INTEGRATED MICROSYSTEM FOR MULTIPLEXED DETECTION OF CARDIAC BIOMARKERS IN BLOOD TOWARDS POINT-OF-CARE DEVICE DEVELOPMENT

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ABSTRACT

A microsystem allowing direct and simultaneous analysis of multiple cardiac biomarkers in blood using an integrated filter chip and silicon nanowire (SiNW) sensor chip is described. The integrated microsystem is composed of the filter chip for plasma separation from blood and the SiNW sensor chip for protein detection. These two chips were fabricated into one via back-to-back integration. The SiNW sensor, spotted with three different antibodies, enabled us to detect three cardiac biomarkers, cTnT, CK-MM and CK-MB, simultaneously. The system is able to attain a low detection limit of 1 pg/ml for the three cardiac biomarkers from 2 µl blood in 45 minutes.

KEYWORDS: Integrated microsystem; Cardiac biomarker; Blood; Detection

INTRODUCTION

Heart disease is by far the #1 killer in the U. S. and more than 1 in 3 (81 million) U.S. adults currently live with one or more types of cardiovascular disease. Electrocardiogram (ECG) is a first test when a patient suffering from chest pain sees a doctor, however, it is lack of the required sensitivity.

Cardiac biomarkers, like troponin-T and creatinine kinases, are proteins used for heart attack diagnosis. Troponin and creatinine are constituents of the cardiac muscle cells that are released into the blood when the cells and tissues are injured after a heart attack. Hence elevated levels of troponin-T or creatinine kinases in the blood alert the doctors that a heart attack has taken place. Enzyme-linked immunosorbent assay (ELISA) is nowadays a common method for identification of the biomarker level in the hospital. However, the method is laborious and also time consuming. Moreover, it is only located in the central hospitals and labs. To assist the doctor in making a decision in an accurate and rapid manner, an analysis of multiple biomarkers simultaneously with fast speed which is capable of monitoring change of the biomarker level in a short period (<1 h) is highly desirable.

Silicon nanowire (SiNW) biosensor has emerged as a promising point-of-care device for sensitive and rapid detection of protein biomarkers[1-3]. Furthermore, this sensor allows multiplexed detection of analytes with a nanowire array format. More importantly, the silicon-based sensor is capable of integrating itself with electrical circuits for miniaturization. In this presentation, a rapid and sensitive integrated microsystem to test for three specific cardiac biomarkers simultaneously in blood was demonstrated, pointing to development of a point-of-care (POC) device.

EXPERIMENTAL

The SiNWs were produced through conventional optical lithography, etching and oxidation, and thus are fully compatible with CMOS technology. Figure 1a shows optical image of the SiNW chip. The chip has 255 individual nanowires fabricated in 4 groups. Group A, B and C, respectively, has 12 clusters (5 nanowires in one cluster) with a distance of 300 µm inbetween. Differently, group D has 15 nanowire clusters.

The filtration chip on the bottom side of the integrated chip is able to extract the test plasma from the whole blood sample. The optical image of the filtration chip is illustrated in Figure 1b. Working principle is basically the size-based exclusion of cells through crossflow filtration. To do so, a submicron vertical pillar gap structure for plasma/blood separation was fabricated. Only plasma can be allowed to pass through the submicron vertical pillars, which are located tangential to the main flow path of the blood sample. In the design, several narrow channels (10 µm in width, 300 µm in length) have been introduced at the downstream of main blood flow channel to build up sufficient pressure and thereby enhance separation speed of plasma.

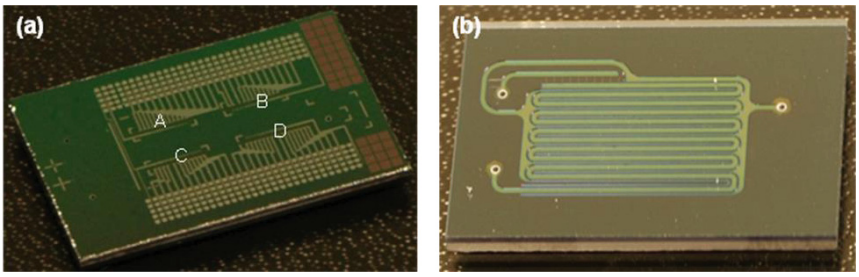

Figure 1. (a) Optical image of SiNW array sensor chip for protein detection. (b) Optical image of filtration chip for plasma separation from blood.

