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Effect of Al₂O₃ Passive Layer on Stability and Doping of MoS₂ Field-Effect Transistor (FET) Biosensors

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Abstract: Molybdenum disulfide (MoS₂) features a band gap of 1.3 eV (indirect) to 1.9 eV (direct). This tunable band gap renders MoS₂ a suitable conducting channel for field-effect transistors (FETs). In addition, the highly sensitive surface potential in MoS₂ layers allows the feasibility of FET applications in biosensors, where direct immobilization and detection of biological molecules are conducted in wet conditions. In this work, we report, for the first time, the degradation of chemical vapor deposition (CVD) grown MoS₂ FET-based sensors in the presence of phosphate buffer and water, which caused false positive response in detection. We conclude the degradation was originated by physical delamination of MoS2 thin films from the SiO2 substrate. The problem was alleviated by coating the sensors with a 30 nm thick aluminum oxide (Al₂O₃) layer using atomic layer deposition technique (ALD). This passive oxide thin film not only acted as a protecting layer against the device degradation but also induced a strong n-doping onto MoS₂, which permitted a facile method of detection in MoS₂ FET-based sensors using a low-power mode chemiresistive I-V measurement at zero gate voltage ($V_{gate} = 0$ V). Additionally, the oxide layer provided available sites for facile functionalization with bioreceptors. As immunoreaction plays a key role in clinical diagnosis and environmental analysis, our work presented a promising application using such enhanced Al₂O₃-coated MoS₂ chemiresistive biosensors for detection of HIgG with high sensitivity and selectivity. The biosensor was successfully applied to detect HIgG in artificial urine, a complex matrix containing organics and salts.

Keywords: MoS₂; metal oxide; biosensor; chemiresistive; field-effect transistor; chemiresistor

1. Introduction

Due to a high surface-area-to-volume ratio and up to centimeter-scale lateral dimension, 2D materials are ideal for integration with current nanofabrication technologies and highly promising transduction materials in sensor technologies that rely primarily on surface interaction [1]. A prominent example of functional 2D nanomaterials is graphene, which was the first 2D material isolated from its bulk structure in 2004 and has been extensively investigated. Despite its extraordinary properties, such as high optical transparency, thermal conductivity up to 3000 W m⁻¹ K⁻¹, high charge carrier concentration, and mobility [2], the lack of an energy gap between the conduction and valence bands limits its application in electronic devices. Attempts to address this deficiency have led to several approaches to engineer a bandgap in graphene. However, most of the successful

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Copyright: © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). methods require lengthy and complicated processes of fabrication using e-beam lithography or unstable doping treatment with harsh chemicals [3,4]. These intensive processes often lead to a significant decrease in carrier mobility and an increase in the number of defects in graphene. This challenge has encouraged researchers to find alternative 2D materials, and transition metal dichalcogenides (TMDCs) have become an emerging field of research. Unlike graphene, TMDCs possess a direct bandgap in the range of 0.2 to 3 eV in their single-layer (SL) structure [5]. This intrinsic band gap suggests that TMDCs are suitable for electronic/transistor applications. Molybdenum disulfide (MoS2) is one of the most studied TMDC compounds. It consists of one layer of molybdenum atoms in between two layers of sulfur atoms. SL-MoS₂ exhibits a direct bandgap of 1.87 eV, which transitions to an indirect bandgap as the number of layers increase. Therefore, a fieldeffect transistor (FET) using SL-MoS₂ as a semiconducting channel is expected to have a high on/off current ratio of up to 10⁸ and excellent subthreshold swing of 74 mV/decade [6]. In addition, due to a planar structure confined to a few angstroms thickness, MoS_2 is ultrasensitive to chemical and electrostatic perturbations at the surface. These advantages make MoS₂ a great choice for transduction or sensing material in FET-based biosensors.

