The ZnO-FET Biosensor for Cardiac Troponin I

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Abstract. This paper investigates the influence of substrate-gate coupling on the ZnO-FET biosensor's sensitivity for detection of cardiac troponin I (cTnI), a 'gold standard' biomarker for acute myocardial infarction (AMI). The FET-based device with introduction of substrate-gate coupling on *p*-type silicon-on-insulator (SOI) substrate is fabricated using conventional lithography processes. An *n*-type zinc oxide (ZnO) thin film deposited via electron-beam evaporator is used as transducer for bridging the source and drain regions. Surface modifications via functionalization with 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GA) as chemical linkers, followed by immobilization of cTnI monoclonal antibody (MAb-cTnI) as bio-receptor on the ZnO thin film allow different concentration of cTnI detection with high selectivity. The device's sensitivity increases up to 9 %·(g/ml)$^{-1}$ with the increase of the substrate-gate voltage (V_{SG}) up to -10 V at very low limit of detection (LOD) down to 1.6 fg/ml.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide with 17.3 million casualties in 2008 [1]. Of those, a large proportion are caused by heart attacks, also known as acute myocardial infarction (AMI). AMI can be defined as a process where heart muscle cells died due to insufficient blood flow when small arteries are blocked causing sudden complete blockage of coronary arteries [2,3]. The permanently damaged heart cells released several cardiac biomarkers (i.e. myoglobin, creatine-kinase, cardiac troponin, and etc.) into the bloodstream [4]. Among these biomarkers, cardiac troponin I (cTnI) is considered as the 'gold standard' due to early concentration to reach peak (2-4 h) [5] and prolonged release in the blood (more than 10 days) [6]. A normal patients exhibits 1 pg/ml of cTnI in their blood, but can escalates to 100 ng/ml during AMI [7] as in Figure 1.

Field-effect transistor (FET)-based biosensors (Figure 2) are presently at the centre of interest for biomolecules detection [8]. The channel's surface, which is directly exposed to the surrounding can sense different surface potential upon biological interaction and modulates the current from drain to source region [9]. Specific immobilized bio-receptors (i.e. antibody, deoxyribonucleic acid (DNA), or enzyme) on the channel through covalent binding with suitable chemical linkers allows biomolecules detection with high selectivity [8]. The influence in electrical behaviour of the channel depends

whether positive or negative charge biomolecules and the properties of the semiconductor material used as channel, which caused increment or reduction in current flow from source to drain [8].

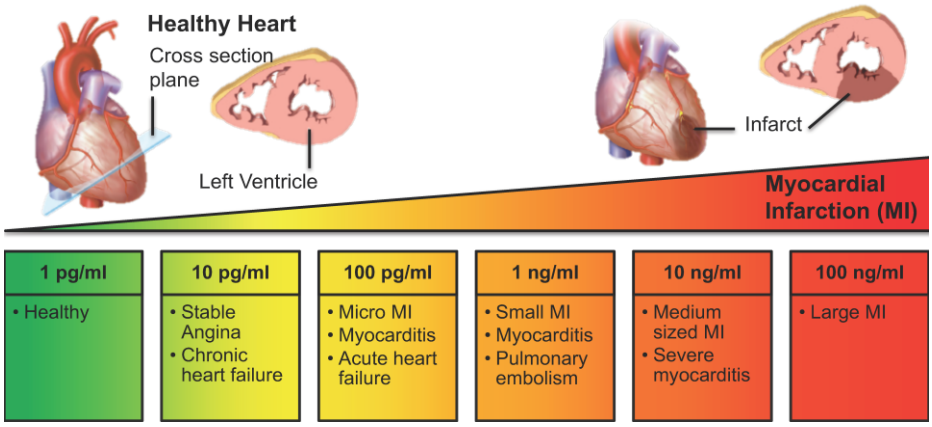

Figure 1. Severity of AMI at different concentration of cTnI [10].

