Design, fabrication and performance evaluation of interdigital capacitive sensor for detection of Cardiac Troponin-I and Human Epidermal Growth Factor Receptor 2

Divya Mahalingam[1], Yasar Gurbuz[1]
[1]Faculty of Engineering and Natural Sciences, Sabanci University, Orhanli 34956, Tuzla, Istanbul, Turkey
E-mail: yasar@sabanciuniv.edu

Anjum Qureshi[2], Javed H. Niazi[2]
[2]Sabanci University Nanotechnology Research and Application Center, Orta Mahalle 34956, Tuzla, Istanbul, Turkey

***Abstract*—An interdigital capacitive biosensor has been designed and fabricated for label free detection of cardiac troponin-I (cTn-I) and Human Epidermal Growth Factor Receptor 2(HER2), cardiovascular disease (CVD) and cancer biomarkers respectively. CVD and cancer comprises of many disorders/types and various protein markers indicate the risk of the disease. In order to facilitate simultaneous multiple detection, a sensor chip, which has 2 rows of 6 interdigital capacitor (IDC) arrays was designed and fabricated. The chip was designed using simulation software (Sonnet) and fabricated on borofloat substrate using image reversal photolithography (for pattern transfer) and DC sputtering (for gold deposition). The IDC surface was functionalized using 2% 3-glycidoxypropyl trimethoxysilane (GPTMS) for antibody immobilization. The antibody-antigen binding on the IDC surface results in change in the capacitance of the IDC sensor. This change is directly related to the concentration of the antigen binding to the surface which helps in the quantification. Highly specific and sensitive detection of HER2 and cTn-I protein in real human serum was captured by anti-HER2 and anti-cTn-I antibody immobilized on IDC surface with a detection range of 5 pg/mL – 5 ng/ml and50pg – 1500 pg/ml respectively has been demonstrated.**

Keywords—Biosensor; Cancer biomarker; Cardio vascukar diseases biomarker; cTn0-I; HER2; Capacitance

I. INTRODUCTION

According to the World health organization (WHO) statistics, CVD and cancer are the major causes of death globally. It has been reported that cTn-I and HER2 are the major risk predictors of heart failure[1]and breast cancer respectively. Thus, their detection is important in early diagnosis of disease.

Various methods like piezoelectric microcantilever[2, 3], quartz crystal microbalance [4], surface plasmon resonance SPR [5], fluorescence [6], amperometric[7], have been reported on detection of cardio vascular disease (CVD) and cancerbiomarkers. Few of the above mentioned methods utilizing SPR and fluorescence have exhibited effective detection but they still hold several drawbacks concerning portability, sensitivity and cost efficiency. Another method that has been used in detection of CVD biomarker is electrochemical impedance spectroscopy (EIS). It is highly sensitive, low cost, reliable with label free detection tool, convenient to construct and can be miniaturized [8, 9]. Thus, EIS sensor is extensively being studied.

II. EXPERIMENTAL METHODS

A. Reagents

Human serum (male; blood type, AB) was purchased from PAN™ Biotech, GmbH and reconstituted by appropriate dilution in phosphate buffered saline (PBS, pH 7.4) prior to use.3 glycidoxypropyltrimethoxysilane (GPTMS) was purchased from Sigma–Aldrich, Germany.

B. Fabrication of interdigital capacitor chip

Interdigital capacitor array was fabricated using lift off process. AZ5214E photoresist was coated on clean borofloat substrate using a spin coater and patterned using standard photolithographic techniques. Then a 30 nm adhesion layer of Tiand 150 nm Au were deposited onto the patterned photoresist by DC sputtering. The gold that was not adhered on the substrate was lifted off by soaking it in acetone.

C. Immobilization of anti-HER2 and anti- cTn-I antibody on the IDC sensor surface

The IDC sensor surface was activated by formation of a self-assembled monolayer (SAM) of 3-glycidoxypropyltrimethoxysilane(GPTMS). The anti-HER2 and anti-Tn I (Cat. No. 10C-CR4036M5, Fitzgerald) antibodies were immobilized separately onto 2 different sets ofsurface activated IDC sensor array by bonding with their amino group and active epoxy group on the surface activated IDC sensor. The procedure followed is as follows: The chip with IDC array was thoroughly washed using ethanol and dried under N2 flow. A SAM was formed on the IDC surface by immersing the chip in 2% GPTMS in toluene for 4 hrs at room temperature. The surface activated chip was rinsed with toluene and dried using N2 gas. 2 µl of 100 µg/ml anti-HER2

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and anti- Tn I antibody in phosphate buffered saline (PBS) was incubated on each IDC for 3hrs at 4°C. The chip was then washed using sterile PBS and dried under N2. The formation of SAM and immobilization of the antibody were confirmed using FTIR.