In the field of biosensor research, label-free affinity-based biosensing is a desirable attribute for biosensor systems due to its simplicity. Amongst the different transducers used for label-free affinity biosensors, FETs and chemiresistors based on 1D and 2D nanomaterials are receiving a great deal of attention because of their high sensitivity, rapid response, low power consumption, viability for miniaturization, scalability, and integration on a chip. While methods of electrical detection either with an applied gate (FET characteristic measurement) or without an applied gate (chemiresistive measurement) have been demonstrated for biosensors using semi-metallic graphene, semiconducting graphene derivatives and carbon nanotubes [7–10], MoS₂-based electrical biosensors heavily rely on FET techniques, in which a source-drain current (Ids) is measured at a constant bias while varying the applied gate voltage to modulate the Ids. This mandatory gate voltage serves as an amplifier of the small current by enhancing the carrier mobility, originated by the nature of low charge mobility in semiconducting MoS₂ and high Schottky barrier at the interface of MoS₂/metal contact [11,12]. In addition, because the applied back-gating effect plays a key role in FET-based measurement, it requires a reliable and direct physical contact between MoS₂ (the conducting channel) and the dielectric substrate. Nonuniform and unstable contact at the materials interface can result in unreliable electrical modulation via gating effects, further leading to degradation of signal integrity and reliability during sensing.

A necessary step in the fabrication of a label-free MoS₂-based biosensors is the immobilization of a biomolecular receptor to the surface of MoS₂ to achieve biospecific responses. This biofunctionalization step is achievable via covalent and non-covalent conjugation strategies, such as hydrophobic interaction, silanization, and sulfide/disulfide bonding [13–15]. However, these methods either employ weak van der Waals bonds or significantly rely on the number of defects in MoS₂, which mainly comprises sulfur vacancies, to create such attachments. As a result, MoS₂-based biosensors are commonly fabricated using MoS₂ synthesized via chemical/physical exfoliation or hydrothermal reaction. MoS₂ synthesized via these methods produce a great number of defects permitting the direct functionalization of biomolecular receptors. On the other hand, biosensing applications using MoS₂ grown by chemical vapor deposition (CVD) are relatively limited due to an inadequate number of the defect sites in CVD-grown MoS₂ (CVD-MoS₂) crystal lattice. Despite this hinderance, CVD-MoS₂ exhibits a higher crystallinity and is amenable to mass production. For these reasons, CVD-MoS₂ deserves more attention in research for biological and chemical sensing applications.

In this work, we report a systematic investigation to identify causes of signal instability of CVD-MoS₂ FET-based biosensors and develop a strategy to mitigate the effects of physical degradation of the device and enhance the overall device stability. Specifically, we observed that CVD-MoS₂-based biosensors degraded, indicated by dramatic change in their electrical outputs during mandatory incubation steps of the biofunctionalization process in 10 mM phosphate buffer (pH = 7.4) and deionized water (pH = 7). We conclude the observed continual shifts in device FET characteristics was attributed to physical delamination of MoS₂ film from Si/SiO₂ substrate, which decreased the gating effect and negated the usage of FET characteristic transfer curve as a mean for detection. This issue was mitigated by stabilizing the device with a 30 nm thick Al₂O₃ coating. In addition, the oxide layer provided available sites for antibody functionalization via surface chemical functionalization with (3-aminopropyl) triethoxysilane (APTES) and glutaraldehyde. Such sensors demonstrated a great performance to detect HIgG with high sensitivity and a limit of detection of 83 ng/mL.

2. Experimental Section

2.1. Materials and Agents

Si wafer with a 300 nm thermal grown oxide layer were purchased from Ultrasil (Hayward, CA, USA). Sulfur powder, molybdenum trioxide (MoO₃), and polystyrene (PS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monoclonal anti-human immunoglobulin G (HIgG) antibody, HIgG protein, bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All organic solvents, salts, and components of artificial urine were purchased from Fisher Scientific (Hampton, NH, USA).