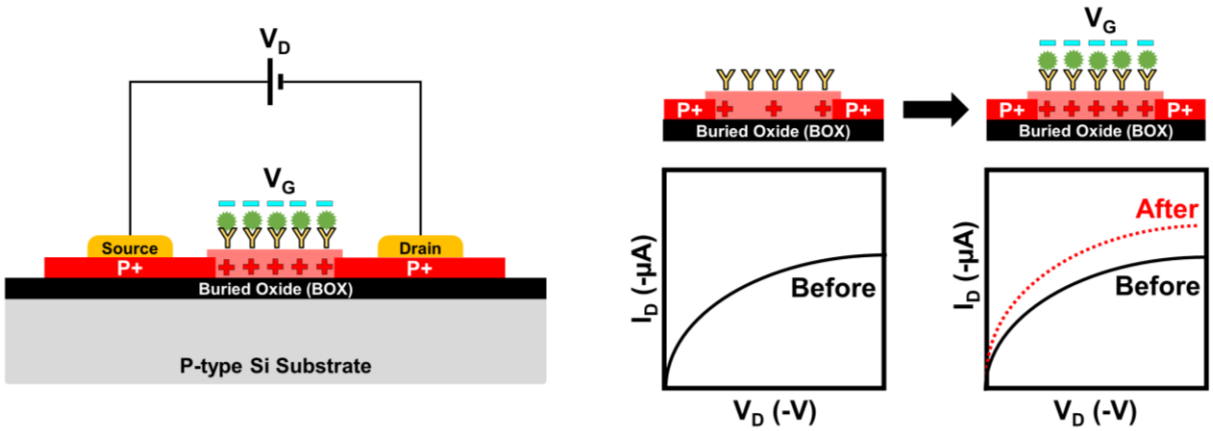

Figure 2. The architecture of the FET-based biosensor with the electrical characteristics during operation [9].

In the recent years, several reports on integration of additional gate in FET-based biosensor had been demonstrated for enhancing performance in biomolecules detection with higher sensitivity [11–14]. Thus, this work presents the impact of the substrate-gate coupling on the sensitivity of zinc oxide (ZnO)-FET biosensor for cTnI target biomarker detection. The device was fabricated on a *p*-type silicon-on-insulator (SOI) substrate through conventional photolithography processes. The surface of *n*-type ZnO thin film as channel was immobilized with cTnI monoclonal antibody (MAb-cTnI), which allow specific capturing of cTnI target biomarker at various concentration. By sweeping the substrate-gate voltage (V_{SG}) with negatively value, the drain current (I_D) values before and after detection of several concentration of cTnI biomarker are measured and analysed to compare the sensitivity of the device at selected V_{SG}. Limit of detection (LOD) of the biosensor is then determined based on the V_{SG} which demonstrates the best sensitivity.

2. Research methodology

2.1. Fabrication of biosensor

The biosensor was fabricated on a 2 × 2 cm silicon-on-insulator (SOI) substrate with thickness of 70 nm, 145 nm, and 1 mm for top-silicon (Si), buried oxide (BOX), and Si substrate layers, respectively

as shown in **Error! Reference source not found.**(a). The source and drain regions of the FET biosensor were formed through conventional lithography processes (**Error! Reference source not found.**(b)). It was started with positive photoresist (PR) spin-coating on the surface of the SOI substrate via a spin coater at 3000 rpm for 40 s. The coated substrate was then soft-baked on a hot plate at 110°C for 40 s to improve the PR adhesion towards the surface of the SOI substrate. The process was continued by aligning and transferring the source and drain patterns on the chrome mask to the coated substrate using ultraviolet (UV) light for 10 s via a mask aligner system. Then, the substrate was dipped into a PR developer solution for 35 s and rinsed with deionized (DI) water in order to develop the source and drain patterns. The substrate was hard-baked on the hot plate for 2 min at 110°C to evaporate the remaining moisture and increased the PR adhesion after development process. By using inductive couple plasma reactive ion etching (ICP-RIE), the top-Si layer was dry etched for 27 s to form the source and drain regions according to the previously patterned photoresist. The process was followed by stripping the PR from the substrate by using acetone, and subsequent rinsing with DI water.

The substrate-gate region of the biosensor was exposed from the top side of the substrate (**Error! Reference source not found.**(c)). Thus, another photolithography processes were repeated by using a second chrome mask for exposing the substrate-gate region. This time, the etching process was replaced by using wet etching inside buffered oxide etch (BOE) solution for 150 s to selectively remove the undesirable BOX layer, followed by rinsing with DI water.

