Direct Label-Free Electrical Immunodetection of Transplant Rejection Protein Biomarker in Physiological Buffer Using Floating Gate AlGaN/GaN High Electron Mobility Transistors

Fahmida S. Tulip*, Edward Eteshola, Suchita Desai, Salwa Mostafa, Subramanian Roopa, Boyd Evans, and Syed Kamrul Islam

***Abstract*—Monokine induced by interferon gamma (MIG/CXCL9) is used as an immune biomarker for early monitoring of transplant or allograft rejection. This paper demonstrates a direct electrical, label-free detection method of recombinant human MIG with anti-MIG IgG molecules in physiologically relevant buffer environment. The sensor platform used is a biologically modified GaN-based high electron mobility transistor (HEMT) device. Biomolecular recognition capability was provided by using high affinity anti-MIG monoclonal antibody to form molecular affinity interface receptors on short N-hydroxysuccinimide-ester functionalized disulphide (DSP) self-assembled monolayers (SAMs) on the gold sensing gate of the HEMT device. A floating gate configuration has been adopted to eliminate the influences of external gate voltage. Preliminary test results with the proposed chemically treated GaN HEMT biosensor show that MIG can be detected for a wide range of concentration varying from 5 ng/mL to 500 ng/mL.**

***Index Terms*—AlGaN HEMT, immunoFET, interferon gamma, label-free, monokine, self-assembled monolayer (SAM).**

I. Introduction

ALTHOUGH organ and tissue transplantation is an increasingly important patient treatment option in a number of disease conditions (for example, kidney, liver, skin, heart, etc.), the rate of allograft rejection episodes is still quite high, occurring in approximately 20% of the recipients [1]. Thus, rejection of transplanted organs or tissues constitutes impedance to the successful long-term treatment of some of the disease conditions. Chemokines are a family of cytokines that have been shown to be crucial mediators of immune reactions leading to transplant or allograft rejection. Specifically, evidence from various studies have clearly shown that monokine induced by interferon-gamma (MIG/CXCL9) plays a central role among the chemokine proteins implicated in rejection of heart, skin and kidney tissue allografts. MIG, a member of the CXC subfamily of chemokines, is an inflammatory chemokine that plays an important role in cell activation and is produced during T-cell-mediated responses to inflammation including allograft rejection episodes [2], [3]. Consequently, MIG is investigated as a target immune response biomarker in transplant applications [2], [4], [5]. The range of MIG concentration in normal and pathophysiological disease states (including allograft rejection episodes) in humans varies from $\approx 0.2 - 3$ ng/mL and 10–400 ng/mL, respectively [6]. Due to the important role of MIG as a biomarker in transplant rejection, there is a great need to develop biosensor technology platforms to sensitively detect MIG for early monitoring of possible transplant rejection. This paper reports preliminary results towards development of biochemically modified field-effect transistor (BioFET) sensor platform aimed for minimally invasive or noninvasive detection of MIG as an early warning system in transplant rejection.

Micro- and nano-electronic biosensors represent a rapidly growing research area due to the ability of these sensors to combine high specificity of biological affinity reagents for label-free and direct electrical detection of charged biomolecules, excellent sensitivity, small dimensions, rapid response, as well as simple detection schemes and possibilities for integration into multi-array biosensor configurations [7], [8]. Label-free detection technique eliminates the need for treating the biomolecules with various labels or reagents (radiochemical, enzymatic, fluorescent) which renders the detection method expensive and complex to implement. One promising approach for the direct label-free and electrical detection of biomolecular interaction is to use field-effect transistors (FETs) because the conductance or the surface potential of a micro- or a nano-sized sensing channel changes upon binding of small quantity of charged biomolecules (e.g., antigens) to complementary receptors (e.g., antibodies) grafted onto the channel surface (i.e., direct electronic readout of biomolecular interactions). A number of different types of semiconductor nanostructures have been employed for FET-based biosensors for detection of biological analytes [9]. For example, silicon-based field-effect transistors (FETs) which are very attractive because of their well known properties and compatibility with the well

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established silicon microelectronic fabrication methods have been greatly explored for this purpose. However, the chemical instability of silicon in aqueous media limits its utility for such bioelectronic devices [10]. In recent years, AlGaN/GaN high electron mobility transistor (HEMT) devices have been the focus of increasing interest as biologically modified field-effect transistor (BioFET) based micro- and nano-electronic sensor platforms. These devices excel over their silicon counterparts because of their inherent properties such as chemically stable bulk and surface properties and the availability of high-density two-dimensional electron gas (2DEG) at the hetero-interface which allows a highly sensitive detection of the surface-charge related phenomena. In addition, as a piezoelectric material, the conductivity of GaN or AlGaN changes upon a change of the surface charge such as that due to binding of antigen onto an antibody-functionalized surface [11]. These very favorable material attributes significantly facilitate amplification of the current change pertinent to the change in the gate potential due to the proximity of the immobilized biomolecules and/or biomolecular binding events between the charged biological molecules (e.g., antigen-antibody or receptor-analyte binding events) at the high charge density sensing channel or the gate surface [12], [13].