D. *Verification of performance of IDC sensor by detecting cardiac Troponin I and Human Epidermal Growth factor Receptor 2*

Various concentrations of c Tn – I (50pg/ml – 1500 pg/ml) and HER2 (50pg/ml – 5000 pg/ml) protein were spiked in real human serum and 2µl of each concentration was incubated on their respective antibody immobilized IDC sensor surface for 1 hr at room temperature. The chip was then washed with sterile PBS and dried under N_2. The capacitance of the IDC sensor was measured after antibody and protein binding. To avoid any non-specific binding on the sensor surface a negative control experiment was conducted before adding the protein by incubating 0.5 % BSA on each IDC for 1 hr at 4°C. Then the chip was thoroughly washed using PBS and dried under N_2 flow.

III. RESULTS AND DISCUSSION

In this study we designed and fabricated an IDC sensor to detect cTn-I protein and HER2 protein, CVD and cancer biomarkers respectively. The layout of the IDC sensor is shown in Fig 1. The specificity of the IDC sensor was obtained by anti-HER2 and anti-cTnI antibodies that specifically bind only with their respective proteins. The antibodies were immobilized on the gold surface of the IDC sensor which captures their respective protein from the human serum. The binding of protein with the antibody was determined by recording the change in capacitance of the IDC sensor.

A. *FTIR confirmation of Antibody immobilization*

The surface activation and the antibody immobilization onto the IDC sensor surface was confirmed using FTIR analysis (Fig2). The ATR-FTIR peaks of the chip treated with 2% GPTMS revealed characteristic peaks at 990 cm^{-1} and 883

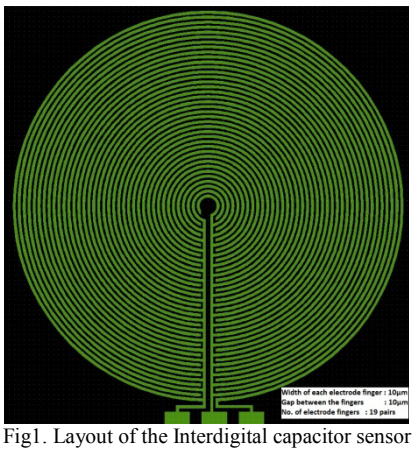

Fig1. Layout of the Interdigital capacitor sensor

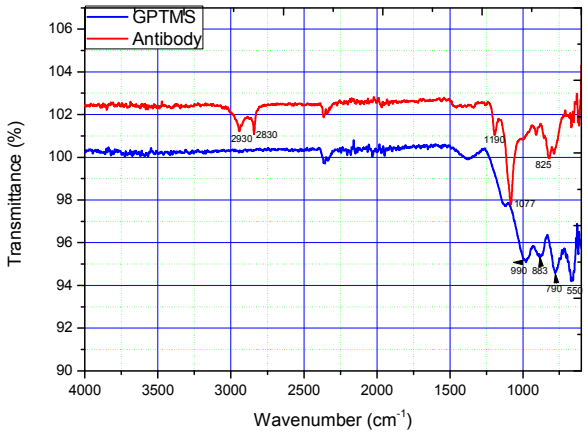

Fig2. FTIR spectra of surface activated and antibody immobilized chip.

cm^{-1} and the bands are assigned in accordance with[10]. The peak seen around 990 cm^{-1} is due to the Si-O-Si asymmetric stretching vibration. The band around 883 cm^{-1} corresponds to the Si-OH asymmetric stretching vibration. The band around 790 cm^{-1} is due to the symmetric Si-O-Si stretching vibration. The band around 550cm^{-1} is attributed to the O-Si-O vibrations.

Antibody was immobilized on the surface activated chips and the FTIR peaks were assigned in accordance with [11] . The bands observed around 2830 cm^{-1} is attributed to N-CH2 stretching. The peak around 825 cm^{-1} corresponds to the NH2 wagging. The bands around 1190 cm^{-1} and 1077 cm^{-1} is due to the C-N stretching. Thus, surface activation and immobilization of the antibody is confirmed.