The ZnO thin film with thickness of 200 nm, which acts as a transducer was deposited on the wafer by using electron-beam evaporator, annealed inside a muffle furnace at 500°C for 2 h, and was slowly cooled down to room temperature for 24 h (**Error! Reference source not found.**(d)). Additional photolithography processes using third chrome mask and wet etch by using hydrochloric acid (HCl) in DI water with ratio of 1:900 were conducted to form the ZnO thin film only at the specific gap between source and drain regions (**Error! Reference source not found.**(e)). The PR was remained on the ZnO thin film in order to protect the film from the next process.

Finally, the nickel/gold (Ni/Au) thin film as electrode pads were deposited on the substrate via a thermal evaporator. The photolithography processes of fourth mask were followed. The Ni/Au thin film was wet-etched by using “aqua regia” solution and rinsed with DI water. The remaining PR was stripped from the surface of the substrate by using acetone and rinsed with DI water (**Error! Reference source not found.**(f)).

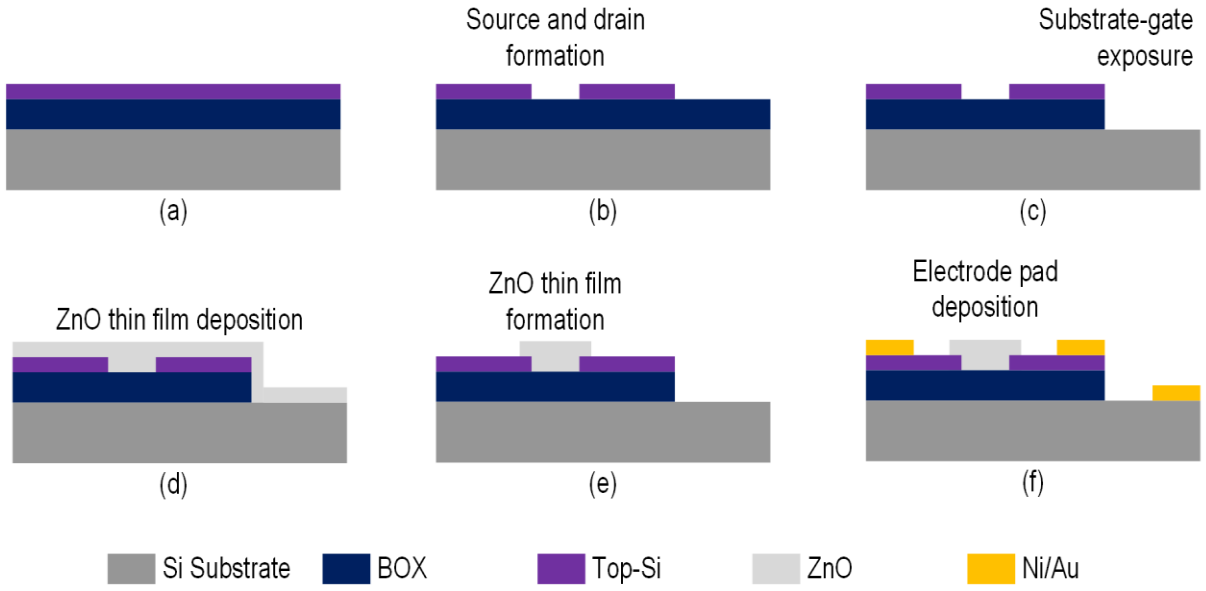

Figure 3. The fabrication process flow of ZnO-FET biosensor via conventional lithography processes. (a) Initial SOI substrate. (b) Source and drain formation via ICP-RIE of Top-Si. (c) Substrate-gate exposure by wet-etching of BOX layer. (d) Deposition of ZnO thin film. (e) Formation of ZnO thin film as channel by wet-etching. (f) Ni/Au deposition and formation as electrode pads.

2.2. ZnO thin film surface modification

The highly selective detection of the cTnI target biomarker requires the surface of ZnO thin film to be modified by using chemistry linkers (i.e. 3-aminopropyltriethoxy-silane (APTES) and glutaraldehyde (GA)) and bio-receptor (cTnI monoclonal antibody (MAb-cTnI)) as in **Error! Reference source not found.**.