Kang *et al.* demonstrated a method of detecting protein immobilized on ungated AlGaN/GaN HEMT devices treated with aminopropyl silane, although specific binding was not confirmed [14]. Eteshola *et al.* showed that streptavidin binding via biotinylated SAM layer results in a strong interface which can withstand environmental impacts but is associated with increased distance from the sensing channel [15]. Gupta *et al.* demonstrated an AlGaN/GaN insulated gate HEMT-based biosensor which can detect biotinylated MIG bound to the gate surface via the already well-known and generally used streptavidin-biotin interaction [16]. The preliminary results of a floating gate AlGaN/GaN HEMT device configuration using a unique interface binding chemistry have been reported by our group [17]. High affinity anti-MIG was used as the specific binding agent to detect MIG in immunoFET platform for the first time [18]. Later, Casal *et al.* demonstrated an immunoFET based on similar binding technology [19]. But apart from the binding agent, the proposed sensor is different from the sensor proposed by Casal *et al.* in terms of the surface chemistry employed to prepare the sensor, the type of device used as well as the operating region of the sensor. While Casal *et al.* uses triethoxy silane (TEA) and aminopropyl triethoxysilanes (APTES) for building the interfacial layers for tethering the biorecognition elements (streptavidin and polyclonal antibody molecules), the sensor platform reported here uses dithiobis (succinimidyl) propionate (DSP) chemistry for modifying the sensing channel surface of a floating gate HEMT FET for the subsequent covalent immobilization of high affinity monoclonal anti-MIG molecules. DSP material is more appropriate for research and development of FET-type biosensor compared to TEA and APTES materials. The bisymmetrical DSP used in our work to form the reactive SAMs for the functional immobilization of monoclonal anti-MIG eliminates chemical activation steps compared to conventional methods, and the short height of the SAMs (spacer arm length of 12.0 Å or 8 atoms) are more

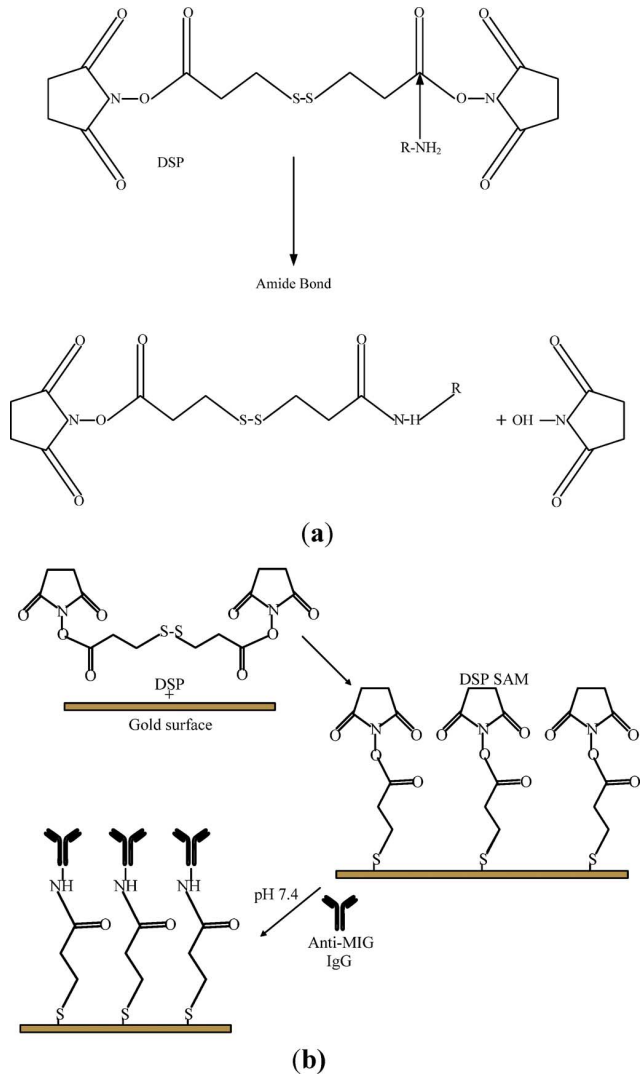

Fig. 1. (a) Schematic illustrating the reaction mechanism between DSP and primary amine groups of protein to form amide bond. (b) Formation of reactive DSP SAM on HEMT gold gate for covalent immobilization of anti-MIG monoclonal IgG.

advantageous for FET sensing architectures. The close proximity of short DSP SAMs from the electrode surface on which the molecules are immobilized has been utilized previously by other investigators to obtain direct electron transfer from redox copper centers of proteins. In addition, the proposed sensor employs a Schottkey gate HEMT which operates in the saturation region as opposed to the sub-threshold region operation of the insulated gate HEMT as demonstrated by Casal *et al.* While the sub-threshold region operation is suitable for low-power applications where power is a constraint, the saturation region operation inherently results in a larger change of current compared to the sub-threshold region operation, which corresponds to higher sensitivity of the sensors.

