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High sensitivity cardiac troponin I detection in physiological environment using AlGaN/GaN High Electron Mobility Transistor (HEMT) Biosensors

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ABSTRACT

In this study, we report the development of a high sensitivity assay for the detection of cardiac troponin I using electrical double layer gated high field AlGaN/GaN HEMT biosensor. The unique gating mechanism overcomes the drawback of charge screening seen in traditional FET based biosensors, allowing detection of target proteins in physiological solutions without sample processing steps. Troponin I specific antibody and aptamer are used as receptors. The tests carried out using purified protein solution and clinical serum samples depict high sensitivity, specificity and wide dynamic range (0.006–148 ng/mL). No additional wash or sample pre-treatment steps are required, which greatly simplifies the biosensor system. The miniaturized HEMT chip is packaged in a polymer substrate and easily integrated with a portable measurement unit, to carry out quantitative troponin I detection in serum samples with < 2 µl sample volume in 5 min. The integrated prototype biosensor unit demonstrates the potential of the method as a rapid, inexpensive, high sensitivity CVD biomarker assay. The highly simplified protocols and enhanced sensor performance make our biosensor an ideal choice for point of care diagnostics and personal healthcare systems.

1. Introduction

Cardiovascular diseases (CVDs) are the leading causes of mortality including premature death worldwide for both men and women (2000; Benjamin et al., 2017). Studies from the past two decades show that better disease management by CVD risk assessment and improved treatment methods have caused a decline in CVD related deaths, limited to certain regions of the world (2016; Roth et al., 2015). The burden of CVDs in developing countries contributes a greater share to the global statistics than the developed countries (Yusuf et al., 2014; Lee et al., 2017). This suggests that it is imperative to develop better risk assessment and treatment methodologies for prevention and control of CVDs and make them accessible and available to everyone, across the globe. Additional risk stratification methods such as CVD biomarker detection can dramatically improve early disease diagnosis and prognosis not only in 'high-risk' patients, but also in people without prior medical history or incidence (de Lemos and Llyod-Jones, 2008).

Myocardial infarction (MI) is an essential factor of global CVD burden. In the epidemiology of MI, the diagnostic biomarker has a critical role to play (Roger, 2007; Mythili and Malathi, 2015). Since the 1980s, Troponins have been used as clinical biomarkers for Acute Myocardial Infarction (AMI) and other cardiac muscle damage related diseases (Mythili and Malathi, 2015; Babuin and Jaffe, 2005). Troponin I is cardio-specific and is released into the blood in a short time following muscle damage. High sensitivity troponin I (hs-cTnI) assays are designed to be very sensitive to closely monitor the damage to the myocardial tissue (Shah et al., 2015). Therefore, hs-cTnI tests can detect the presence of troponin I earlier than 3 or 6 h after onset of AMI, which is the time mark for first generation assays and also identify other reasons for cardiac muscle injury (Christenson and Azzazy, 2006; Keller et al., 2009; Diercks et al., 2012).

Several techniques have been widely investigated for troponin I detection including, fluorescence, colorimetric, electrochemical and

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surface plasmon resonance (Song et al., 2011; Cho et al., 2009; Choi et al., 2010; Bhalla et al., 2012; Kwon et al., 2011). Currently existing commercial assays mostly employ fluorescence, ELISA or a combination of fluorescence and paramagnetic techniques (Tate et al., 2012). The use of additional reagents for labeling, multiple wash steps, large sample volume requirement, longer reaction times, high background interference and bulky bench top equipment settings are some of the disadvantages associated with the current assays. Such laboratory based biomarker detection techniques are very expensive and inconvenient thereby limiting their availability and accessibility globally.

Field effect transistor (FET) based biosensors have the capability to provide high sensitivity, low detection limit and easy signal read out capabilities (Liu and Guo, 2012; Shen et al., 2014). However, the phenomenon of charge screening or Debye screening in high salt concentrations such as physiological fluids limits the clinical applications of this technology as the assay requirements include extensive sample pre-treatments (Zheng et al., 2005; Stern et al., 2010; Lei et al., 2017). Recently, our group developed an electrical double layer (EDL) gated FET sensor design that can detect target proteins in high ionic strength solutions such as 1x PBS and human serum without any sample pre-processing, thus offering detection beyond the Debye length (Chu et al., 2017). AlGaN/GaN High Electron Mobility (HEMT) transistor was used for the implementation of EDL FET design in light of its unique electrical, chemical and thermal properties (Zeggai et al., 2014). Using this technology, high sensitivity immunoassays can be performed for potentially fatal diseases such as CVDs, cancer and infectious diseases. At present, clinical diagnostics rely on expensive, bulky and bench-top instruments that often require trained laboratory personnel to carry out operations. As a result, diagnostic facilities are costly and scarce. If early and timely diagnosis and continuous disease monitoring are our goals, personal healthcare has to be improved in terms of affordability and accessibility of diagnostics.