B. *HER2 andcTn-I protein detection using IDC sensor*

The antibody immobilized IDC sensor chips were employed to test detection of various concentrations of HER2 and cTn-I proteins in human serum. A series of concentrations of the target proteins were incubated on an array of their respective antibody immobilized IDC sensor for 1 hr at room temperature. The chips were then washed with PBS and dried under N_2 flow. The capacitance change was recorded using a precision impedance analyzer. The capacitance of the IDC sensor was measured after the following steps and the change was recorded , i)Blank IDC sensor chip ii) After antibody immobilization iii) After BSA binding iv) After the protein binding. The IDC sensor did not show any response after BSA incubation and the value of capacitance after the BSA binding was treated as reference value with which the capacitance values of the IDC sensors incubated with various concentrations of protein was compared and plotted in figs 3&4. The relative change in capacitance of the IDC sensors incubated with different concentrations of cTn-I at a frequency of 3.9 MHz is presented in fig 3. It shows that the relative change in capacitance increases with increase in concentration and beyond 1000

pg/ml the capacitance drops, suggesting the saturation of the IDC sensor surface with the antigen. A similar behavior can be observed in fig 4 which shows the relative change in capacitance against various concentrations of HER2 protein at frequency 3.2 MHz, here the capacitance drops beyond 1000 pg/ml. When the surface of the IDC sensor saturates, all the available ends of antibody to bind with the protein is occupied and when more protein molecule competes to bind to the surface it leads to dissociation of already bound proteins from the IDC sensor surface, resulting in drop in capacitance value. The IDC sensor design could be optimized to detect even higher concentrations by increasing the area of the sensor which would provide room for more antibody binding sites.

The underlying mechanism by which the capacitance change occurred with addition of antibody and protein to the

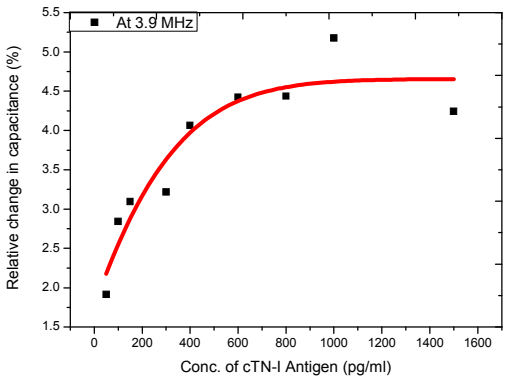

Fig3. Relative change in capacitance of IDC sensor at 3.9 MHz against various concentrations of cTn-I antigen.

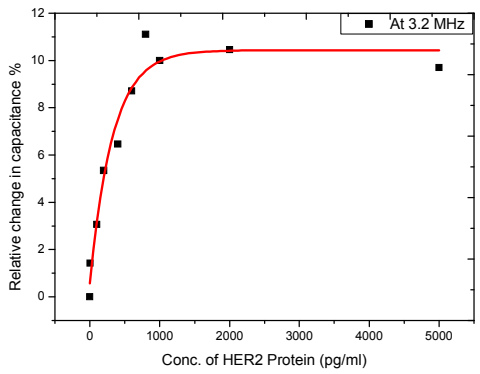

Fig4. Relative change in capacitance of IDC sensor at 3.2 MHz against various concentrations of HER2 protein.

IDC sensor surface is postulated to occur due to the chargespresent on antibody andprotein[12]. The capacitance response was generated as a result of change in total surface charges on IDC sensor surface. When protein binds with the antibody it enhances the capacitance value. Any further changes like increase in the concentration of the protein binding to the antibody has further enhanced the capacitance value. This difference in the capacitance value can be used to quantify the binding of antibody and protein that happened on the IDC sensor surface.

IV CONCLUSION

In this work we developed and demonstrated cancer and CVD biomarker detection capacity of an IDC sensor. The antibodies served as recognition elements that binds only with specific proteins. The performance of the IDC sensor was verified by testing the detection of HER2 and cTn-I proteins, cancer and CVD biomarkers respectively. The relative change in capacitance was measured and recorded using an impedance analyzer. The change in the capacitance value was in accordance with the interaction between the antibody and protein on the IDC sensor surface. Thus the relative change in capacitance value was used to quantify the concentrations of the protein binding to the the IDC sensor surface. Sensitive detection of HER2 and cTn-I proteins by their antibodies was demonstrated in a dynamic range of 5 pg/mL – 5 ng/ml and 50pg/ml – 1500 pg/ml respectively.

The developed IDC sensor has advantages of high senstivity, low cost, label free detection of biomarkers and has potential to be miniaturized. The design could further be used to test detection of other disease biomarkers helping in early diagnosis of disease, to help save lives.

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