From the standpoint of a HEMT based biosensor platform MIG is an attractive analyte. The human CXCL9 cDNA encodes a 125 amino acid residue precursor protein with a 22 amino acid residue signal peptide that is cleaved to yield a 103 amino acid residue mature protein, with a molecular weight of ∼11.7 kDa. MIG contains multiple basic and acidic residues and is highly charged (carries ∼18 cationic or positively charged groups) at

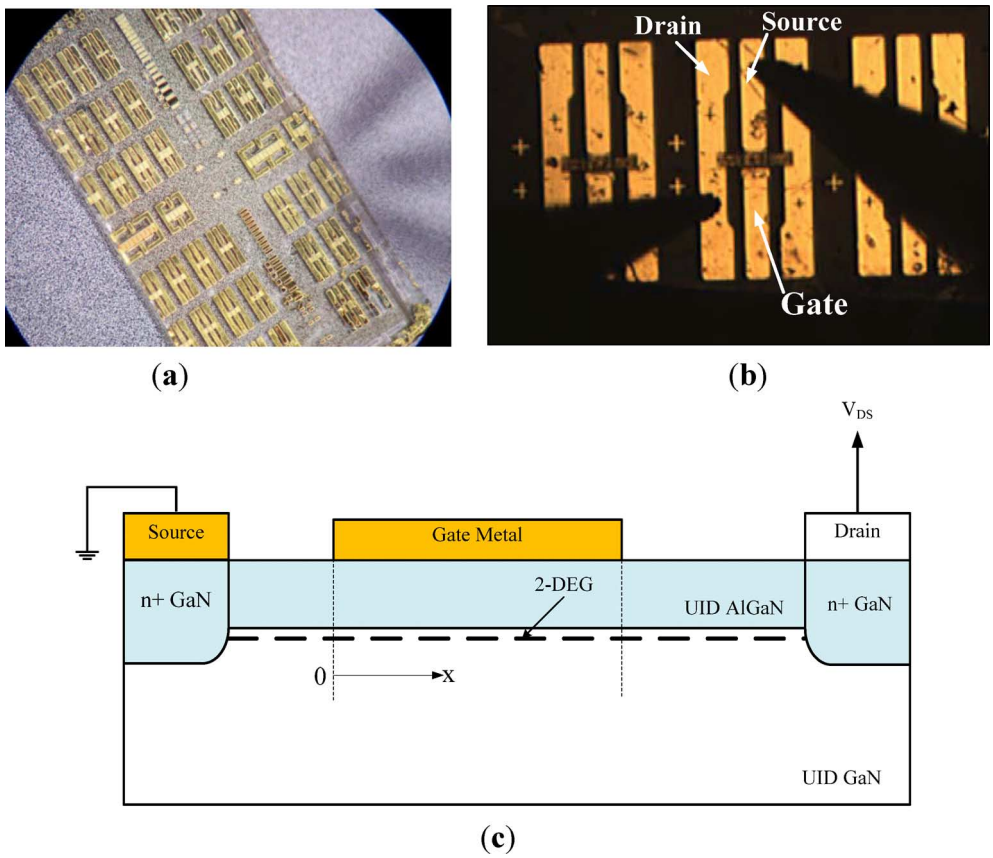

Fig. 2. (a) Photograph of the HEMT devices used for experimentation. (b) Device under test configured in floating gate configuration. (c) Cross-sectional view of a single device.