In here, we report the development of GaN HEMT based biosensor for high sensitivity troponin I detection in physiological samples. The gate electrode of sensor is functionalized with specific receptor (antibody or aptamer) and the binding of target protein causes changes within the EDL gating the transistor dielectric, resulting in concentration dependent drain current change. FET sensor response is highly sensitive to the surface binding events, thus dramatically reducing the reaction time to 5 min. This technique eliminates the need for sample pre-treatments or processing such as washing or labeling. The sensor exhibits detection with high sensitivity and dynamic range, offering extremely low detection limit even in high ionic and protein background such as human sera. The ease of sensor fabrication using conventional semiconductor manufacturing processes, stable operation in biological media, low cost and miniaturized size are some of the added advantages that qualify the sensor to be used as point of care or mobile diagnostics. The sensing technique can be applied to all types of FET sensors that allow us to utilize enhanced electrical characteristics resulting in even higher sensitivity. We have developed a portable biosensor system that can carry out hs-cTnI assay in 5 min with < 2 μl sample volume. The mobile diagnostic system for cardiac troponin-I is intended to be used as a personal healthcare device and point-of-care testing in hospitals, especially in emergency situations that demand immediate medical intervention.

2. Materials and methods

2.1. AlGaN/GaN HEMT fabrication

The AlGaN/GaN epi-wafer consists of 3 μm-thick undoped GaN buffer layer, 150 Å - thick undoped $Al_{0.25}Ga_{0.75}N$ layer, and 10 Å -thick undoped GaN cap layer. Metalorganic Chemical Vapor Deposition (MOCVD) is used to deposit AlGaN/GaN on silicon substrate. The device fabrication starts with the formation of mesa structures on the epi-wafer using inductively coupled plasma (ICP) etching to define transistor active area. A mixture of Cl_2/BCl_3 gases (35 sccm/35 sccm) is used under ICP power of 300 W and RF bias of 120 W at 2 MHz. Ohmic contacts are deposited using electron beam (e-beam) evaporator with a standard recipe: Ti/Al/Ni/Au (200 Å/400 Å/800 Å/1000 Å). Rapid thermal annealing (RTA) is then carried out at 850 °C for 45 s in N_2 environment. The distance between source and drain contacts is 30 μm. Metal interconnects including the gate electrode is deposited consisting of Ti/Au (200 Å/1200 Å) using e-beam evaporator. The gate metal electrode is positioned at fixed distances of 65 μm, 265 μm and 465 μm from the transistor channel and the typical distance used is 265 μm. Before conducting measurement in solution, the entire device is passivated using 2 μm thick photoresist (Shipley S1818) and a 100 × 120 μm^2 and 10 × 60 μm^2 openings are made on the gate metal electrode and transistor channel region respectively, using photolithography.

Packaging technology for HEMT chip has been developed previously (Hsu et al., 2017). Briefly, GaN HEMT chip is fabricated with a 1 mm^2 die area. The chip is embedded in epoxy resin using a PDMS mold and cured at temperatures of 125 °C and 165 °C for 1 h each. Metal interconnects (Ti/Pt/Au: 200/500/2000 Å) are formed using e-beam evaporator and device is passivated using photoresist and sensing region is opened. A simple microfluidic channel is used to allow sample to flow on the sensor surface. The microfluidic channel is self-powered as it uses the principle of capillary action to flow the sample in to the sensing region. The packaged chip is connected to the commercial microSD card reader which is mounted on the portable sensing system.

2.2. Surface functionalization

Monoclonal antibody (mAb) to Troponin I purchased from SantaCruz Biotech (sc-133117) is used as the receptor which is immobilized on the gold gate metal electrode. The native thiol groups in the IgG molecule are used to covalently bind to the Au gate electrode. The immobilization method follows (Karyakin et.al, 2000), with some modifications. The disulfide bonds in the hinge region of the mAb is cleaved using a mild reductant such as 2-mercaptoethylamine (purchased from Sigma Aldrich) in the presence of chelating agents such as EDTA. The mAb (1 μM) and 2-mercaptoethylamine (MEA) (10 mM) are mixed together and incubated in 37 °C in water bath for 15 min. The mixture is then dropped on the device and incubated in room temperature for 1.5 h followed by 12 h incubation period in refrigerator at 4 °C. Afterwards, the device is rinsed in 1 mL 1X PBS to remove unbound antibodies.

Troponin I specific aptamer is purchased from Sigma Aldrich and is thiolated. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1 mM) is added to the aptamer solution (1 μM) in Tris-EDTA buffer to reduce the dithiol bond to yield free sulfhydryl groups. The solution is heated to 95 °C and cooled down to room temperature and incubated on gold gate electrode to allow SAM formation at ambient conditions for 24 h. Afterwards, the device is rinsed in 1 mL 1X PBS to remove unbound aptamer.

2.3. Purified protein samples in 1X PBS

Troponin I protein is purchased from Abcam (ab9936) and diluted to physiological concentrations in 1X PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4 with NaOH) with 4% Bovine serum albumin (BSA).

2.4. Clinical human serum samples

Clinical samples of human serum containing Troponin I are obtained as per Institutional review Board (IRB) of National Cheng-Kung University Hospital (NCKUH) with approval (IRB. No. B-ER-21 104–116) and under supervision by National Tsing Hua University IRB

approval (10405HE014). The concentration of cardiac Troponin I (cTnI) in the clinical samples is determined using Siemens Centaur Ultra diagnostic system. The cTnI concentrations are in the range of 0.006–148 ng/mL. The clinical serum samples are directly used for electrical detection without any sample pre-processing.

2.5. Measurement of sensor

The source drain current characteristics are measured using Agilent B1530/B1500A semiconductor parameter analyzer system. A 50 μs wide short duration pulse is applied as gate voltage. The drain to source is biased with a fixed voltage of 2 V. The sampling rate is 10 ns.

