Real-Time Detecting Concentration of Mycobacterium Tuberculosis by CNTFET Biosensor

Hsiao-Wei Wang, Jung-Tang Huang, and Chun-Chiang Lin

Abstract—Aptamers are useful tools in microorganism researches, diagnoses, and treatment. Aptamers are specific target molecules formed by oligonucleic acid molecules, and are not decomposed by alcohol. Aptamers used to detect Mycobacterium tuberculosis (MTB) have been proved to have specific affinity to the outer membrane proteins of MTB. This article presents a biosensor chip set with aptamers for early detection of MTB with high specificity and sensitivity, even in very low concentration. Meanwhile, we have already made a modified hydrophobic facial mask module with internal rendering hydrophobic for effectively collecting M. tuberculosis.

Keywords—Aptamers, CNTFET, *Mycobacterium tuberculosis*, early detection.

I. INTRODUCTION

BECAUSE the traditional method for MTB culture requires several weeks for confirmation, several studies dedicate to early detection by molecular methods. Fan Chen et al. discovered aptamers binding to virulent strain M. tuberculosis with high affinity and specificity [1]. In this study, we compared MTB with *Candida albicans* (*C. albicans*) which is a dimorphic fungus that exists as colonization of warm-blooded animals including humans. *C. albicans* colonizes mucosal surfaces of the oral cavities and the digestive tract and is also able to cause a variety of infections. The MTB and *C. albicans* isolates were all collected from sputum samples, and went through respective culture processes.

II. PROCEDURE

Experimental substrate was a 4 inch wafer after APTMS (3-aminopropyl)-trimethoxysilane forms SAM and then the substrate was immersed in NMP (N-methyl pyrrolidinone) solution with SWCNTs after pickling. Used lithography process and sputtering process to make the comb-shaped electrodes on the substrate. Modify SWCNTs with the sensing probes (Aptamers) [2]-[4]. EDC–NHS coupling chemistry was utilized for aptamers integration onto the SWCNTs. Dispersing in PBS buffer was used for the

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attachment of the aptamer. Briefly, 1 µl of NK2 aptamer was activated using 6 µl of 400 mM EDC and 6 µl of 400 mM NHS [5]. In order to protect the surface without reacting with aptamer, we employed DGME and Tween20 to cover the surface of COO- and surface after pickling for decreasing noise.
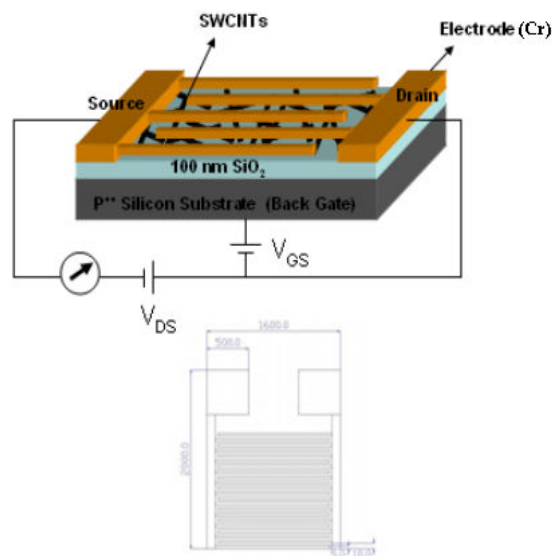

Fig. 1 The structure of Biochip

III. RESULT AND DISCUSSION

The structure of biosensor chip is shown in Fig. 1. For MTB detect experiment, a safe P3 laboratory must be required. It is a containment facility that enables the isolation and manipulation of dangerous biological materials for various research purposes. The MTB liquid concentration was 0.3 O.D and we recorded the response until the signal was stabilized. We compared MTB signal current curve from PBS buffer as shown in Fig. 2. Compared to the baseline of PBS buffer at approximately zero current, the MTB signal showed the peak value at 20 µA and saturated on 18 µA. The obvious difference indicated strong affinity reaction between our biosensor and MTB isolates.

