Semi – automated System Microfluidic Machine for Microfilarial Detection

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***Abstract* — Lymphatic filariasis, the disease caused by filarial nematodes consists of *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* and transmitted by mosquitoes. The distributions of the disease are Thailand-Myanmar border and southern Thailand. The gold standard technique for filarial detection is thick blood smear staining technique, which has low efficiency and time-consuming. Recently, microfluidic based devices design strategies for pathogen detection. Microfluidic technique offers many advantages for pathogen detection such as miniaturization, small sample volume, portability, shorten the detection time and point-of-care diagnosis. In this study, we develop a semi-automated microfluidic system to detect microfilariae of filarial parasites. The system contains a peristaltic pump with multichannel tank for sample loading and multichannel microfluidic chip. For the semi-automated microfluidic system testing, microfilariae were detected in the microfluidic chip. In conclusion, semi-automated microfluidic system provides a simple, rapid and convenient diagnostic device for microfilariae detection.**

Index Terms — microfluidic device, lymphatic filariasis, microfilariae, peristaltic pump

I. Introduction

Lymphatic filariasis (LF) is one of the neglected tropical diseases which is the second leading cause of permanent and long term disability worldwide [1]. The human filarial infection is caused by the filarial nematode parasites i.e. *Wuchereria bancrofti, Brugia malayi*, and *B. timori* [2]. More than 9 0 % of the infections are infected with *W. bancrofti* that can be found in the tropical and in some sub-tropical areas. *B. malayi* is mostly found in Southeast and Eastern Asia and *B. timori* is found only in Timor and its nearby islands [3].

Understanding of several aspects of filariasis has led to an improved means of controlling the infection. A specific and sensitive assay for case diagnosis would permit accurately longitudinal assessment of the impact of the control, vector eradication, chemotherapy and vaccine program [4].

A thick blood smear staining method is a conventional method which is still being use. The detection limit of this method is between 1 5 -2 5 Mf/ ml blood or > 1 Mf/ slide. It may cause false negative in an infected person with low densities of microfilariae or after the success of GPELF. Moreover, a loss of 1 0 to 5 0 % of microfilariae during the staining process [4]-[6].

Recently, microfluidic based devices and lab-on-chip (LOC) design strategies for pathogen detection with the main focus on the integration of different techniques that led to the development of sample-to-result devices have been developed [7]. Microfluidic lab-on-a-chip (LOC) devices offer many advantages for pathogen detection such as miniaturization, small sample volume, portability, shorten the detection time and point-of-care diagnosis.

In Thailand, Amrit and colleagues developed a faster and reliable testing technique to count and identify nematode species resided in plant roots. This work proposes utilizing a multichannel microfluidic chip with an integrated flow-through microfilter to retain the nematodes in a trapping chamber [8]. From this proposed microfluidic chip technology for the plant parasitic nematode. It can apply to develop the diagnosis tool for the infection of human parasites.

Filarial infections are among many diseases that pose particular problems in diagnostic aspect, thus, it could potentially benefit from the introduction of such microfluidic based diagnostic methods. Thus, the objective of the present study is to develop a semi- automated microfluidic system to enable an efficient diagnosis of the filarial infection in both human and reservoir hosts.

This semi-automated microfluidic system as an alternative method for effective diagnosis of filarial infection. It provides a simple, rapid and convenient diagnostic device for microfilariae detection. Moreover, this diagnosis device may helpful for management of the disease, both at the level of individual patient care and at the level of disease control in populations.

II. EXPERIMENT

A. The fabrication of sample-loaded tank and chip socket

The sample-loaded tank was designed by using engineering drawing program and constructed by Digital Light Processing technique (DLP). In brief, the designed tank was cast with 50 microns per layer and cure time for 4.5 sec. After that, rinsed with isopropylalcohol (IPA) and

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brought to cure with UV for 2 hours. And then, the support was removed from the sample-loaded tank.

For the fabrication of chip socket, a filament plastic was injected to form a socket by using Fused Deposition Modeling (FDM 3D Printer). This chip socket was designed to proper with the microfluidic chip and to provide the microscope USB for parasite detection. The model of sample-loaded tank and chip socket was shown in Fig. 1.