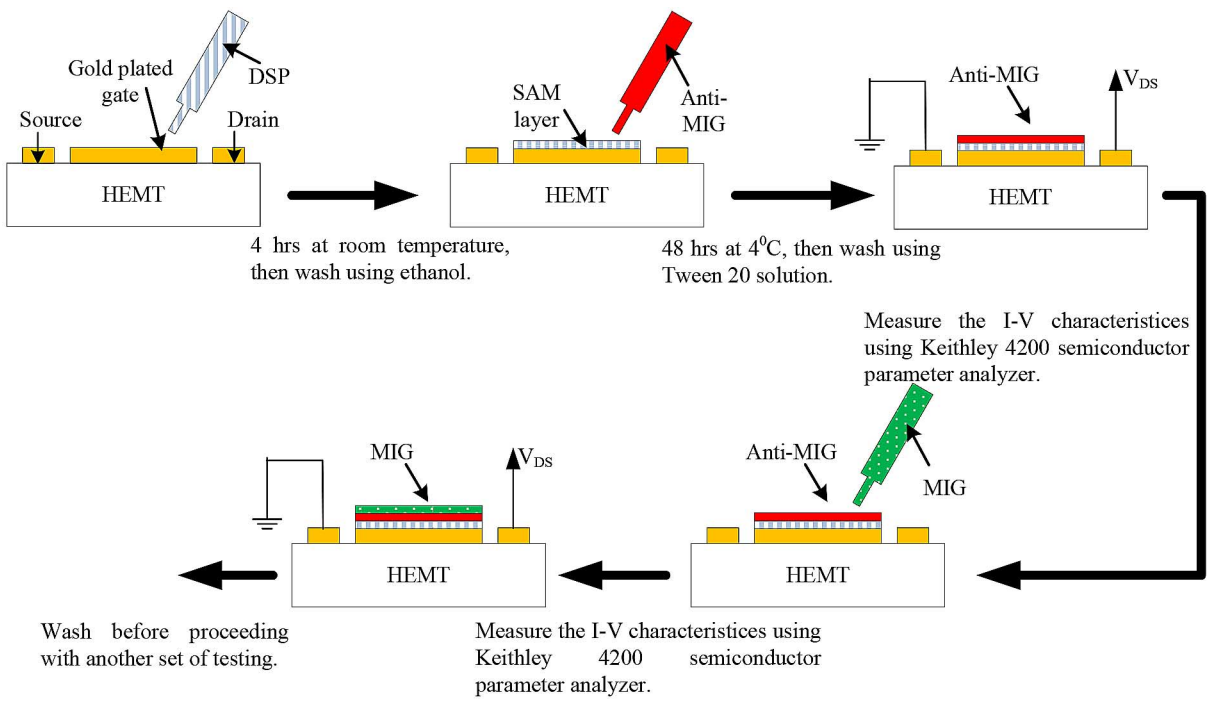

Fig. 3. Step by step procedure for testing using GaN/AlGaN biosensor.

neutral pH [20] making it highly adaptable to a HEMT based biosensing platform.

The sensing gate channel of the HEMTs has gold contact deposition which is suitable for creating self-assembled monolayers (SAMs) to immobilize the biomolecules. The self-assembly method for anchoring of the biorecognition molecules have a number of advantages relative to other approaches, namely, high reproducibility, molecular level control, as well as proximity to the surface thereby allowing direct electron/charge transfer [21]. Dithiobis (succinimidyl) propionate or DSP is

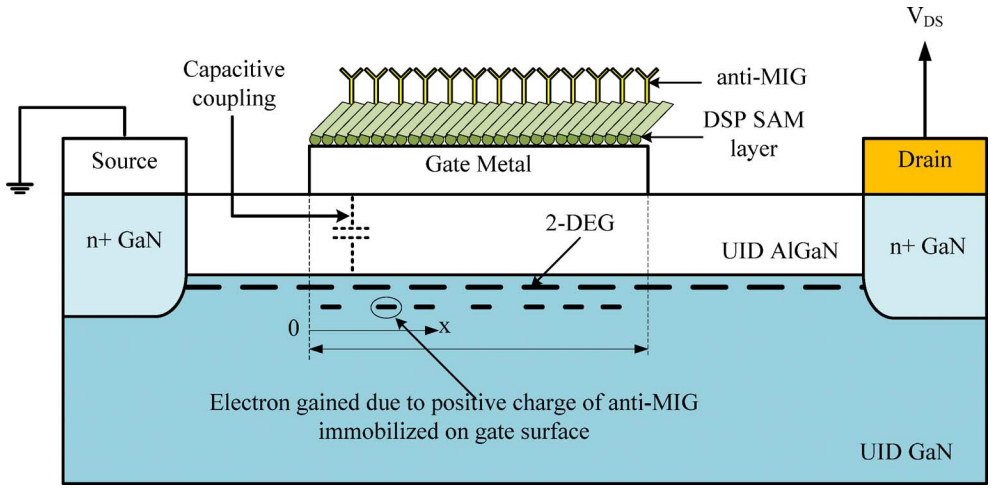

Fig. 4. Binding of anti-MIG IgG molecules with the SAM layer. The drain current increases due to the positive polarity of the anti-MIG molecules adjacent to the gate surface.