The SiNW chip was treated with 3-Aminoproplyltriethoxysilane and glutaraldehyde, respectively. Afterwards, solutions of three monoclonal antibodies to CK-MB, CK-MM, cTnT, and control BSA, were spotted onto the SiNW arrays, and incubated in a moist environment for 2 h at room temperature. After the incubation, the unbounded antibodies and BSA were washed off. Subsequently, passivation of the unreacted aldehyde surface groups was carried out with amino-PEG overnight.

To make the microfluidic manipulation automated, an electrofluidic packaging system is required for liquid exchange as well as electrical interconnection. As shown in Figure 2, the SiNW chip was located in the housing and four chambers were made on the four different NW groups. Blood was added to the bottom chip for separation of the plasma from the blood. Afterwards, the separated plasma was collected in the group D through a drilling hole between the two chips. Buffer could be exchanged through the tubes connected to the group D for washing after incubation with the plasma.

The original design having 4 chambers is that the other chambers can be for positive and negative control in case the real sample from patients is involved. In the work, only chamber D was used for the experiments because this is the first demonstration of using the integrated chip for protein analysis directly in blood.

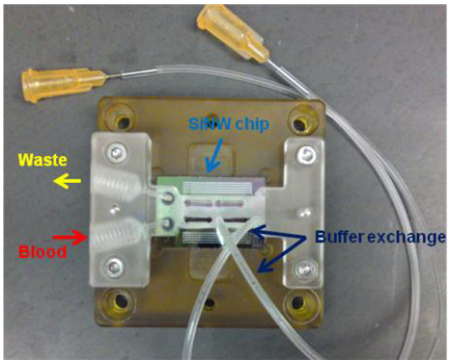

Figure 2. Micro-electro-fluidic packaging to fix the SiNW chip inside the housing and allow addition of blood, plasma separation and injection of

Electrical measurements were made between the source and drain electrodes of the SiNW based FET using the Alessi REL-6100 probe station (Cascade Microtech, Beaverton, OR). The sensing experiments were carried out in an aqueous environment of 0.01×PBS buffer solution. These measurements obtained after the immobilization of antibody are known as Ro measurements. The readings obtained after the addition of target protein is known as R measurements. A higher value of R indicates that binding has occurred because the proteins contain negative charges at neutral PBS buffer solution.

Following Ro measurement, filtration of blood spiked with the target proteins was carried out and the measuring buffer was replaced by the serum containing the target proteins. (The blood had been previously diluted and spiked with the target protein to make concentrations as needed). The chip was then incubated with the plasma for 20 mins following which R measurements were carried out using the same 0.01×PBS measuring buffer.

RESULTS AND DISCUSSION

The potential POC device is designed to be composed of a back- to-back integrated chip including filtration chip for plasma separation, a silicon nanowire biosensor for biomarker detection, and an interface readout application specific integrated circuit (ASIC), as shown in Figure 3. The in-built filtration chip on the bottom side of the integrated chip is able to extract the test plasma from the whole blood sample. An array of SiNW chips is fabricated on the top of the integrated chip, as above mentioned. The ASIC finally records concurrent and immediate signal-readout from the multiple SiNW sensors. To separate plasma from blood sample, a continuous flow plasma/blood separator was fabricated using submicron sized pillar gap structure. The nanowire chip for bio-sensing is designed with 4 groups divided by 51 clusters. Each cluster includes 5 individual nanowire sensor. Such a design allows Robot to dispense one droplet of antibody to one cluster for purpose of simultaneous detection. Once developed, such an integrated microsystem will be able to be used as a point-of-care device for diagnosis of cardiovascular disease.

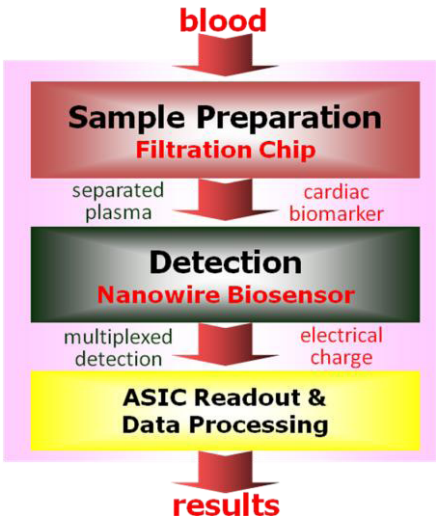

Figure 3. A work flow for an integrated microsystem for multiplexed detection of cardiac biomarkers in blood for the POC device development.

