons, New Hyde Park, NY) via 1/16" OD Teflon tubing. Two syringe pumps (Harvard Apparatus, Holliston, MA) were utilized to drive liquids within the microchannels at constant flow rates. One pump was connected to the central inlet of the microfluidic chip for cell loading and the second pump was connected to the outer two inlets for buffer loading via a T-connector. A Leica inverted microscope (McBAIN Systems, Simi Valley, CA) was used to monitor the process of cell trapping and lysis. Images were captured using a Sony CCD camera and IC Capture imaging acquisition software (Charlotte, NC). Image processing was carried out using nEO iMAGING.

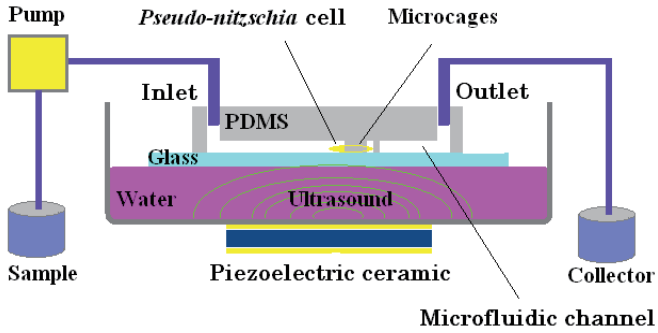

Figure 2. Schematic illustration of the experimental setup.

III. Results and Disscusion

A. *Influence of Hydrodynamic Focusing on Cell Trapping*

Pseudo-nitzschia is a cosmopolitan genus of pinnate diatoms restricted to marine environments [2] and healthy cells are typically long and slender in shape. To control the cell's motion within the microchannel, the two symmetrical outer inlets were loaded with buffer solution and utilized for hydrodynamic/cell focusing. Consequently, the position and orientation of the cells could be controlled within the microchannel, enabling for precise alignment. As a result, cells could be accurately guided toward the center of the microcages located further downstream the microchannel.

Before the cells were loaded into the chip, the cell concentration was adjusted to 10^5 cells/mL. This density was chosen to minimize cell blockage within the microchannel, while still allowing for trapping events to occur within a timely manner. Once the concentration was stabilized, the cells were loaded into the center inlet and buffer solution was loaded into the outer two inlets (Fig. 1). The profile of the flow could be adjusted by changing the flow rates of the cell and buffer solutions. With different degrees of hydrodynamic focusing, the cell's position within the channel could be adjusted, influencing the cell trapping performance (Fig. 3). When no hydrodynamic focusing was applied, the cells tended to flow around the microcages, making cell trapping nearly impossible. With low hydrodynamic focusing (cell and buffer solution flow rates of 5 µL/hr and 10 µL/hr respectively), the cells were focused toward the center of the channel; however, their orientations were random, which caused them to become trapped outside of the cages (Fig. 3 (b)). With high hydrodynamic focusing (cell and buffer solution flow rates of 5µL/hr and 15 µL/hr respectively), cells were horizontally oriented in the center of the microchannel and could be fully trapped inside the cages (Fig. 3 (c)).

These results indicate that cell orientation and positioning within the microchannel can be precisely controlled by

hydrodynamic focusing. High hydrodynamic focusing is necessary to effectively trap the cells within the microcages. However, it was observed that if the flow rate of the buffer solution was much larger than the cell solution (i.e., buffer flow rate >20 µL/hr), the buffer solution would restrict the cell solution from flowing within the microchannel; thus impeding the performance of the device.

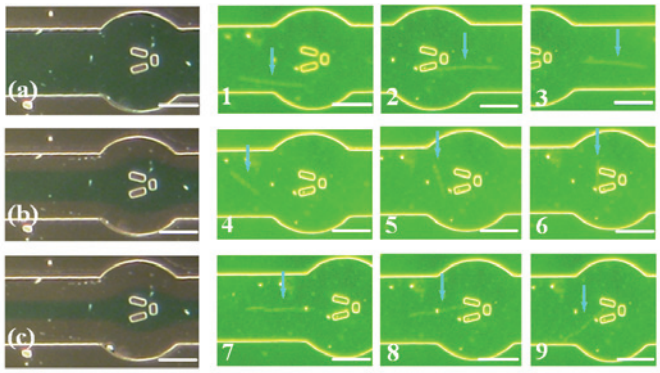

Figure 3. Influence of hydrodynamic focusing on cell trapping; (a) without flow focusing, (b) with minimal flow focusing, (c) with high flow focusing. The time interval between each inset is 1 second. The picture color was modified to achieve higher contrast for improved visualization. (Blue arrows denote the cell's position; scale bar = 100 µm)

B. Single and Multi-cell Trapping

At present, the understanding of DA production by *Pseudo-nitzschia* is unclear due to a high degree of variability in DA production among individual cells and different species, as well as various environmental factors. Specifically, the role of individual nutrients in the metabolism and toxin production of *Pseudo-nitzschia* is poorly understood. Conventional methods for studying DA production involve large populations of cells (hundreds to thousands) and these averaged results can often conceal important information. Therefore, this work is focused on the extraction of DA from individual and small population of cells, which can provide new insight into understanding the biological mechanisms of DA production.

