

INTEGRATED MICROFLUIDIC PLATFORM FOR ALGAL TOXIN ANALYSIS

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ABSTRACT

A simple microfluidic chip to detect the algal toxins was fabricated by polydimethylsiloxane (PDMS). A heterogeneous immuno-enzyme assay was constructed inside the microfluidic device for rapid and automatic analysis of the algal toxins. Within this device, microcystin can be analyzed in 15 minute with detection limit at 0.02 ng/ml. The designed device is highly automatic, more efficient and economic compared with conventional techniques, which is not only suitable for rapid toxin analysis, but also has potential applications in fast protein analysis in biomedical fields.

KEYWORDS: microfluidics, PDMS, microcystin, algal toxin

INTRODUCTION

With rapid urbanization and consequential eutrophication in our aqueous environment, harmful algal blooming (HAB) has become a more and more serious problem around the world. HAB not only damages the aqueous eco-system, but also create various algal toxins, which has already affected the living and health of residents. Among all the toxins, microcystin, saxitoxin and cylindrospermopsin are the most common ones reported for related water and food poisoning around the world. Traditional methods for algal toxins include HPLC, LC-MS, ELISA, and even mouse bioassay. Among all the methods, algal toxin analysis in laboratories mainly relies on high performance liquid chromatographic (HPLC) methods which require expensive equipment and highly qualified personnel due to the high variability of toxin structures. HPLC also involves lengthy analysis time and pretreatment of the collected samples. Moreover, the bulky size and delicate structure make it impractical for field analysis. Additionally, its detection limit is relatively high, especially for the trace analysis of algal toxins in water environment.

Other method, such as traditional enzyme linked immunosorbent assay (ELISA) performed on microwell plates is usually used in laboratory, which involves the step-by-step reagent introduction procedure and more than 1 hour analysis time at least. ELISA reader is also needed to final results readout. Generally, a rapid and simple analysis method for algal toxin analysis are in great need for field or on-site application.

THEORY

Microfluidic devices are designed to control and manipulate microliter or nanoliter volumes of reagents in an integrated chip design^[1]. Such chips contain functional elements such as valves, pumps and columns that allow the precise delivery, mixing and movement of reagent solutions. They offer high surface area-to-volume ratio, fast mass and heat transfer and improved local control. Due to these advantages, microfluidic techniques offer substantially improved performance over conventional bench-top systems and are increasingly used in biomedical analyses. So far, there has been few reports about utilizing microfluidic device to analyze algal toxins, especially highly automatic integrated microfluidics. Herein we developed a simple and automatic microfluidic device which is capable of rapid and multiple toxin analysis.

EXPERIMENTAL

DI water (Milli-Q, Millipore, Bedford, MA) was used through the whole experiments. Microbeads were purchased from Bang's Laboratory. Microcystins and antibodies were purchased from Enzo Life Sciences.

The chip was fabricated using multi-layer soft lithography.^[2] Two different molds were fabricated by photolithographic processes to create the fluidic channels and the control channels for actuating the valves located in the respective layers of the microfluidic chip. The mold used to create the fluidic channels was made by spin-coating a layer of 25 micron thick positive photoresist (AZ 50XT) on the silicon wafer. After UV exposure and development, the wafer was heated to create round the surface profile. After heat treatment, this mold is about 25 micron high and 200 micron wide. The control channel mold was made by 25 micron thick photoresist (Microchem SU8-2025) pattern on a silicon wafer. Afterwards, poly(dimethylsiloxane) PDMS (GE, RTV 615 A and B in a 5:1 ratio) will be mixed, degassed in a vacuum and poured onto the mold placed in a Petri dish to give 5 mm fluid layers with their respective microchannels. The PDMS will be cured in an 80 °C oven for 50 minutes. The PDMS will be later removed from the mold and the individual devices cut apart. Holes will be introduced onto the fluidic layer at the end of the channels for access of the various experimental solutions. The fluid layer will then be placed on a PDMS (GE, RTV 615 A and B in a 20:1 ratio) coated glass slide (2000 rpm, 60s, ramp 15 s) and incubated overnight at 80 °C. Metal pins (23 gauge, New England Small Tubing Corp.) will be used to connect the microchannels and Tygon tubing (Cole Palmer) for introducing solutions

The control system was home-made, which consists of 24 electromagnetic solenoid valves (SMC) driven a relay board (785B, Advanced Tech.) The relay board was controlled by a USB 6501 digital I/O card (National Instruments).