2.6. Protein elution process

Sample of solution (1X PBS with 4% BSA and clinical serum) is incubated on the device for 5 min prior to electrical testing. After every measurement, the device is washed in ample quantity of mild protein elution buffer (pH 6.6) containing very high salt concentration to disrupt the antibody-antigen binding and remove all the proteins bound via electrostatic interaction. The elution buffer is then washed away using 1X PBS before using the device for subsequent cTnI detection.

3. Results and discussion

3.1. Characteristics of EDL gated AlGaN/GaN HEMT biosensor

Fig. 1(a) and (b) shows the schematic structure and top view of GaN HEMT sensor. The gate electrode which is similar to the reference electrode in traditional FET design is placed at a process controlled distance away from the transistor channel. The sensing region comprises of the open area on the transistor channel and the gate electrode. The open area on the transistor channel is 20 times less than that on gate electrode, to provide an asymmetric electric field from gate to channel when supplied with a gate bias voltage. The gate electrode is functionalized with either anti-Troponin I antibody or Troponin I specific aptamer. In the case of immobilized antibodies, the disulfide bonds in the hinge region of the IgG molecule is selectively cleaved using a mild reductant such as 2-mercaptoethylamine resulting in free thiols that can form self-assembled monolayers on the gold gate electrode. The covalently linked antibody immobilization strategy has several advantages over the conventional immobilization strategy using linker and EDC-sulfo NHS coupling: proper binding site orientation, increases the dynamic range of detection, utilizes stable Au-thiol chemistry and process can be carried out at physiological pH. Thiol

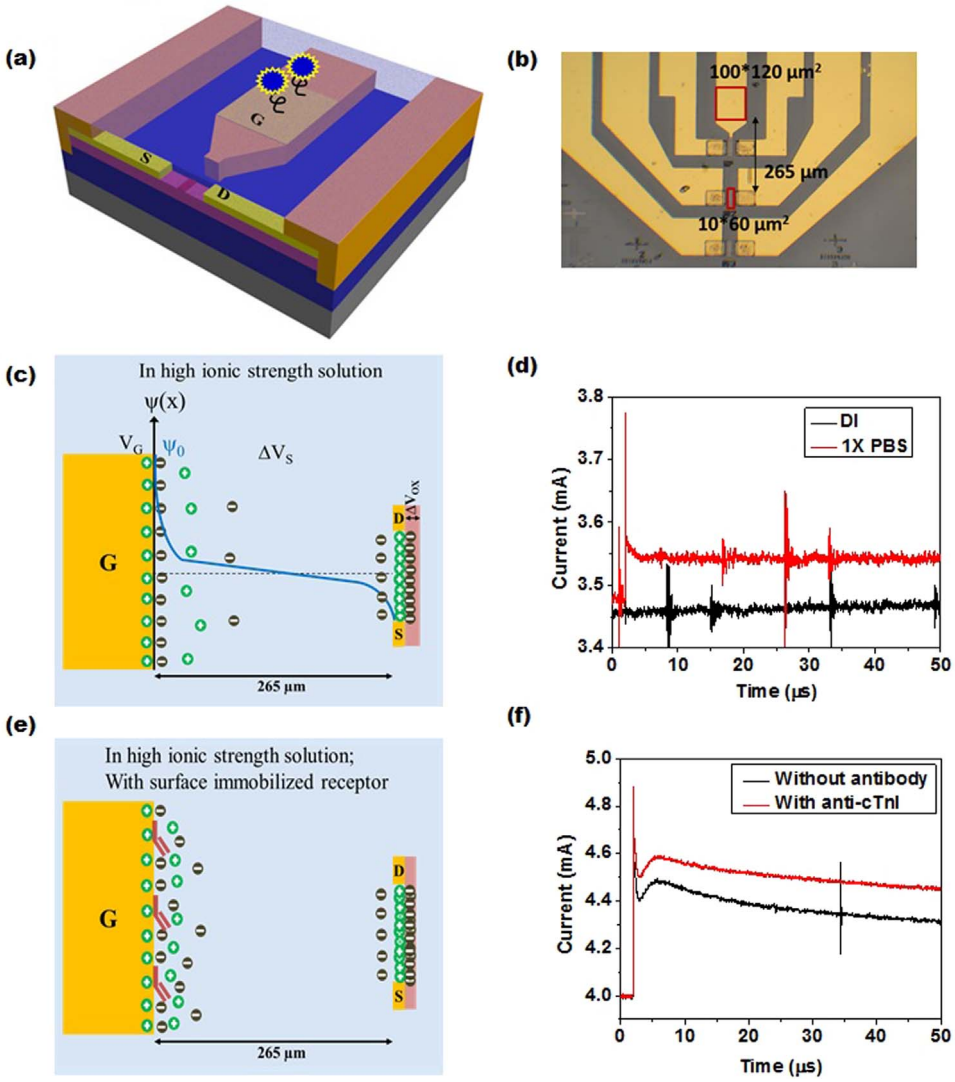

Fig. 1. (a) Schematic illustration of the AlGaN/GaN HEMT sensor. (b) Top view image of HEMT sensor depicting the sensing region consisting of gate electrode opening and channel opening separated by a fixed distance. (c) Potential distribution across the solution when gate voltage and transistor bias are applied. (d) Sensor response in low and high ionic strength solutions. (e) Illustration of charge distribution in the EDL gated HEMT structure when receptor is immobilized on the gate electrode area. (f) Sensor response with and without antibody immobilized on gate electrode area.