2.2. Synthesis of MoS₂

MoS₂ was synthesized via CVD method with sulfur and MoO₃ as the precursors and Si/SiO₂ as the substrate. A total of 0.01 g of MoO₃ was centered in a ceramic boat with the Si/SiO₂ substrate positioned on top of the MoO₃ source. The growth took place at the center of a two-zone 1-in quartz tube furnace at 650 °C while the tube was saturated with sulfur vapor by heating 0.1 g of sulfur at 170 °C and using a nitrogen carrier gas at a flowrate of 50 sccm. The temperature was maintained at 650 °C for the complete reaction and the entire system was naturally cooled down to room temperature by removing the tube from the furnace.

2.3. Fabrication of FET Devices

First, 3% PS in toluene was spin-coated over the as-grown MoS₂ film, and the PS coated MoS₂ film was then baked at 60 °C for 1 min. The PS/MoS₂ film was isolated from the substrate via simple wet etching of Si/SiO₂ in 1 M KOH solution. The isolated film was washed several times with deionized (DI) water and transferred on a pre-cleaned Si/SiO₂ substrate. The film was dried in ambient air and annealed at 80 °C for 15 min to increase the contact between MoS₂ and Si/SiO₂ substrate. PS layer was removed in toluene at room temperature.

Buffered oxide etch (BOE) 6:1 (Sigma-Aldrich, St. Louis, MO, USA) was used for etching the back side of the wafer, and a 10 nm/100 nm of Cr/Au was deposited on the silicon side of the Si/SiO₂ as a back-gate. The source and drain terminals with a gap size of 10 μ m × 10 μ m were patterned using conventional photolithography followed by electron-beam evaporation of 10 nm/100 nm of Cr/Au.

2.4. Atomic Layer Deposition (ALD) of Aluminum Oxide Layer

Aluminum oxide layers were deposited using Cambridge Nanotech Savannah 100 from Veeco Instruments, Inc. (Plainview, NY, USA) at 250 °C. Trimethylaluminum (TMA) and water, as precursors, were pulsed in the system periodically for 0.03 s. The thickness of the oxide layer was varied by changing the number of cycles, estimated at about 1 Å/cycle. Aluminum oxide of 10 nm and 30 nm thickness were grown using 100 and 300 cycles.

All optical images were taken using a Hirox KH-7700 digital microscope from Seika Machinery, Inc. (Los Angeles, CA, USA). Al₂O₃ thickness was measured by Jobin Yvon UVISEL model M200 Ellipsometer from Horiba (Kyoto, Japan). Both Raman spectra and PL measurements were collected by Horiba LabRam HR (Kyoto, Japan) using a green laser (λ = 532 nm) and 100× objective (NA = 0.9). A minimal power of 5 mW was used to avoid local heating and possible damage to the materials. SEM images were taken by a Zeiss STEM Gemini 1540xb (Oberkochen, Germany).

FET characteristics curves were obtained by Keithley 2636 source meter from Tektronics (Beaverton, OR, USA). A constant $V_{ds} = 5$ V and $V_{ds} = 0.1$ V were applied while sweeping the back-gate voltage from 0 V to 40 V.

2.6. Biofunctionalization and Biosensing of HIgG

The Al₂O₃ passivated MoS₂-based device was immersed in 5 mL of 20% ammonium hydroxide for 30 min at room temperature (RT) ensuring that -OH groups were sufficiently activated on the surface and improving the formation of the homogeneous layer. The device was then carefully rinsed with DI water and dried with nitrogen gas. Subsequently, the device was incubated in 1 mL of APTES for 1 h and washed thoroughly with ethanol to form a self-assembled monolayer providing amino groups for covalent attachment of anti-human immunoglobulin G (HIgG) antibody, which interacted selectively with HIgG. APTES amino groups were, first, activated by placing the device in 4 mL of 25% glutaraldehyde in DI water for 2 h to form a Schiff base between -NH₂ of APTES and -CHO of glutaraldehyde at RT. This was followed by overnight incubation at 4 °C with 20 μ L of 50 μ g/mL anti-HIgG in phosphate buffer (PB) to form a Schiff base between the 2nd -CHO of glutaraldehyde and -NH₂ of anti-HIgG. Lastly, the device was reacted with 20 μ L of 10% ethanolamine to quench unreacted -CHO groups of glutaraldehyde.