Hydroxyl functional groups available on the surface of ZnO were allowed to covalently bind with silane groups from 2% APTES in DI water via immersion for 120 min in room temperature. The surface was then rinsed with DI water to remove remaining unbound APTES. Next, 1 µl of 2.5% glutaraldehyde (GA) in DI water was dropped on the previously produced amine-terminated surface and left in room temperature for 1 h, allowing the free end of APTES contained amine functional groups to covalently bind with aldehyde group in GA. Again, the surface was rinsed with DI water to remove remaining unbound GA.

Finally, 1 µl of highly selective 10 µg/ml MAb-cTnI in 0.01 M phosphate buffered saline (PBS) as bio-receptor was dropped on the functionalized ZnO thin film and left at room temperature for 1 h, thus allowing the amine functional group from the antibody to covalently bind with aldehyde-terminated surface on the ZnO thin film. To remove remaining unbound MAb-cTnI, the surface of the thin film was rinsed with 0.01 M PBS. The biosensor was stored in PBS at 4°C before use in experiment.

2.3. Detection of cTnI

The biosensor was dropped with 1 µl of cTnI target biomarker at several concentrations ranging from 10 pg/ml to 1 µg/ml. Each drop required a waiting time of 10 min prior to washing the device with 0.01 M PBS to remove any unbound cTnI biomarker.

2.4. Electrical characterization

The current versus voltage (I-V) characteristics of the device before and after detection of different cTnI target biomarker concentrations were measured by using Keithley 4200-SCS semiconductor parametric analyser. The drain voltage (V_D) was bias with -1 V, the substrate-gate voltage (V_{SG}) was sweep from 0 to -10 V at step voltage of 0.1 V, while the source was grounded as in Figure 5. The I_{DS} were measured and analysed to understand the effect of (V_{SG}) on the sensitivity of the device's detection.

3. Results and discussions

3.1. Electrical characteristics

The electrical detection of several cTnI target biomarker concentrations by using ZnO-FET biosensor was demonstrated as in Figure 6. By sweeping the V_{SG} from 0 to -10 V, the output I_D had significantly increased.

This is related to the device architecture, which utilized *p*-*n*-p junctions of drain, channel, and source, respectively from FET devices. The current can only flow from p-type drain to p-type source region when hole conduction layer forms in the n-type ZnO thin film [15,16]. The *p*-*n*-p junctions allow the detection of biomolecule charges at the n-channel region and increase the current modulation with the aid of gate bias (i.e. substrate-gate) and, hence amplify the output signal.

With the introduction of negatively V_{SG} to the substrate-gate region [11–14], electron carriers accumulate at the BOX/Si substrate interface, attract hole carriers in the ZnO thin film, and follow by formation of hole conduction layer across the channel as in Figure 7(b). This event allows more current flow from drain to source region with the increase of V_{SG} [17,18].

Upon detection of different concentration of cTnI biomarker concentrations, significant reduction in I_D can be observed from -5.04 to -1.35 µA at V_{SG} = -10 V and V_D = -1 V with the increase target concentrations from 10 pg/ml to 1 µg/ml, compared to the initial I_D before detection (I_{D0}) at 7.55 µA. The cTnI target biomarker has an isoelectric point (P_I) of 9.87, which exhibits positive charge when diluted in PBS solution at pH 7.4 [19]. As the cTnI was captured by MAb-cTnI (the bio-receptor, which was previously immobilized on the ZnO thin film), it caused repulsion of hole carriers and diminished the hole conduction channel in the thin film as in Figure 7(c). As a result, the increase concentration of cTnI target biomarker causes reduction of I_D.