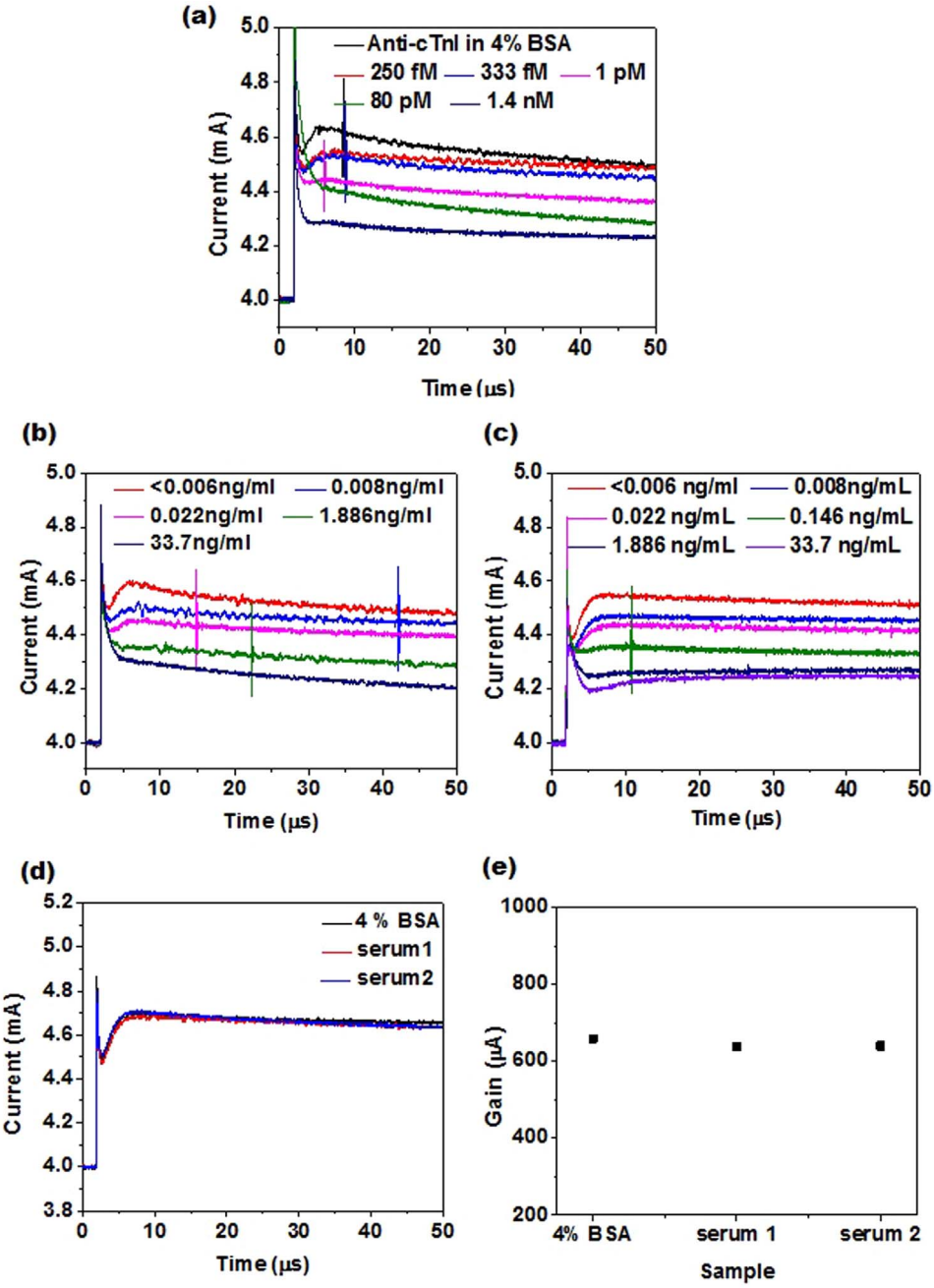

Fig. 2. Current versus time graph for the antibody based detection of Troponin I (a) purified proteins prepared in 1XPBS containing 4% BSA, (b) and (c) in clinical human serum samples, (d) and (e) Response of sensor without receptor to 4% BSA and serum samples.

modified aptamer is also immobilized on the gate electrode area using the same surface chemistry to obtain stable and improved sensor performance. The advantages of using aptamer over antibodies are sensor stability, longer shelf life, lesser variation compared to differing sources of antibody and cost. The lesser immunoreactivity or affinity and lack of availability of aptamers for all the disease biomarkers are some of the present shortcomings that prevail antibodies as the major choice for receptor. The device baseline measured before and after surface modification yields sensor response that corresponds to the successful immobilization of antibody on the gate electrode, as shown in Fig. 1(d).