The biofunctionalized devices were applied to detect HIgG antigen in both PB and AU solution. A 30 μ L customized PMMA cell was employed for the detection. A pair of inlet/outlet tubes was connected to the test cell and used to deliver a series of reagent fluids, including PB (10 mM, pH 7.4), AU (pH 7.4) solution, and analyte solutions of HIgG. A volume of 200 μ L of solution was injected into the sensor test cell for each run while the dynamic change in electrical resistance of the sensor was monitored continuously at an applied source-drain voltage of 1 V (with no gate voltage), using a Keithley source meter.

3. Results and Discussion

The optical image in Figure 1a (inset) shows the MoS₂ synthesized by CVD was a continuous film that remained a continuous film after the transfer. The similarity of color contrast across the film illustrates the growth of MoS₂ was uniform and the transfer was residue-free.

The film thickness and electrical bandgap of the synthesized MoS₂ were confirmed by different techniques such as Raman spectroscopy, photoluminescence (PL) under a green laser with spot size $\approx 1 \mu m$. The presence of two signature peaks of E_{2g} at 385 cm⁻¹ and A_{1g} at 404 cm⁻¹ in Figure 1a confirms the successful growth of MoS₂. In addition, the difference in wavenumber of 19 cm⁻¹ between the two peaks indicates the as-grown MoS₂ was single-layer [16]. Due to the intrinsic direct bandgap in SL MoS₂ structure, the photoluminescence spectrum in Figure 1b shows a significantly high peak at 1.87 eV.

A back-gated FET using CVD-MoS₂ as the semiconducting channel was prepared using conventional photolithography process. As shown in Figure 1c, the FET characteristics curve shows a typical n-type behavior with a high on/off current ratio of 10⁴ in ambient condition. Under the applied positive back-gate voltage, the device was turned on, i.e., dramatic increase in current due to the accumulation of electrons in MoS₂. The field-effect mobility was calculated to be 20.5 cm²V⁻¹s⁻¹, using the linear FET characteristics reported elsewhere [17]. This low charge mobility is mainly caused by charge trapping at the interface between the SiO₂ substrate and MoS₂ [18,19] and high contact resistance at metal/MoS₂ interface [11]. Because of such challenges, electrical measurement methods in



MoS₂-based devices are limited to a FET characteristics curve with applied gate voltage [13,20,21].

Figure 1. (a) Spectroscopic properties of CVD-MoS₂: Raman spectrum and optical image of MoS₂ device (inset). (b) Photoluminescence of CVD-MoS₂ showing a direct band gap of 1.87 eV. (c) Field-effect transistor characteristics curve using MoS₂ as the conducting channel (V_{ds} = 5 V).

Due to the significant role of the FET measurement in MoS₂-based sensors, the stability of as-fabricated FET devices becomes critical. A stability test was carried out initially in ambient air and later in both biocompatible media of phosphate buffer (PB, pH = 7.4) and DI water (pH = 7). As shown in Figure 2, the FET characteristics curve experienced a measurable decrease in current after 30 min in ambient air, which was due to the interaction between MoS₂ film and oxidizing gas molecules in air [22]. However, after 30 min and for an additional 90 min under the same condition, the FET characteristics curve remained unchanged, which indicates physical and chemical pseudo-steady-state in the device.



Figure 2. Stability of MoS2 device in ambient air.