As a proof-of-concept study, three different cardiac biomarker antibodies involving MAb creatine kinase-MM, MAb creatine kinase-MB and MAb troponin T and BSA were separately spotted on the chip array. 2 µl of blood in 198 µl of PBS buffer spiked with proteins underwent the filtration chip and the plasma was subsequently collected for nanowire detection. Specificity of the microsystem was investigated by selectively binding separate cardiac biomarker (cTnT, CK-MM and CK-MB) to the antibodies and measuring the resistance change before and after the binding. Because these three cardiac biomarkers are all negatively charged in a neutral PBS buffer, binding of the three proteins to the n-type SiNW sensor leads to an increase in resistance. Results in Figure 4 show that distinguishable resistance change was obtained to each corresponding specific antibody and BSA when 100 ng/ml of cTnT, CK-MM and CK-MB was spiked in blood.

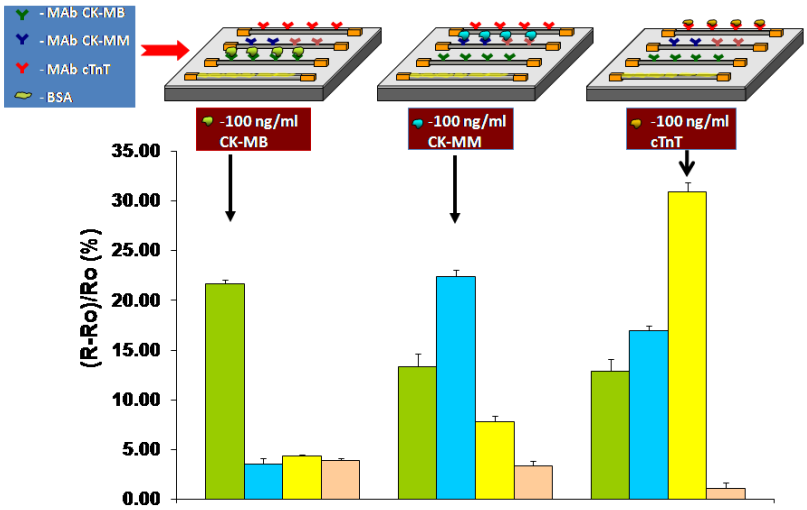

Figure 4. Specificity of the integrated chip showing that individual biomarker could be distinguishable from the blood. The SiNWs immobilized with BSA exhibit negligible response to the three proteins, respectively.

To investigate the sensitivity, blood spiked with the three proteins at various concentrations was injected the integrated chip. Figure 5 shows a clear trend of resistance reduction along with decrease of protein concentration in blood. Blood without proteins indicated negligible resistance change. The results show that 1 pg/ml of three biomarkers was detected by using the integrated chip, which is 1 order of magnitude lower than the conventional ELISA technique.

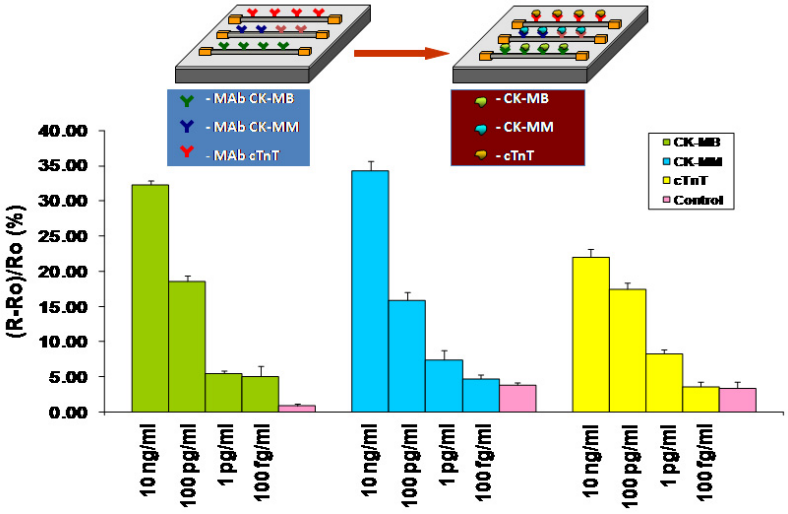

Figure 5.Response of the integrated chip to various concentrations of three cardiac biomarkers spiked in blood. In control, blood doesn't contain proteins.

CONCLUSION

In conclusion, the integrated chip can attain a low detection limit of 1 pg/ml for the three cardiac biomarkers from finger-prick blood sample in 45 minutes. The sensitivity is 1 order of magnitude higher than the well-established ELISA method. The next step is to conduct system characterization aimed at developing the POC device for screening and diagnostic applications.

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