The test sample is placed above the sensing region and provided with a constant drain source bias and pulsed gate bias for 50 µs. The resulting source drain current is a function of the net potential drop across the solution. The sensing mechanism has been elucidated in our previous work (26). Briefly, the net potential drop across the solution is the sum of potential drops in the EDL formed at the gate electrode, bulk solution V_s and the EDL formed at the transistor channel, thus affecting the voltage across the transistor dielectric V_{ox}, as shown in Eq. (1). The net potential drop across the solution creates a potential gradient through the solution, as shown in Fig. 1(c). When we operate the sensor in this EDL FET design with transistor to gate electrode gap of 265 µm and V_g is applied, solution capacitance C_s is formed with EDL on both the gate electrode and transistor channel and the net potential drop modulates the capacitance of the transistor dielectric, resulting in change in drain current. The electric field gradient generated using this EDL FET design is similar to that of electrophoresis (Chu et al., 2017; Jameson and Alvarez-Tostado, 1939; Holmes and Stellwagen, 1990). Thus the change in voltage across the transistor dielectric can be expressed as a function of solution capacitance, as

shown in Eq. (2) where C_{ox} is the dielectric capacitance, resulting in change in drain current. When solution capacitance increases, it increases the effective V_g applied to the transistor.

$$V_g = \Delta V_s + \Delta V_{ox} \tag{1}$$

$$\Delta V_{ox} = \frac{\frac{1}{j\omega C_{ox}}}{\frac{1}{j\omega C_{ox}} + \frac{1}{j\omega C_s}} \times V_g = \frac{C_s}{C_{ox} + C_s} \times V_g \tag{2}$$

Where j and ω are current and angular frequency, respectively. ΔV_s and ΔV_{ox} are the gate voltage drops in solution and in dielectric, respectively. C_s and C_{ox} are the solution capacitance and the dielectric capacitance, respectively.

Ion density in the solution, surface charge modifications such as surface functionalization by antibody or aptamer and receptor-ligand binding can result in change in solution capacitance and thus the drain current response. This is depicted in Fig. 1(d) and (f). In Fig. 1(d), the sensor's drain current response with and without mobile ions is recorded. When there are very less mobile ions as is the case of de-ionized (DI) water, the drain current quickly relaxes to the baseline as there are no ions to generate EDL distribution. On the other hand, when 1X PBS is used as the test solution which consists of high concentration of mobile ions, the drain current responds to the change in solution capacitance as the ions form EDL on the gate electrode and transistor sides when applied with a V_g. The difference of drain current with and without gate bias is considered as the transconductance gain of the sensor. Similarly in Fig. 1(e) and (f), the functionalization of sensor with receptor on the gate electrode and corresponding drain current response is depicted. The receptor immobilization increases the overall solution capacitance, resulting in an increased gain of the sensor. Therefore, a change in number of mobile ions and surface modification with receptor respectively, bring about distinct change in drain current response, elucidating the working mechanism of the ion gated EDL FET. This net change in solution capacitance is also observed during receptor-ligand binding, helping us study the interaction of the binding entities.

When the receptor on the gate electrode captures the target protein, the change in charge distribution in and around the EDL on the gate electrode is mirrored in the EDL on the transistor channel, thus modulating the drain current or 'sensing' the concentration of the target protein in the test sample. This way, we can detect charged or un-charged proteins because the sensor mechanism relies not only on the potential change brought by receptor-ligand binding, but the overall change in ionic strength in the local region of the receptor-ligand binding moiety. The EDL gated FET design takes advantage of the high k dielectric property attributed to EDL. Thus, in solutions with higher ionic strength, such as human blood, EDL provides higher transconductance gain leading to higher sensitivity.

3.2. Troponin I detection in physiological buffer solution

The detection of purified Troponin I in 1X PBS with 4% BSA is depicted in Fig. 2(a). The physiological concentration of BSA is added to 1X PBS to mimic human serum as albumin is the most abundant protein found in blood. The drain current response for 1X PBS with 4% BSA is noted as the control or sensor baseline. By allowing only 5 min for sample incubation on device, the non-specific interaction from the high background protein concentration is significantly reduced. Several clinically relevant concentrations of purified Troponin I are tested: 250 fM, 333 fM, 1 pM, 80 pM and 1.4 nM. The transconductance gain or simply 'gain' of the device keeps decreasing with increasing Troponin I concentration. Between each concentration, the device is washed in near neutral protein elution buffer to remove the bound and non-specific proteins by interrupting the electrostatic interaction. In this way, the accumulation of non-specific binding is prevented and sensor surface is regenerated for further testing. The sensor exhibits high sensitivity with an extremely low detection limit of 250 fM (6 pg/mL) (from experimental observation) when measured using purified proteins prepared in 1X PBS with 4% BSA.

3.3. Troponin I detection in human serum

Clinical human serum samples were also used to demonstrate the high sensitivity troponin I detection capability of the GaN HEMT sensor. The samples were collected from different patients who were hospitalized owing to relevant symptoms. The serum samples are calibrated using laboratory standard diagnostic equipment. Our sensor is used to test different serum samples containing varying concentrations of Troponin I: < 0.006 ng/mL, 0.008 ng/mL, 0.022 ng/mL, 0.146 ng/mL, 1.886 ng/mL and 33.7 ng/mL. The drain current response from two different sensor chips is depicted in Fig. 2(b) and (c). By comparing Fig. 2(a), (b) and (c), it can be conceived that the results from testing in purified buffer system and human serum samples are very similar and thus the sensor can target the specific antibody-antigen binding at the gate electrode interface. Even in the presence of high ionic strength and high background protein concentration contained in blood serum, the sensor can detect the binding of target protein with high sensitivity and specificity. The smallest troponin I protein concentration that can be detected by the laboratory standard diagnostic equipment is 0.006 ng/mL and we may use our sensor's standard curve to derive and predict the unknown concentration of troponin I contained in the serum sample denoted as < 0.006 ng/mL.