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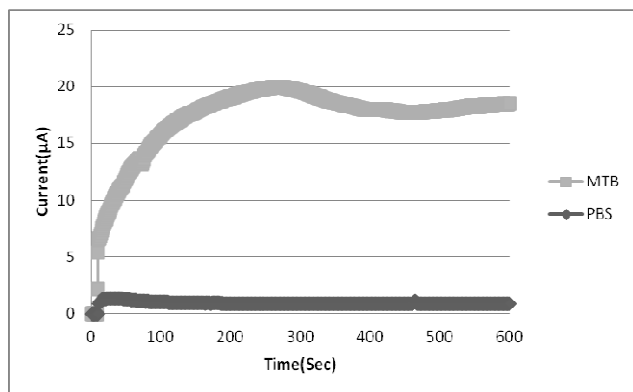International Journal of Biomedical and Biological Engineering
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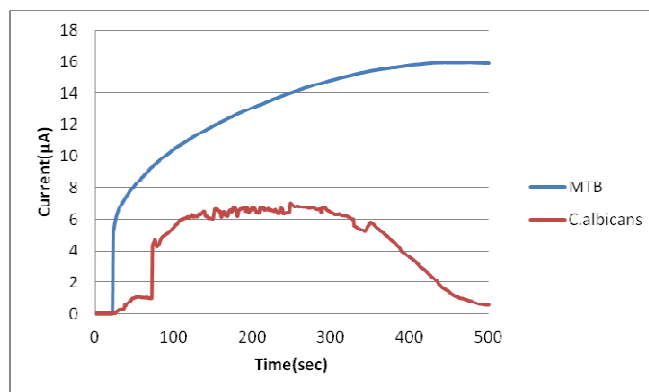Fig. 2 MTB and PBS buffer comparison

Fig. 5 MTB and *C. albicans* comparison

We titrated the same volume of deionized water after the MTB signal was stable, which was shown in Fig. 3. The post-titration red line proved that half of the concentration causing half current change. We titrated 75% alcohol solution after the stable MTB signal was shown in Fig. 4. The ability of alcohol to decompose MTB cells was certificated by rapid dropping red line, and the specificity of aptamers to outer membrane of MTB cells was also established. In Fig. 5 we compared the signals from MTB and *C. albicans* isolates. The red line showed the lack of stability of the signal, which confirmed the lack of effective affinity between aptamers and *C. albicans*.
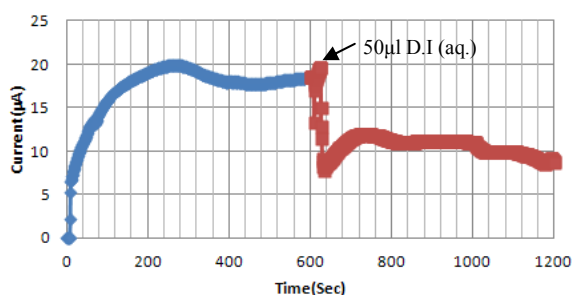
In the second experiment we dropped only 5μl on the chip. Adding the target MTB caused a sharp increase in the source-drain current and then a gradual saturation at 0.6μA. Fig. 6 shows the time dependence for source-drain current of the MTB aptamer modified CNT-FET device at the source-drain bias of 3V and the gate bias of 3 V after the introduction of target MTB (a) 0.3 O.D (b)0.03 O.D (c) 0.003 O.D, respectively. The different concentration of MTB versus current signal response with error bar is shown in Fig. 7.
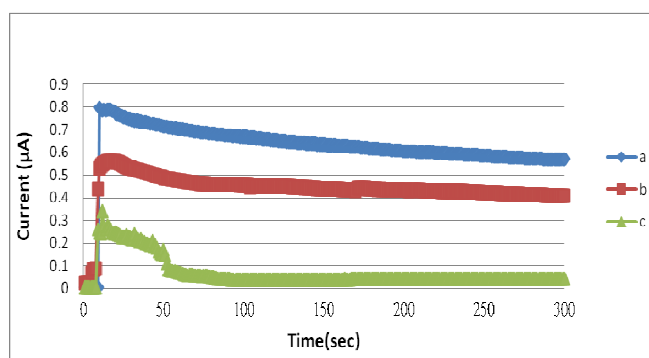