Control lines from the device are connected to the corresponding channels. The LabVIEW program allows for manual control of individual valves and for semi-automation of the whole experiment processes.

Light and fluorescent micrographs were taken by inverted microscope (Olympus IX51, Japan). All fluorescent images employed for protein repelling investigation were taken using the same objective with an identical exposure time.

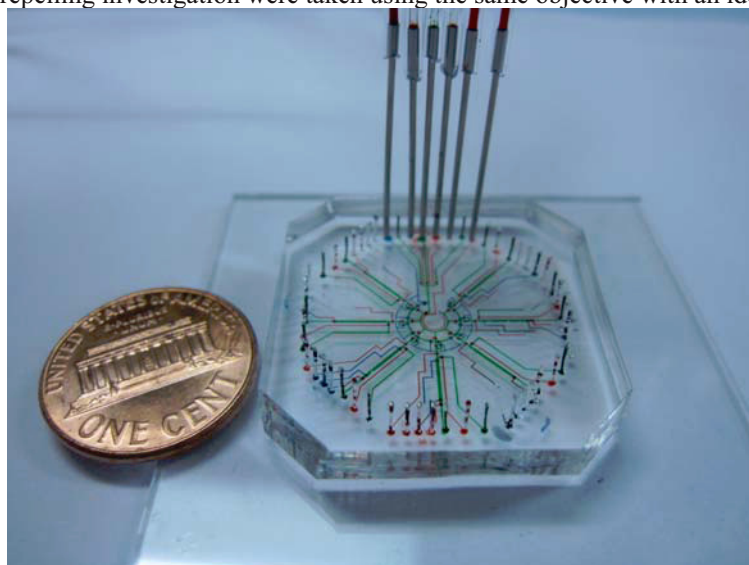


Figure 1: Picture of the microfluidic chip. The channels are filled up dyes of different color to show the relative positions of channels in the chip. A coin is shown aside to show the real size of the microfluidic chip.

RESULTS AND DISCUSSION

Herein we developed an integrated microfluidics platform capable of rapid parallel multiple toxin analysis. The platform includes a core microfluidic chip, cartridges for reagents, digital camera, control system, digital I/O and a laptop computer. The core microfluidic chip is designed by autoCAD and fabricated from Polydimethylsiloxane (PDMS), based on validated microfluidic components and engineering methods. Seven microfluidic columns for algal toxin concentration and following immunoanalysis, are integrated into single microfluidic chip (shown in Figure 1), in which sample input, reagent loading, toxin analysis and following cleaning can all be controlled by a laptop. The columns are filled with micro beads, on the surface of which different antibodies are immobilized for competitive immunoanalysis. The concentrations of various targeting toxins are read out by measuring the fluorescent intensity of the micro columns. Each column can be individually addressed for different operations and different toxin analysis.