The sensor's electrical response to background proteins is evaluated in Fig. 2(d) and (e). The current gain response of the device is very similar for 4% BSA and different serum samples obtained from multiple patients, when there is no receptor on the device. All the samples are incubated on the device surface only for 5 min before testing, just as is the protocol for target analyte detection. This evidence suggests that there is negligible effect of background proteins on the response of the sensor. This is because the change in current gain is not majorly a result of variation in the bulk of the solution, which is formed by the background proteins. The specific interaction of receptor-ligand binding is the contributing factor to the change in EDL charge distribution. As seen in Fig. 2, the sensor calibration curve depicts a strong dependence on analyte concentration, in the test for serum samples. This is also validation for the selectivity of the GaN HEMT sensor. Although the serum samples obtained from different sources have a variety of non-target proteins, the calibration curve demonstrates highly sensitive Troponin I dependent signal change. This enables us to develop the immunoassay by eliminating the need for blocking and washing, potentially simplifying the assay protocols for the development of mobile diagnostics and increasing its reliability.

Fig. 3 shows the standard curves for the Troponin I sensor. The device gain is denoted as the change in drain current when V_g is turned on. Thus the change in gain with respect to control is defined as the signal of the sensor. The signal vs concentration graph for tests using purified Troponin I in 1X PBS with 4% BSA is shown in Fig. 3(a). The receptor ligand binding is a reversible reaction denoted as

$$[Ab] + [Ag] \overset{K}{\longleftrightarrow} [Ab - Ag] \tag{3}$$

Where $K = 1/K_D$, K_D being the dissociation constant.

Estimation of K_D can be carried out using one binding site model equation:

$$\Delta G = \Delta G_{max} * [Ag]/(1 + K_D * [Ag]) \tag{4}$$

Where ΔG is the sensor signal, ΔG_{max} is the saturation signal and [Ag] is the concentration of Troponin I.

Fig. 3(a) shows the fitted curve following the one binding site model. The derived K_D is 1.2 pM Following Eq. (2), K_D can be extracted from calibration curve for testing in human serum samples as well, as shown in Fig. 3(b). The calibration curve is derived from multiple tests

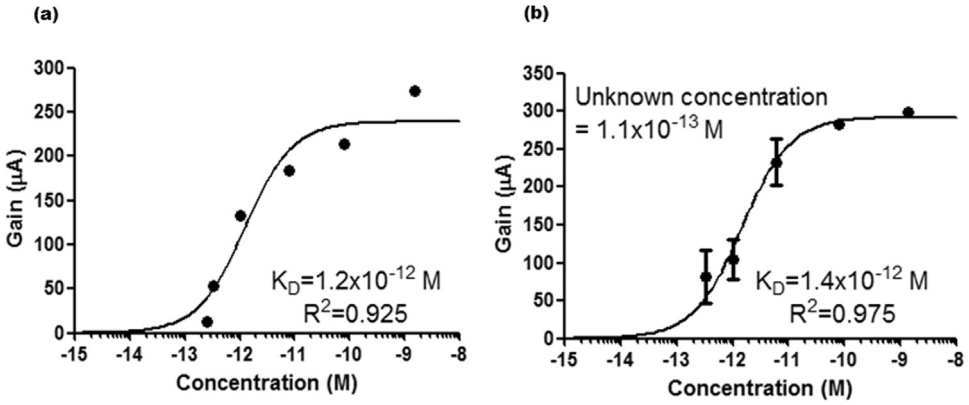

Fig. 3. (a) Binding site model for the detection of purified Troponin I samples. (b) Binding site model for the detection of Troponin I in human serum samples.

performed on multiple sensor chips. The extracted K_D is 1.4 pM Thus, binding site model illustrated for detection of troponin I in purified buffer and clinical serum samples are in good agreement. The standard curve in Fig. 3(b) is used to estimate the unknown troponin I concentration in serum to be 2.62 pg/mL, which is lower than the LOD for almost all the existing commercial assays (Tate et al., 2012; Amundson Beret and Apple Fred, 2015). This emphasizes the ability of the sensor to detect low concentrations of Troponin I which suggests variety of clinical applications and not limited to early detection of AMI such as cardiotoxicity, unstable angina, cardiac muscle injury and renal failure analysis (Christenson and Azzazy, 2006; Kanderian and Francis, 2006).

3.4. Troponin I detection using aptamer based sensor

The characteristics of Troponin I specific aptamer based sensor is studied and reported in Fig. 4. The aptamer development and selection and affinity is studied previously (Jo et al., 2015) and we have chosen aptamer sequence 5′- CGCA TGCC AAAC GTTG CCTC ATAG TTCC CTCC CCGT GTCC –3′ as the receptor to bind with cardiac troponin I present in clinical serum samples from patients. Aptamer is ssDNA that can form secondary structure and bind to target proteins much like the lock and key mode of interaction observed in antibody-antigen binding. Therefore, our sensor can monitor the binding of aptamer to protein as it effectively changes the solution capacitance. Fig. 4(a) shows the immobilization of aptamer on HEMT chip. Fig. 4(b) displays the drain current versus time graph for aptamer-troponin I binding observed for varying concentrations from < 0.006 ng/mL (estimated concentration is 0.00262 ng/mL), 0.033 ng/mL, 0.146 ng/mL, 1.886 ng/mL, 33.7 ng/mL and 148 ng/mL. The sensitivity of sensor measurement can be enhanced and random noise can be removed by calculating the total charge accumulated on the device surface by integrating the drain current through time. The total charge versus time graph is shown in Fig. 4(c). As the concentration of target proteins increases, the current