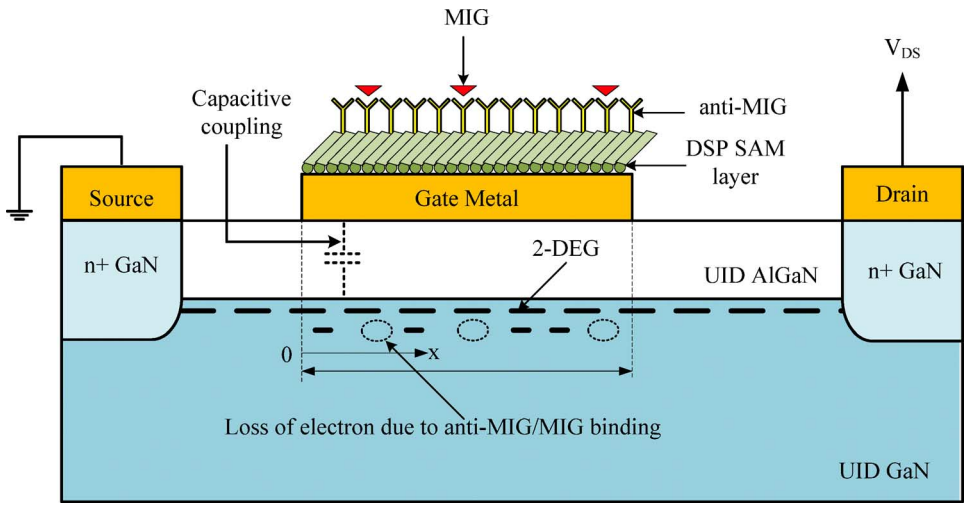

Fig. 5. Reduction in drain current after the device was exposed to MIG.

a water insoluble, homobifunctional N-hydroxysuccinimide (NHS) ester that can form a self-assembled monolayer on a gold surface due to its disulphide group [22]. The NHS group has primary amines as principle targets and as a result it has found applications for the immobilization of biomolecular species (proteins, etc.) containing primary amines to gold surface [23]. The bisymmetrical DSP links to ε-amine groups on the α-amine position of N-terminal amino acids. The succinimidyl ester of DSP is an ideal leaving group to electron donating primary amines. Attack occurs at the electron deficient carbon of the ester on DSP by a primary amine to create an amide bond in suitable pH range for the covalent immobilization of the protein on the gold surface. The great advantage of building DSP SAMs in comparison with conventional methods [24], [25] is the elimination of the chemical activation step in the covalent attachment of biomolecules containing primary amines to create an amide bond as shown in Fig. 1.

In this work DSP was used for the preparation of reactive SAMs for the functional immobilization of high affinity anti-MIG monoclonal IgG molecules on the surface of the HEMT sensing channel or the gate. The vicinity of short DSP SAMs from the electrode surface has been used previously to obtain direct electron transfer from redox copper centers [26]. It has been reported that DSP has spacer arm length of 12.0 Å or 8 atoms [27]. The short height of the SAM and the elimination of additional layers of chemical activating groups are advantageous for FET sensing architectures where proximity of the binding antibody-antigen pair to the sensing gate surface is crucial for sensitive detection of the binding event.

II. Experimental Section

A. Devices and Materials

A photograph of the devices with varying gate widths and lengths used for testing is shown in Fig. 2(a). The widths of devices vary from 50 μm to 150 μm with an increment of 50 μm, while the gate lengths vary from 4 μm to 12 μm with an increment of 2 μm. The test results presented here were achieved by using a device having gate dimensions of 100 μm $\times$ 8 μm which is shown with the test setup in Fig. 2(b). The cross-sectional view of a single device is shown in Fig. 2(c). The HEMT structure consists of a 20 nm undoped AlGaN layer on top of a 2 μm undoped GaN layer. Before performing each set of experiments, the current-voltage (I-V) characteristics of the devices were measured to properly ensure their functionality.