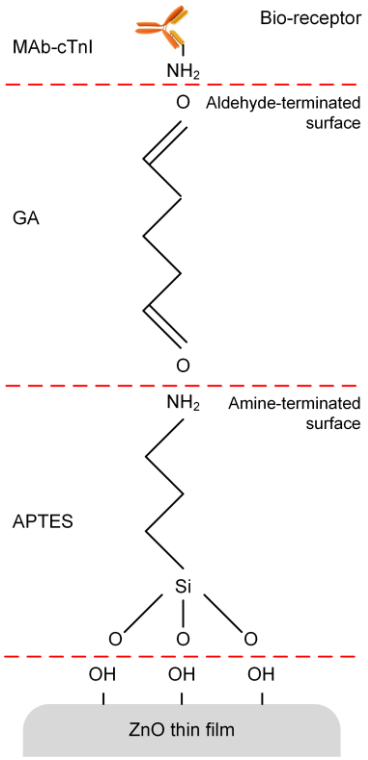

Figure 4. Surface modification of the ZnO thin film with chemical linkers (APTES and GA) and bio-receptor (MAb-cTnI).

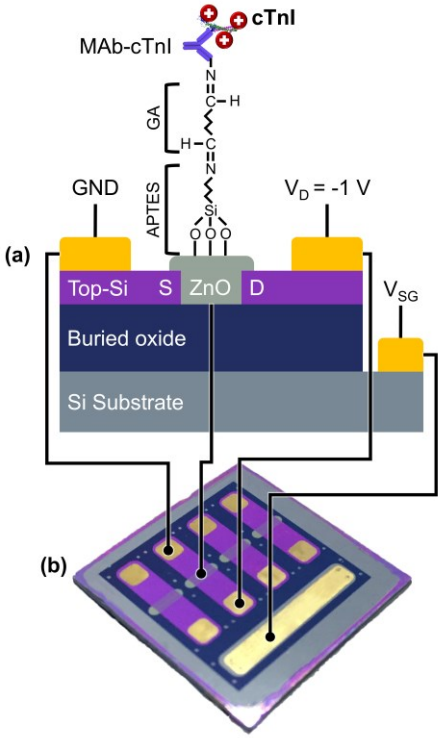

Figure 5. Device architecture of ZnO-FET biosensor. (a) Cross-section view of the device with surface modification. (b) The fabricated biosensor in array.

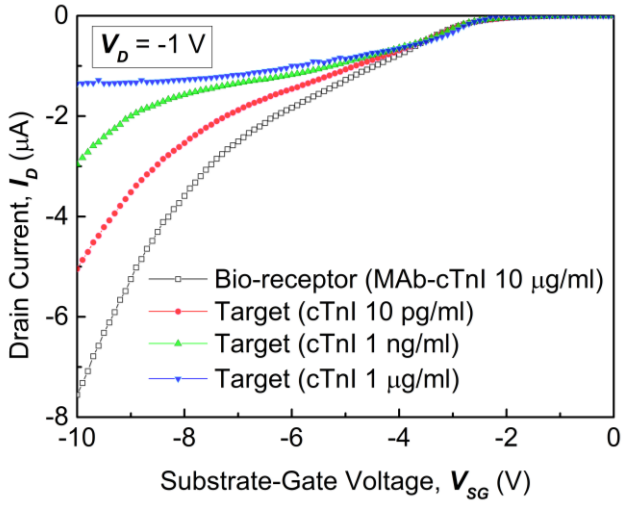

Figure 6. I_D versus V_{SG} of different cTnI target biomarker concentrations ranging from 10 p to 1 μg/ml at V_D = -1 V.

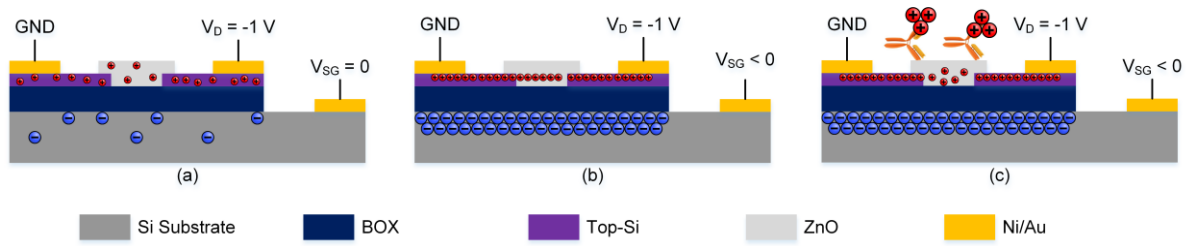

Figure 7. The impact of V_{SG} on the formation of hole conduction layer at the channel region. (a) Without V_{SG}, (b) With $V_{SG} < 0$ V, and (c) during detection of positively charged cTnI target biomarker with $V_{SG} < 0$ V.