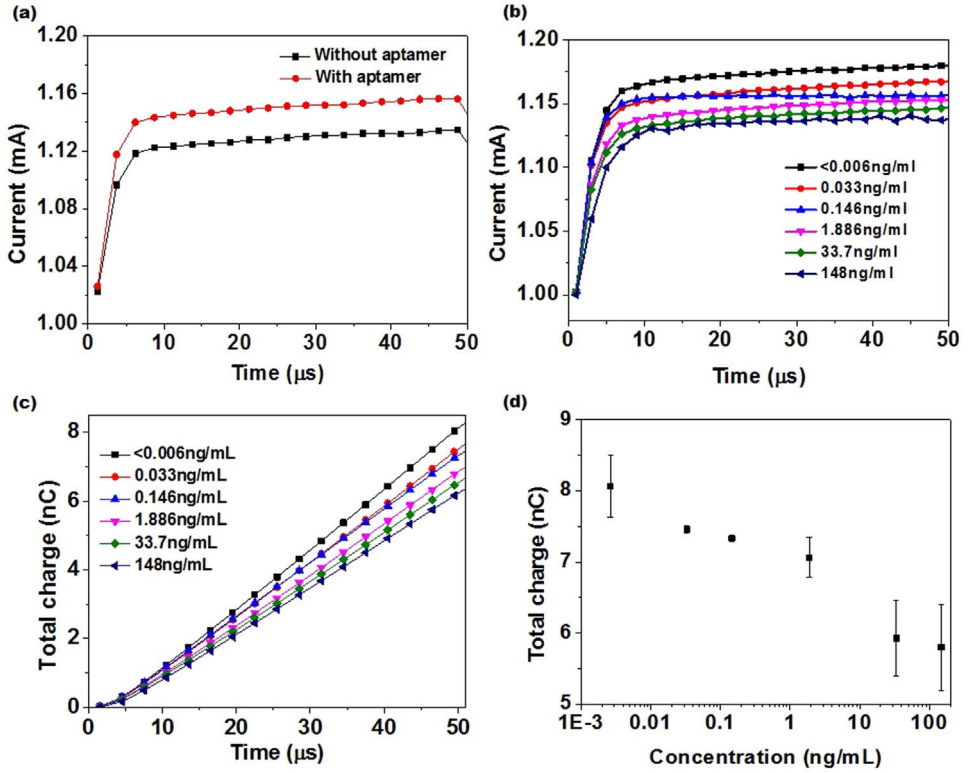

Fig. 4. (a) Sensor response with and without aptamer immobilized on gate electrode area. (b) Current versus time graph for the aptamer based detection of Troponin I in clinical human serum samples. (c) Total charge accumulated versus time graph for the aptamer based detection of Troponin I in clinical human serum samples. (d) Aptamer based sensor calibration curve for the detection of Troponin I in clinical human serum samples.

gain and hence total charge decreases. Thus total charge can be used as sensor index to generate a calibration curve depicting good sensitivity for aptamer based Troponin I detection in clinical serum samples from different patients (shown in Fig. 4(d)), proving the utility of aptamers as potential candidates for receptors in place of diagnostic antibodies. By comparing the antibody and aptamer based sensors (Figs. 2 and 4) we can see that both type of sensors can achieve similar detection limits, based on experimental observation. Aptamer based sensors showed a wider dynamic range which is relevant to the current clinical concentration range of cTnI. Both antibody and aptamer based sensors using EDL gated HEMT demonstrate high sensitivity due to the high field operation of EDL gated HEMT sensors. Thus, both type of sensors have the potential to be used for high sensitivity cTnI assays and therefore can be considered as strong candidates for clinical and home-care diagnostics.

3.5. Portable biosensor system for high sensitivity cTnI assay

The primary focus of this research is to develop a high sensitivity assay intended for personal healthcare and point of care diagnostics. The increased device scalability of semiconductor based miniaturized sensors and stable and mature semiconductor fabrication processes provide opportunities to integrate semiconductor based biosensors to portable or mobile diagnostics. AlGaN/GaN HEMT chip fabricated on a 1 mm × 1 mm die area is shown in Fig. 5(a). The chip is embedded in a thermocurable epoxy resin and metal interconnects are laid to connect the chip to a commercial microSD card reader (shown in Fig. 5(a)). The reader connected to the chip is mounted on a portable electrical measurement unit, shown in Fig. 5(b), which is interfaced with laptop to display the test results. The simple self-actuated microfluidics working on capillary action can flow the sample into the transistor sensing region instantly. The sample volume required is as less as 1.5 µl. After sample incubation for 5 min, electrical measurements are carried out by applying drain and gate voltages using the interfacing software in the laptop. Quantitative results obtained for testing clinical serum samples from different patients using the prototype portable biosensor unit are depicted in Fig. 5(c) and (d). The figures show calibration curves of cardiac troponin I detection, obtained by testing two different sensor chips. The change in current gain shows high sensitivity in clinical sera and is in good agreement with the sensor performance depicted in Figs. 2 and 3. As explained in Fig. 2(d) and (e), there is no need of additional washing or blocking steps which greatly simplifies the assay protocols for the end user and thereby eliminating the need for laboratory personnel to perform the tests. A comparison of existing commercialized CVD sensors and traditional FET based CVD sensors with our EDL gated HEMT CVD sensor is summarized in Table 1, to highlight the features of present work. These features enhance the desirability of our sensing technology as an ideal candidate for personal healthcare and disease management.