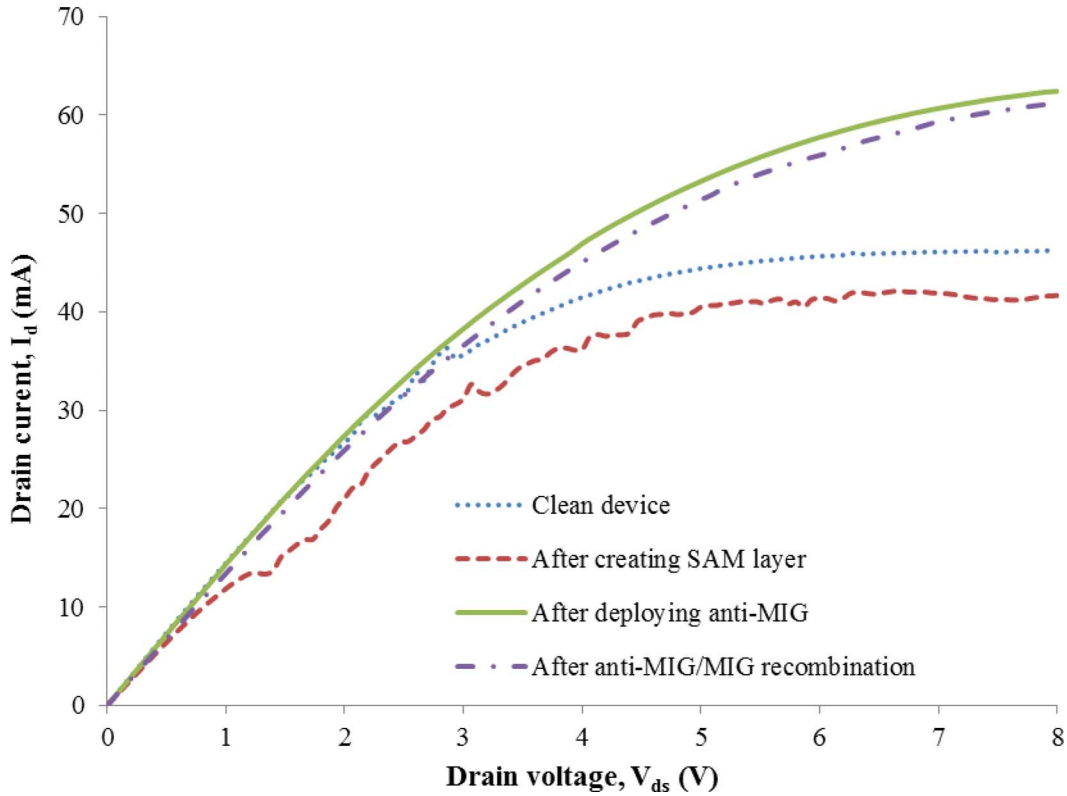

Fig. 6. Detection of MIG by the change in current due to anti-MIG/MIG recombination.

The chemicals used for testing purposes are DSP (1 mM solution in acetone), Phosphate Buffer Saline or PBS (150 mM solution, pH 7.4), anti-MIG (solution in PBS, concentration 2.5 μg/mL) and human MIG (of varying concentrations). Besides, acetone, absolute ethanol, DI water and Tween 20 solution (0.1% v/v) were used for cleaning purposes. DSP (Lomant's reagent, 404.42 g/mole) and PBS (Dulbecco's Phosphate Buffer Saline 1x) were purchased from Pierce Protein Biology Products and Invitrogen, respectively. Acetone (A. C. S., spectrophotometric grade, 99.5%), and ethanol (absolute, 200 proof, $\geq$99.5%, A. C. S. reagent) were purchased from Sigma-Aldrich. Tween 20 solution was purchased from Hyclone Laboratories (Logan, Utah). Recombinant human MIG (CXCL9) was purchased from PeproTech (Rocky Hills, NJ, USA) and Mouse anti-MIG (recombinant human MIG) monoclonal antibody was purchased from BD Biosciences (San Jose, CA, USA).

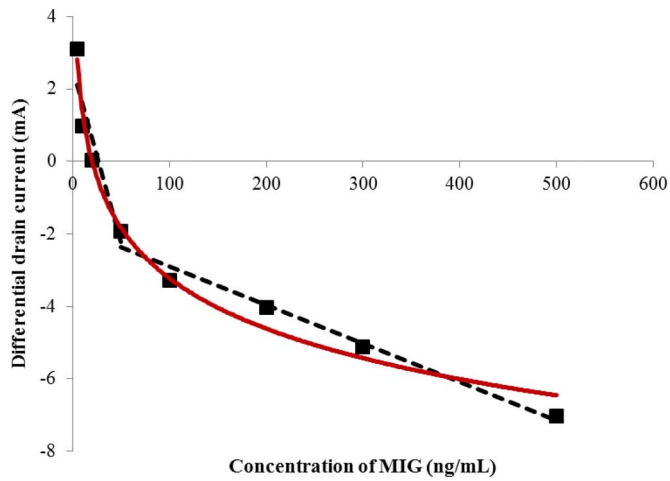

Fig. 7. Decrease in differential drain current with increase in MIG concentration. V_{ds} is kept fixed at 2 V.

B. Preparation of DSP SAM and Interface Receptors on HEMT Gold Gate Surface for Human MIG Detection

Prior to any experimental work, the device was properly cleaned with acetone and ethanol and dried in nitrogen flow. The devices were characterized using a Keithley 4200 semiconductor parameter analyzer, and then the drain current in the floating gate configuration was measured as illustrated in Fig. 2(b). The drain voltage was varied from 0–5 volts, and the gate terminal was left open. To create the SAM layer, the gold gate region was exposed to 1 mM solution of DSP for two hours at room temperature. The device was then washed again using acetone to remove the weakly absorbed DSP. The reactive property of the created SAM layer was exploited to covalently bind the anti-MIG monoclonal IgG molecules through its primary amine groups. To achieve immobilization, the gate was exposed to 2 μL of anti-MIG 1gG in phosphate buffered saline (PBS). To achieve maximum immobilization, anti-MIG solution was pipetted at the gate and the sensor was stored at 4 °C for 48 hours. After 48 hours, the weakly bound anti-MIG was washed away by rinsing the device with 0.1% v/v Tween 20 solution in PBS. The low concentration Tween 20 solution also helps prevent the non-specific binding of the proteins in enzyme immunoassay [28]. After the devices were air-dried, the drain current after the immobilization of the anti-MIG was measured in the floating gate configuration using the Keithley 4200 semiconductor parameter analyzer. The