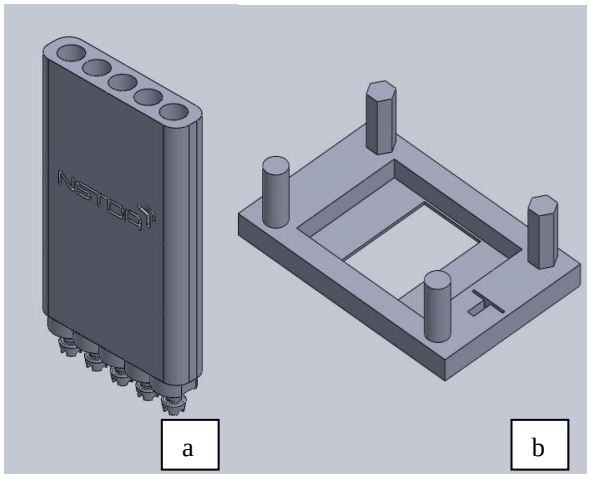

Fig. 1. Model of sample-loaded tank (a) and model of chip socket (b)

B. The fabrication of multichannel microfluidic chip

The five-channeled microfluidic chip was adapted from the previous study [8]. In brief, the microfluidic chip was fabricated from polydimethylsiloxane (PDMS) with patterned Photoresist on Si wafer [9]. The pattern features were created on a silicon wafer through PL and DRIE processes. The resulting Si master is a molding template for casting PDMS. A 10:1 mixture of PDMS prepolymer and curing agent was cast with the silicon master and cured the polymer at 75 °C for 2 h., then removed the PDMS replica from the master mold and cut to the required shape using a sharp cutter. Inlet and outlet ports are done by punching holes through the PDMS chip using the desired hold puncher. The PDMS replica is sealed to a PDMS substrate after interface bonding with oxygen plasma process. For this microfluidic chip, five samples can be run simultaneously. The model of semi – automated microfluidic machine was shown in Fig. 2.

C. The constriction of semi – automated microfluidic machine

The semi – automated microfluidic machine was constructed with two important parts consist of sample injection part by the peristaltic pump which connected to multichannel microfluidic chip and parasite detection part by microscope USB. The machine can adjust the flow rate for sample injection and alarm when the process is complete.

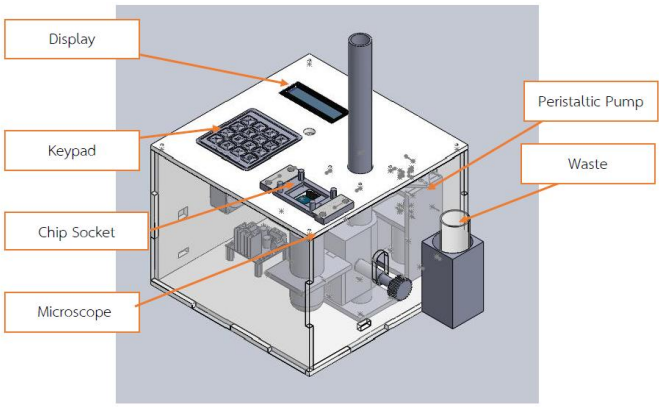

Fig. 2. Model of semi – automated microfluidic machine

D. Testing of semi – automated microfluidic system

For testing of a semi-automated microfluidic system, EDTA blood was washed with blood cell lysis buffers and then loaded into the multichannel tank. Turn on the peristaltic pump; each sample was then introduced into the microfluidic device. The microfilariae were trapped in the microfluidic chip while the remaining solution flew out of the microfluidic chip via the outlet port, into the waste tube. Then, the trapped microfilariae were inspected by using USB microscope.

III. RESULTS AND DISCUSSION

A. The fabrication of sample-loaded tank and chip socket

The multichannel sample-loaded tank contains 5 channels. Each channel can load 180 µl maximum volume of sample. The tank can fixable connected with a multichannel microfluidic chip.

The chip socket was used as a state to place the microfluidic chip which fixes the area with Microscope USB for the result inspection. The multichannel sample-loaded tank and chip socket was shown in Fig. 3.