4. Conclusion

In this paper, we have discussed the design and development of a high sensitivity troponin I assay using AlGaN/GaN HEMT, demonstrated in physiological salt environment such as 1X PBS with 4% BSA and clinical human serum samples. The EDL FET design enhances the current gain of the sensor in high ionic strength solutions, resulting in increased sensitivity and specificity in detection of Troponin I, even in clinical serum samples. The calibration curves depict Troponin I concentration dependent current gain change, with ultra-low detection limit that equals and surpasses prevailing Troponin I detection techniques. This assay has a major clinical relevance because early detection of Troponin I can significantly alter the prognosis of AMI. Also ascertaining the concentration of Troponin I in cardiac muscle is now being investigated for other cardiovascular diseases. Due to the high sensitivity of the EDL FET design, assay turn-around time is reduced to just 5 min. The short sample incubation time and capacity to detect target proteins directly in high salt environment greatly minimizes the effects from non-specific background proteins, which is a major bottleneck in the existing clinical diagnostics. Apart from high sensitivity, dynamic range and stable operation, our GaN HEMT sensor has demonstrated within realm of possibility, great capabilities to be

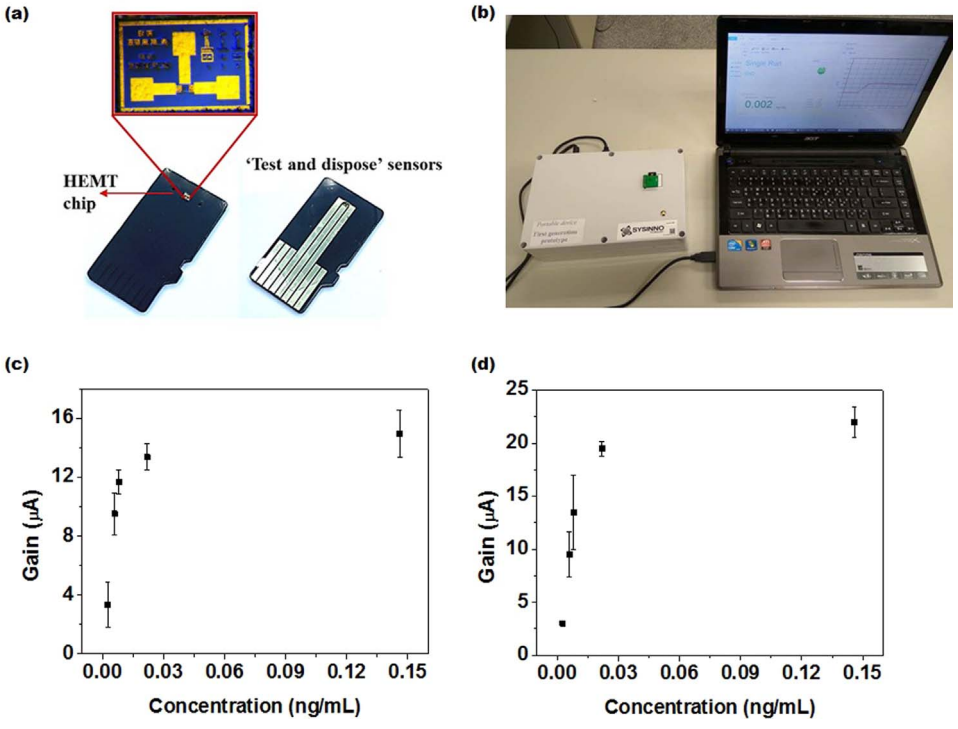

Fig. 5. (a) Miniaturized AlGaN/GaN HEMT sensor packaged in polymer based substrate. (b) Portable measurement unit interfaced with laptop to display the measurement results. (c) and (d) Current gain versus concentration graph for the detection of Troponin I in clinical human serum samples obtained using the portable biosensor system.

Table 1
Comparison of CVD sensor technologies (Tate et al., 2012; Zheng et al., 2005; Stern et al., 2010).

Sensor comparison	Commercialized CVD sensors	Traditional FET based CVD biosensors	EDL gated high field HEMT based CVD biosensor
Type	Optical based immuno-detection system (ex: ELISA, electrochemiluminiscence, turbidimetry)	Affinity type sensor; Ungated FET	Affinity type sensor; EDL gated high field FET
Sensitivity	Sensitive; Low detection limit	Poor sensitivity in physiological salt concentration	Highly sensitive in physiological salt concentration; Low detection limit
Response time; sample volume	10–20 mins; Large sample volume (2–3 mL)	5–20 mins; Varying sample volume requirements	5 mins; < 2uL sample volume
Labels	Needs labeling	Label-free	Label-free
Simplicity	Repeated washing steps required	Extensive sample pre-treatments needed (Dilution/desalting)	No wash or sample pre-treatments needed
Size/Mobility	Bench top detection system	Miniaturized sensors	Miniaturized sensors and systems for portable and mobile diagnostics
Work environment	Lab, Hospital, Diagnostic centres	Depends on the whole system	Anytime, Anywhere
Cost	Very expensive	Cost effective	Cost effective

used as a point-of-care or mobile diagnostic system.

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