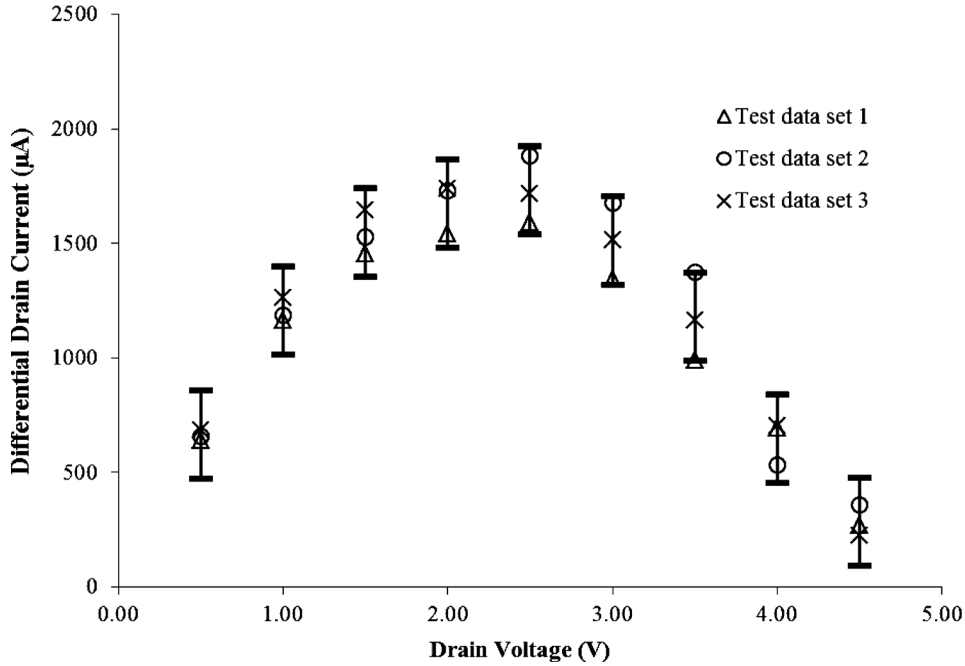

Fig. 8. Standard error bars demonstrating the reproducibility of the results. The concentration of MIG was maintained at 5 ng/mL.

resulting molecular affinity of the interface IgG receptors are then used for binding and detection of the MIG. To accomplish this, the biofunctionalized gate was exposed to 1 μL of human MIG in PBS (150 mM) and the change in the drain current due to the anti-MIG/MIG binding interaction was measured in a manner similar to the measurement scheme employed when anti-MIG molecule was immobilized. The step by step testing procedure is elaborated in Fig. 3.

III. Results and Discussion

A. Detection of MIG

After the formation of the SAM layer, the device current was observed to be slightly lower compared to that of the clean device. This slight decrease in current may be due to the change in the gate surface after the SAM layer was created. However, exposure to anti-MIG IgG changed the drain current significantly. The anti-MIG molecules immobilized on the gate surface of the device impart specific biorecognition properties to the HEMT BioFET sensor. It has been reported in the scientific literature that antigens elicit antibodies of opposite charge and that the antigen-binding sites dominate the interface properties of IgG antibodies [29], [30]. Therefore, it is assumed that the positive polarity of the anti-MIG IgG molecules lies adjacent to the gate surface as shown in Fig. 4. The positive charge end of the anti-MIG which is closer to the gate surface attracts electrons in the buried HEMT channel via capacitive coupling, resulting in the increase in the drain current.

The recombinant human MIG exposed to the device sensing gate binds specifically with the previously immobilized anti-MIG IgG molecules. The corresponding changes in the net charge of the MIG/anti-MIG complex modulate the electrical properties of the HEMT FET sensing channel. These results in loss of the electron gained in the previous anti-MIG immobilization and cause a decrease in the drain current. This is schematically shown in Fig. 5.

The experimental results shown in Fig. 6 confirm the sensitive detection of MIG by the anti-MIG monoclonal antibody. The intrinsic specificity and high affinity of this anti-MIG monoclonal IgG for MIG have previously been demonstrated through surface plasmon resonance (SPR) measurements (results not shown here). On the basis of the SPR results, it is anticipated that there would be a reduced chance of non-specific binding in the *in vitro* PBS environment in which this work was carried out.