On the other hand, upon incubation in PB for 30 min by drop casting of 20 μ L of PB onto the sensing area, the device showed a significant decrease in overall Ids current across the entire range of applied gate voltages as seen in Figure 3a. In fact, the current at the gate voltage of 40 V dropped by 33% after 30 min of incubation, as compared to further 1.6–5% drop after additional 90 min. Similar deterioration in electrical behavior was observed when the device was incubated in DI water (Figure 3b). Thus, in considering the similar magnitudes of diminution of Ids current for the device in the ionic PB environment and deionized water environment, this suggests the presence of water molecules as the major contributing factor for the observed electrical behavior of the device, instead of the buffer salt ions. Three possible mechanisms for how water could diminish the performance of a FET MoS₂-based device were considered:

- 1. Water altered the chemical properties of MoS₂ during the incubation via -O or -OH bonding;
- 2. Water intercalated between gold electrodes and MoS₂ and increased the contact resistance;
- 3. Water intercalated between MoS₂ and the substrate and decreased the gating effect on the MoS₂ channel.



Figure 3. FET characteristics curve of MoS₂-based device in (**a**) PB (pH = 7.4) and (**b**) DI water (pH = 7).

Raman spectrometry was employed to examine the properties of MoS₂ after 30 min incubation in DI water [23]. As illustrated in Figure S1, the absolute intensity of the two signature peaks of MoS₂ remained unaffected after the incubation, indicating there was

no oxidation taking place. As a result, the mechanism (1) of water induced chemical effect on MoS₂ film was disregarded.

To determine if the phenomenon of water intercalation between MoS₂ and gold electrodes was occurring, controlled experiments were conducted where MoS2-electrode interfacial regions of the FET device were physically isolated (via passivation with photoresist) from the immediate buffer environment. A thin layer of positive photoresist S1813 was spin-coated on top of the device, and a small photoresist area of 10 μ m × 10 μ m at the contact between MoS₂ and gold electrodes was removed using photolithographic patterning. An optical image of such a schematic can be found in Figure S2a. This polymeric layer behaved as a hydrophobic layer that prevented water from intercalating between the gold contacts and MoS₂ but still allowed the possibility of water inserting between MoS₂ film and Si/SiO₂ substrate due to the open window of 10 μ m × 10 μ m. Figure S2b shows the resulting FET characteristic curves of pre-photoresist, post-photoresist, and after device incubation from 30 to 120 min in DI water. As a protecting layer, S1813 film minimized the interaction between MoS_2 and oxidizing gas molecules in the ambient, such as O_2 and water vapor, and thus increased the electrical outputs of FET devices as observed by the increase in the FET current. Similar FET transfer curves collected after 30, 60, 90 and 120 min indicates the device was more stable than the one without the photoresist but still suffered from the previously observed degradation. Therefore, this experiment series removed the possibility of mechanism (2) of the resistance increasing due to water intercalating between gold and MoS2 and confirmed mechanism (3), i.e., water intercalated between conducting channel and the Si/SiO₂ substrate causing the delamination of MoS₂ film. This resulting poor contact between MoS_2 and the dielectric SiO₂ layer decreased the gating effect onto the conducting channel in FET measurement, yielding the false positive response in detection. In fact, the delamination phenomenon is a commonly used method for isolating CVD-grown MoS² from its growth substrate. Due to the high hydrophobicity of the substrate and MoS₂, water is used as a medium for a clean, complete lift-off for MoS₂ as reported elsewhere [24,25].

Even though the as-synthesized MoS_2 appeared as a continuous film as shown in Figure 1a, it is believed that the film consisted of many single MoS₂ crystals with distinct grain boundaries. Therefore, further investigation on grain boundaries of the as-grown CVD-MoS₂ was conducted to understand in more depth the delamination phenomenon that resulted in the instability of MoS2-based device. Work function of a similar CVD-SL-MoS₂ was measured at 4.90 eV in our previous work [8] while the absolute potential of Au³⁺/Au is 5.64 eV. The difference in the work functions creates an intrinsic internal force driving electrons from n-type MoS₂ to Au³⁺ ions and reduced the ions to Au nanoparticles (NPs). This reduction happens at the reactive sites on MoS₂'s lattice, which concentrates at its edge and grain boundaries [26–28] and, hence, MoS₂ grain boundaries become evidently detectable due the presence of formation of gold NPs. Figure S3 shows scanning electron microscopic (SEM) images taken at the same location before and after an incubation of MoS₂ in 5.9 mM AuCl₃ solution for 10 min. The grain boundaries that were indistinguishable originally became evident due to the formation of Au NPs. This indicates the as-grown continuous MoS₂ film, in fact, consisted of many coalescing MoS₂ crystals. These existing large grain boundaries permitted water molecules to severely penetrate through MoS₂ film and delaminate it from the substrate.