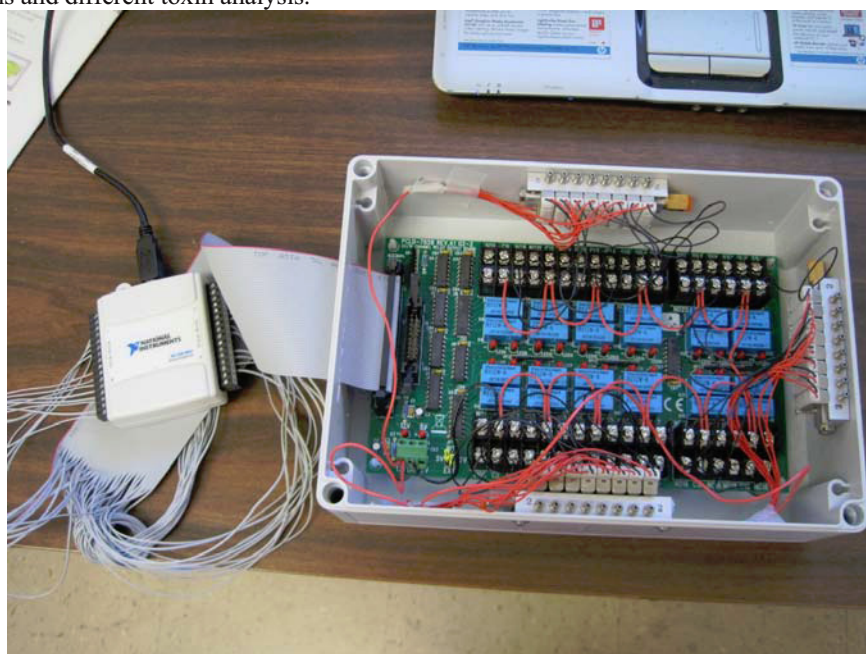


Figure 2: View of home-made control system.

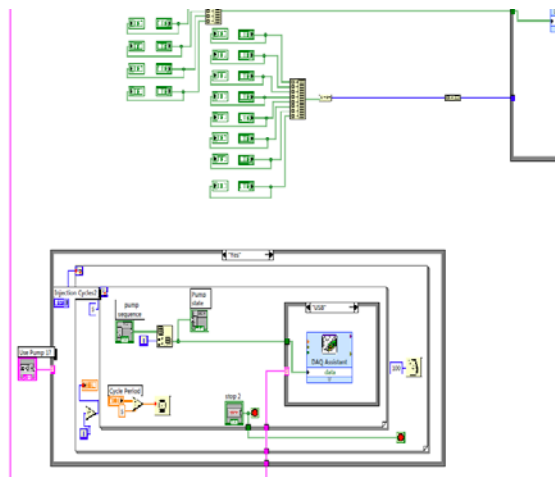


Figure 3: LabView Program interface.

The control system (shown in Figure 2) is home-made from USB based digital I/O (National Instruments), relay-board, and three solenoid micro valve manifolds. LabView are both used for the programming (shown in Figure 3). The control system can be designed as manual, semi-automatic, or fully automatic. Microcystins are utilized to validate this microfluidic platform. Fluorescent images of the micro columns for toxin at different concentration are shown in Figure 4. Generally, the detection limit can reached less than 0.02 ng/ml for each toxin. Reagent consumption has decreased to less than 40 nanoliter, and total analysis time has decreased to less than 15 minutes. Compared with conventional ELISA kit, our platform can save 75% time and consumes 3 orders less samples. Moreover, the whole analysis process can be controlled from laptop computer.

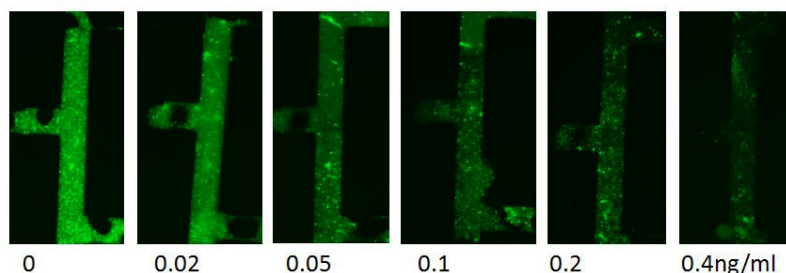


Figure 4: Fluorescent images of the columns inside the microfluidic chip loaded with microcystin (LR) at different concentrations.

CONCLUSION

We successfully designed and fabricated a microfluidic device for rapid microcystin analysis. The microfluidic device is more efficient and economic compared with conventional techniques, which is not only suitable for rapid toxin analysis, but also has potential application in fast protein analysis in biomedical fields.

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