Fig. 3 50μl TB signal (Blue line) and then DI dropping

Fig. 6 Different dilution MTB concentration (a) 0.3 O.D (b) 0.03 O.D (c) 0.003 O.D

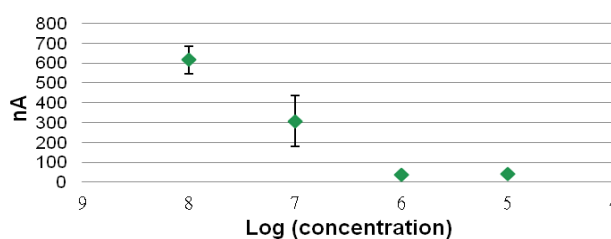Fig. 4 Titrate the 50μl alcohol (liq.) (Red line) after MTB test

Fig. 7 The different concentration in CFU of MTB versus current signal response

We compared several other bacteria to prove the specificity of the MTB aptamer-modified biosensor. The current rate of change signal is shown in Fig. 8. Obviously MTB outperformed the response from other bacteria.
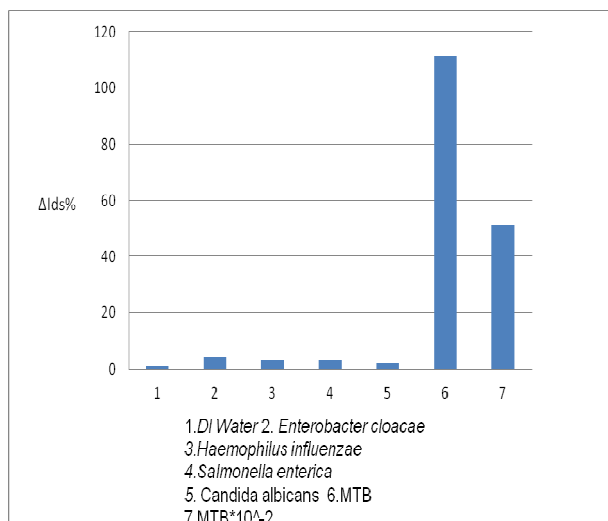
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Fig. 8 Different bacteria Current rate of change signal

IV. CONCLUSION

In our experiment, we successfully used a CNTFET sensor to detect MTB isolates in our biosensor chip, in which CNT channels were modified with aptamers. Our aptamer-based CNTFET device is a promising candidate for the development of an integrated, high-specific, multiplexed real-time biosensor.

The next step is to detect MTB with airflow simulation. To avoid the risk of contamination of staff and equipment, we have designed a remote data transmission system using Bluetooth models. Meanwhile, in order to collect MTB cells from patients' respiratory airflows, we have built a facial mask module containing biosensor chips, Bluetooth models, and batteries (Fig. 10). The internal rendering hydrophobic surface of facial mask helps accumulation of the very few MTB cells within respiratory airflow, and avoids possible spread of MTB cells. This module will significantly reduce the time needed for MTB detection and diagnosis currently.

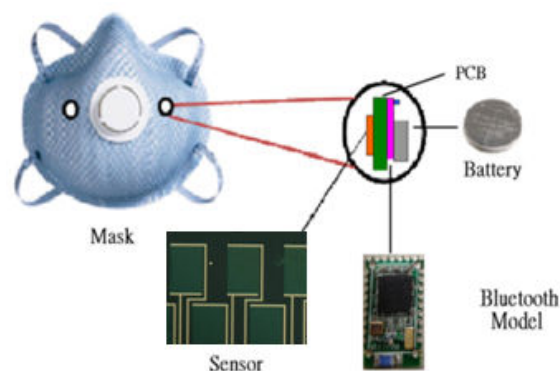Fig. 9 The schematic structure of Bluetooth system in P3 lab

Fig. 10 The schematic equipment of mask-based Lab chip

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