This finding suggests that a physical passivation layer is required to minimize the intercalation of water molecules through MoS₂ grain boundaries leading to delamination of MoS₂ in aqueous solutions. Although a hydrophobic polymeric layer such as S1813 proved to alleviate the degradation of the FET device characteristics, its chemical inertness to surface chemical modification inhibits the subsequent biofunctionalization. On the other hand, thin metal oxide layers, such as hafnium oxide, silicon oxide, and aluminum oxide, are more commonly used as passive layers in logic electronics to protect the underneath material from being oxidized in the ambient [29,30]. In addition, the oxide layer, in presence of ambient and/or water vapor, produced sufficient hydroxyl groups making the

device's surface amenable for biofunctionalization. In this work, the aluminum oxide layer was deposited on the sensors using an ALD method [31]. This deposition process involved two steps, in which the first step provided hydroxyl seeding by casting water vapor on the device's surface while the second step was a chemical reaction between -OH groups and TMA to form the Al₂O₃ layer. The schematic of MoS₂-based FETs with a Al₂O₃ passive layer is shown in Figure S4. Figure S5 shows the different color contrast of a device before and after Al₂O₃ deposition confirming the successful deposition of an oxide layer on the device. The thickness of the Al₂O₃ layer on the SiO₂ substrate was measured at 30 nm by ellipsometer, which can be found in Figure S5c. The oxide layer's thickness was optimized by measuring the device's stability after incubation in DI water. Figure 4 demonstrates a device with a 10 nm thick Al₂O₃ layer suffered from the degradation. On the other hand, the delamination phenomenon subsided in the device coated with a 30 nm thick oxide layer, as proven by the stable FET transfer curve of the device after 120 min. It is important to highlight that a strong n-doping effect of Al₂O₃ onto MoS₂ was also noticed in the FET and drain current measurements, as seen in Figure 5 [32,33]. Due to its intrinsic semiconducting properties and relatively large work function, CVD-MoS₂ exhibits low charge mobility and the high contact resistance at MoS₂/gold interface. However, when the device was passivated by Al₂O₃ film, the insignificant current of 3×10^{-10} A at V_{gate} = 0 V was increased by three orders of magnitude to 3×10^{-7} A, while the field-effect charge mobilities increased by 26-fold, from 14 cm²V⁻¹s⁻¹ to 367 cm²V⁻¹s⁻¹ in the as-grown MoS₂ and Al₂O₃-MoS₂, respectively. Figure 5a indicates the threshold voltage (VTH) of the device also shifted to a more negative threshold voltage (VTH) indicating a strong n-doping effect in MoS₂ by Al₂O₃. This interesting observation renders two possible reasons, including the incomplete oxidation of Al₂O₃ and the passivation of sulfur vacancy defects. During the Al₂O₃ growth, the incomplete reaction between TMA and water vapor resulted in excess positive charged Aln+ ions. The residual positive charges in the oxide layer, hence, attracted additional electrons in the MoS₂ channel and promoted the n-doping effect. This led to the appreciable current found in the Al₂O₃ passivated device at $V_{gate} = 0$ V. On the other hand, as-grown CVD-MoS₂ exhibited a great number of sulfur vacancies at the boundaries and edges, as observed in the previous experiment with gold chloride solution. These vacancies created deep and localized states trapping electrons and, hence, impaired electron mobility in MoS₂ [34,35]. However, the presence of the Al₂O₃ layer allowed its oxygen atoms to fill sulfur vacancies and, in turn, minimized electrons trapping. Such a healing mechanism has been reported previously using other chemicals such as CO, NO, NO₂, and other sulfonate groups [35,36]. To the benefit of device electrical performance, this resulting increase in current and charge mobility provided additional electrical measurement method with less power consumption (no gate and lower Vds) and simpler fabrication and operation due to the elimination of the gate. Figure 5b highlights such an increase in drain current in the gateless FET device with an Al₂O₃ passive layer. At V_{gate} = 0 V, the I_{ds} current is as high as 9.2×10^{-7} A at V_{ds} = 1 V and 5.6×10^{-6} A at V_{ds} = 5 V (with Al₂O₃), as compared to 7.1×10^{-10} A and 1.8×10^{-7} A (without Al₂O₃), respectively.