3.2. Sensitivity

With more V_{SG} supplied to the device from -6 V to -10 V as in Figure 6, the reduction in I_D also become more apparent. Several I_D values at V_{SG} = -6, -8, and -10 V from Figure 6 were extracted and inserted into Equation (1) to calculate the relative change in I_D as plotted in Figure 8.

$$\text{Relative change in } I_D = \frac{\Delta I_D}{I_{D0}} \times 100 \quad (1)$$

ΔI_D represent the difference between I_D and I_{D0}. From the relative change in I_D, the sensitivity of the device at different V_{SG} can be calculated based on the slope of the calibration curve as in Equation (2).

$$\text{Sensitivity} = \Delta(\Delta I_D/I_{D0})/\Delta(\text{cTnI Concentration}) \quad (2)$$

The results are plotted as in Figure 9 for easier interpretation. The sensitivity of the device had clearly increased double from ~4 to 9.57 %·(g/ml)$^{-1}$ with the increase of V_{SG} from -6 to -10 V. This exceptional result suggest that the V_{SG} biasing has significant impact toward the sensitivity of the ZnO-FET biosensor for the detection of cTnI biomarker.

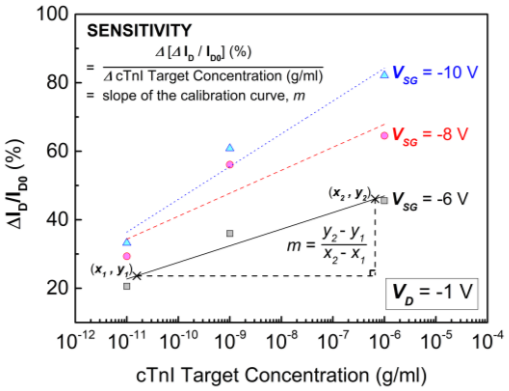

Figure 8. The sensitivity determination of the ZnO-FET biosensor at V_{SG} = -6, -8, and -10 V. The relative change in I_D versus cTnI target biomarker concentrations.

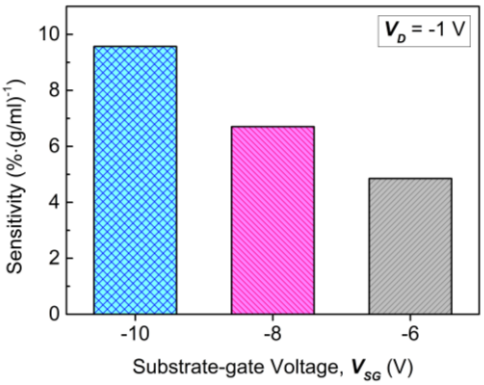

Figure 9. Sensitivity versus V_{SG} of the cTnI target biomarker.

3.3. Limit of detection

The LOD of the device is determined at V_{SG} = -10 V as in Figure 10**Error! Reference source not found.** due to high sensitivity detection as previously demonstrated based on the result from Figure 9. A device with higher sensitivity should give more accurate determination of LOD. It was calculated by replacing the $y_{(LOD)}$ as in Equation 3 into the straight-line equation of the calibration curve as in Equation 4 in order to get the $x_{(LOD)}$, which is the LOD of the device.

$$y_{(LOD)} = 3\sigma \tag{3}$$

$$y_{(LOD)} = m \cdot \log_{10} x_{(LOD)} + C \tag{4}$$

σ, m, and c represent the standard deviation of the y-intercept, the slope, and the y-intercept of the calibration curve, respectively. Thus, LOD of the device is 1.6 fg/ml, which can be considered as among the lowest LOD attained for the detection of cTnI compared to the other previous reports.