In the previous section, we have demonstrated that cells can be effectively focused toward the center of the microcages by applying appropriate hydrodynamic focusing. To enhance the efficiency of cell trapping, the microcages were designed to accommodate for a wide range of cell sizes. The unique reproduction pattern of *Pseudo-nitzschia* results in a smaller cell size with each reproduction cycle. When diminished to about 30% of their original size, the cells undergo sexual reproduction and regenerate back to a larger size [9-11]. Therefore, individual *Pseudo-nitzschia* cells within the same population can range from tens to hundreds of microns in length.

Fig. 4 shows the typical results of cell trapping; Fig. 4 (a), (b), and (c) demonstrate the trapping of single cells of various sizes, and Fig. 4 (d), (e), and (f) demonstrate the trapping of small populations of cells. These results indicate that the effectiveness of this design remains constant for cells of various sizes and various population sizes. The desired number of cells can be precisely trapped by controlling the flow rates and the duration of flow (i.e., longer flow duration results in a greater number of trapped cells). This ability to control the amount of cells that are trapped enhances the versatility of this system, allowing for subsequent biological analysis at the single-cell and multi-cell levels.

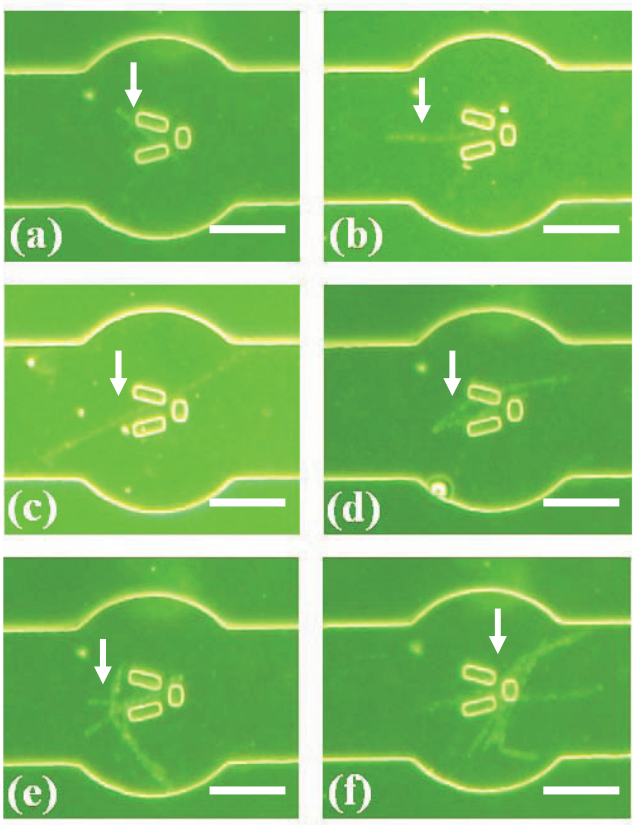

Figure 4. Trapping single and multiple cells of varying sizes; (a), (b), and (c) show single cells of varying lengths being trapped; (d), (e), and (f) show small population of cells being trapped. The picture color was modified to achieve higher contrast for improved visualization. (White arrows denote the cell's position; scale bar = 100 µm)

C. Cell Lysis

Captured cells were lysed by placing the microfluidic chip inside an ultrasonic bath. Ultrasonic waves produced inside the bath propagated within the microchannels, which ruptured the cell's membrane. After only several seconds of ultrasonicaton, cells could be fully lysed. Fig. 5 shows the typical results; (a), (b), and (c) demonstrate single-cell lysis and (d), (e), and (f) demonstrate multi-cell lysis. The time interval between each inset is 3 second; therefore, we can see from Fig. 5 that both single cells and small population of cells can be completely lysed in less than 10 seconds, which is 10x faster compared with conventional methods [3]. Additionally, this method does not require the use of any chemicals or lysing reagents, which could potentially damage the intercellular molecules and influence downstream DA detection. Following cell lysis, the resulting intercellular fluid can be collected from the microchannel and analyzed for relevant biological studies.

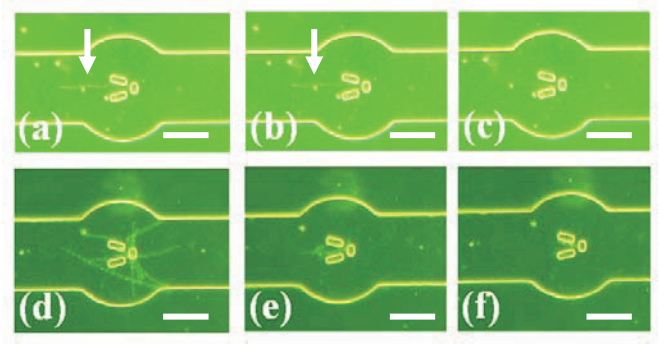

Figure 5. Lysis of trapped cells by ultrasonication; (a), (b), and (c) show a single cell being lysed; (d), (e), and (f) show a cluster of cells being lysed. The time interval between each inset is 3 second. The picture color is modified to achieve higher contrast for improved visualization. (White arrows denote the cell's position; scale bar = 100 μm)

IV. Conclusion

The proposed method provides a simple and efficient tool for *Pseudo-nitzschia* cell lysis at the single and multi-cell level, which is crucial for improving the understanding of the biological mechanisms responsible for DA production. The microfluidic platform utilized in this work can be integrated with on-chip components and sensors for an in field detection system, specifically, for monitoring the DA generation activity of *Pseudo-nitzschia* at the coastal collection regions. Furthermore, the proposed technique can be applied to other algae and non-algae cell types, thus greatly facilitating biological intracellular studies and broadening our understanding of intracellular mechanics.

Acknowledgments

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