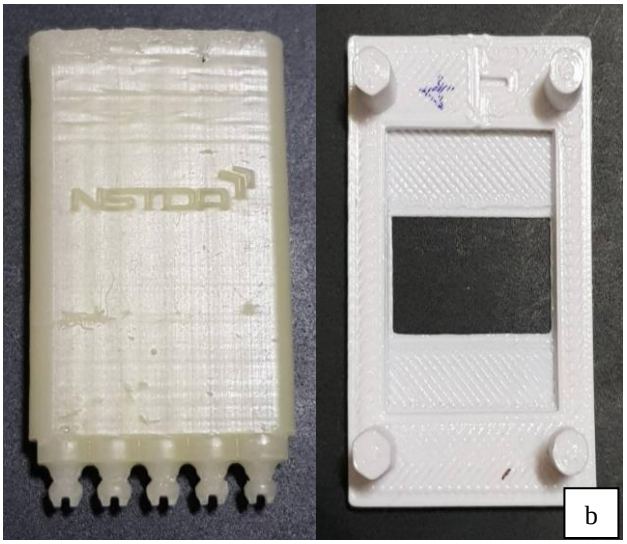

Fig. 3. The multichannel sample-loaded tank (a) and chip socket (b)

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B. The fabrication of multichannel microfluidic chip

The multichannel microfluidic chip contains 5 channels which have 5 inlets and 1 outlet. The multichannel microfluidic chip size is 3.2 cm x 3.2 cm and can be properly connected to the multichannel sample-loaded tank. The multichannel microfluidic chip was shown in Fig. 4.

Fig. 4. Multichannel microfluidic chip

C. The constriction of semi – automated microfluidic machine

The semi-automated microfluidic machine size is 21 x 16 x 21 cm. This machine has two important parts consist of sample injection part by the peristaltic pump which connected to the multichannel microfluidic chip and parasite detection part by microscope USB. The AC 220V power supply was applied to control the flow rate. The semi-automated microfluidic machines has a keypad and display which easy to use for time adjustment and machine control. The Semi – automate microfluidic machines was shown in Fig. 5.

Fig. 5. Prototype of semi – automated microfluidic machine

D. Testing of semi – automated microfluidic system

A semi – automated microfluidic system was developed for parasite detection. Up to five samples with a volume of 150 µl were delivered to the microfluidic chip in parallel using peristaltic pump. Each channel of microfluidic chip contains the microfilter which use for trapping microfilaria in the desired area whereas the other can flow throughout. For the detection, the trapped microfilariae were inspected by microscope USB.

For the semi – automated microfluidic system testing, the microfluidic system was able to detect microfilaria. These microfilariae were clearly detectable under microscope USB.

For further development, the image processing system will develop to helpful for result analysis. This image processing system can detect the trapped microfilariae and automated analyze the result without a trained technician.

The testing of semi – automate microfluidic machines were shown in Fig. 6.

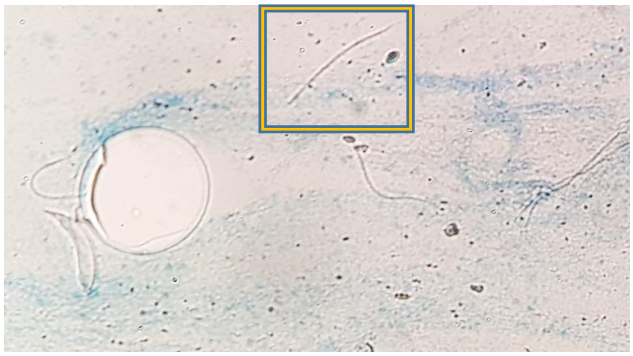

Fig. 6. The trappad Microfilaria in the microfluidic chip

IV. CONCUSSION

The semi – automated microfluidic system was developed with two important parts consist of sample injection part by the peristaltic pump which connected to five- multichannel microfluidic chip and parasite detection part by microscope USB. For the system testing, the microfilariae can be trapped in the microfluidic chip and clearly detectable under microscope USB.

Thus, the semi-automated microfluidic system provides a simple, rapid and convenient diagnostic device for microfilariae detection.

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