The strong polarization field in AlGaN material responds strongly to the charges of the biomolecules immobilized on the gate surface [31]. In addition, the HEMT device used in this work is a depletion mode transistor with inherent channel which can be modulated by the charges present on the gate without the application of any external voltage. The device was configured as a floating gate transistor, with the gate terminal kept open. Elimination of the supply voltage at the gate terminal ensures that the change in the drain current represents purely the effect of anti-MIG IgG immobilization and biomolecular MIG/anti-MIG binding events, and not the effect of the applied gate voltage.

B. Effect of Varying MIG Concentration

Experiments were carried out using various concentrations of MIG, ranging from 5 ng/mL to 500 ng/mL. It was observed that at low concentrations range the increasing MIG concentration decreased the differential current due to anti-MIG/MIG recombination. As the anti-MIG concentration was held constant, increasing MIG concentration results in a gradual increase in the drain current after immobilization due to the presence of the positive charge on the MIG. As a result, the differential current before and after the immobilization of MIG is gradually

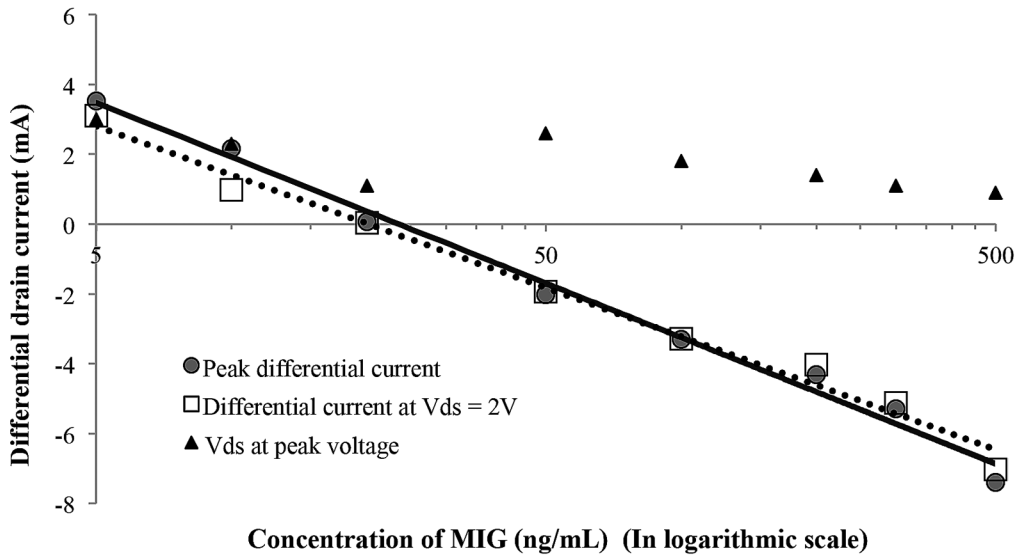

Fig. 9. Variation in V_{ds} at which peak differential current occurs with the increase in MIG concentration. The x axis is shown in logarithmic scale for better comprehension.

decreased as shown in Figs. 6. 7 represents the reproducibility of the result with three sets of test data at 5 ng/mL concentration of MIG that fall within the standard error bars.

As MIG concentration was further increased, the positively charged MIG dominates the net charge at the gate. Due to the dominating positive gate charges, electrons are attracted to the channel resulting in the increase in the drain current. Eventually the current after immobilization of the MIG becomes greater than the current prior to the immobilization. As a result, the differential current increases in the negative direction, which is confirmed by Fig. 8.

It was also observed that at increased MIG concentration, the voltage at which the differential current reaches its peak value had decreased. Increased MIG concentrations means increased net charge at the gate. This is reflected as larger effective voltage at the gate which helps the device to reach saturation at a lower drain voltage. This phenomenon is illustrated in Fig. 9.

IV. Concluding Remarks

The detection of human MIG is vital to organ and tissue transplant since the concentration of MIG can be a useful biomarker for monitoring allograft or transplant rejection. As a result the detection of human MIG in a minimally invasive or noninvasive fashion will be critical for early monitoring and detection of transplant rejection events. The sensitive detection of human MIG as reported here using specific and high affinity anti-MIG monoclonal IgG immobilized via DSP chemistry on the floating gate surface of the highly sensitive AlGaN/GaN HEMT in physiological buffer concentration (PBS, 150 mM) is an important development towards achieving that goal. When fully developed, this platform will have potential applications in the sensitive monitoring of transplant rejection events in a minimally invasive or noninvasive fashion and can be extended to other biomedical applications where detection of MIG or other biomedically charged proteins/carbohydrate biomatters is important.

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