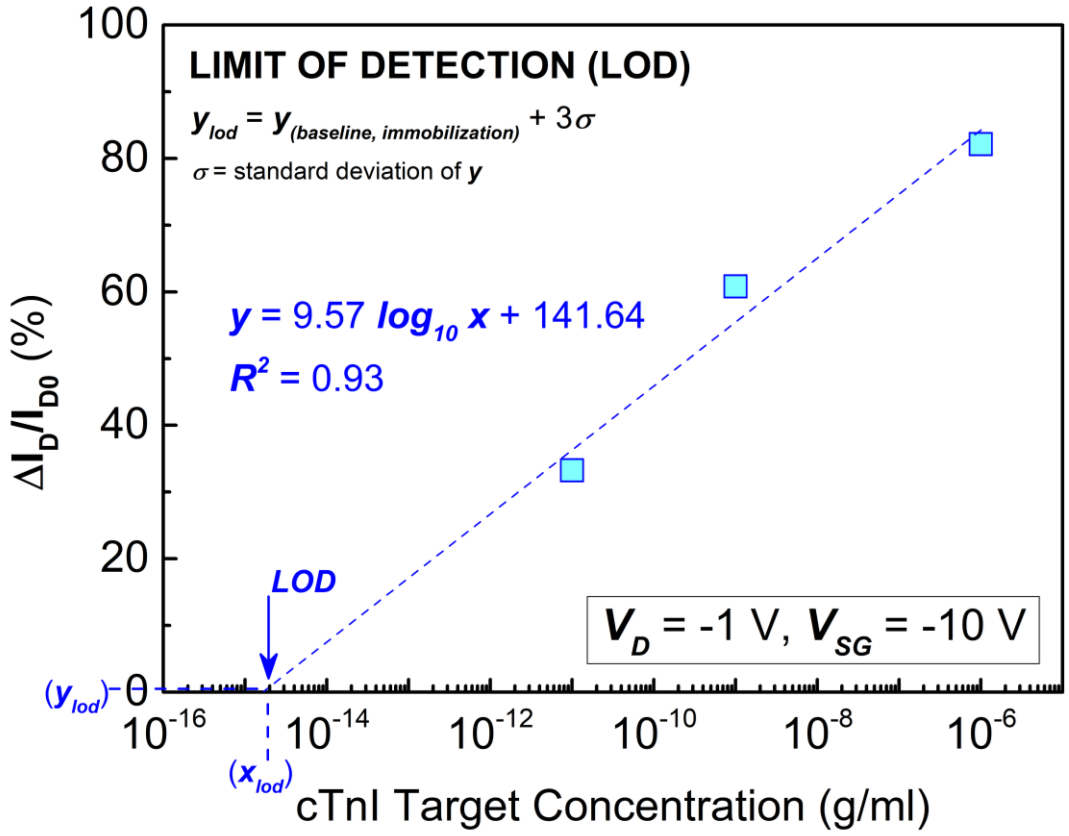

Figure 10. Limit of detection (LOD) of the ZnO-FET biosensor at V_{SG} = -10 V and V_D = -1 V.

4. Conclusion

The substrate-gate coupling has significant impact on the sensitivity of the ZnO-FET biosensor. With the increase of V_{SG} supplied to the substrate-gate region, more current flowed from drain to source region due to formation of better hole conduction layer in the ZnO thin film. As different cTnI biomarker concentrations were captured on the modified surface of ZnO thin film, reduction in I_D were obtained as a result of diminished hole conduction channel due to repulsion with positively charged cTnI captured on the ZnO thin film. The changes are more apparent with the introduction of higher V_{SG}. The device sensitivity had increased double with V_{SG} of -10 V compared to lower V_{SG} of -6 V, suggesting improvement in sensitivity with the introduction of substrate-gate biasing. With better sensitivity, the LOD of the device had managed to reach down to 1.6 fg/ml. This device shows promising potential in detection of cTnI biomarker for determination of AMI.

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Acknowledgments
The authors are grateful to the Department of Higher Education, Ministry of Higher Education, (KPT) for funding this research through the Fundamental Research Grant Scheme (FRGS; with the Grant number 9003-00536). The authors also would like to acknowledge all the team members in Institute of Nano Electronic Engineering (INEE), Universiti Malaysia Perlis (UniMAP) for their guidance and help.