Figure 4. FET characteristics curves of the sensor with (**a**) 10 nm and (**b**) 30 nm Al₂O₃ after incubation in DI water in different time ranges.

Figure 5. (a) FET characteristics curve and (b) drain current characteristics curve ($V_{gate} = 0$ V) of MoS₂-based device with and without passive Al₂O₃ layer.

As hydroxyl groups were available on the Al₂O₃ layers after ammonium hydroxide treatment, anti-HIgG was covalently functionalized on the device via surface chemical reactions using APTES and glutaraldehyde to construct a biosensor to detect HIgG in PB solution. The device was initially primed with PB solution for stabilization. Then, increasing concentrations of HIgG solution were injected into the cell while the resistance of the sensor was monitored for 15 min. The result in Figure 6a shows the time-dependent change in resistance. It was evident that a spike in the readings was due to the physical injection at each injection point and yet the increase in the reading after stabilization was indeed due to increasing amounts of HIgG. It showed a stabilized signal was achieved in an average of 2 min after each addition of the target solution. It is important to note that the oxide layer, due to the strong n-doping effect, permitted electrical measurement without requiring a back-gate potential that allowed a more facile and low-power mode measurement. The biosensor exhibited good sensitivity by showing clear responses to HIgG solution of concentrations down to 100 ng/mL, as well as high selectivity with non-response to PB solutions, 10⁵ ng/mL of BSA, and 10⁵ ng/mL of HSA solutions, showed in Figure 6b. The calibration curve plotted in Figure 6d shows a linear relationship (y =0.0529x - 0.0671; R² = 0.9945) between HIgG concentration and the percentage change in resistance within a wide dynamic range from 10² to 10⁶ ng/mL in PB solution. The sensitivity of 0.0529 per Log₁₀ (ng/mL) was obtained from the slope of the response ($\Delta R/R_0$) as a function of HIgG concentration, where R₀ is the resistance of the device established with PB solution in the priming process and ΔR is a resistance change caused by affinity binding of HIgG antigen-antibody interaction. From the noise data of the PB baseline in Figure 6a, root-mean-square value of $\Delta R/R_0$ noise of ~0.034 was obtained. The limit of detection (LOD), defined as 3 × SD/m (where SD is the standard deviation of the blank and m is the slope of calibration plot), was estimated to be 83 ng/mL. Additionally, the small error bars demonstrate good reproducibility, which assures a reliable consistency in detecting HIgG.



Figure 6. (a) Real-time monitoring of the changes in resistance for HIgG sensing and the priming process with 10 mM PB ($V_{ds} = 1 V$ and $V_{gate} = 0 V$). (b) Control sensing experiment with PB solution and high concentration nonspecific antigens of BSA (10⁵ ng/mL) and HSA (10⁵ ng/mL). (c) Real-time monitoring of the changes in resistance for HIgG sensing in AU with 10 mM PB wash ($V_{ds} = 1 V$ and $V_{gate} = 0 V$). (d) Calibration curves of Al₂O₃ passivated MoS₂-based sensor for HIgG detections in PB and AU solutions. Data points are average of 3 measurements and error bars represent ±1 SD.

A biosensor that can be used directly with physiological samples is much favored and very demanding. The sensor was further used to detect the HIgG in an artificial urine (AU) matrix (pH = 7.4), which simulated the real-life human body fluids sensing environment [37]. Since the AU has high ionic strength due to a lot of salts, it reduced the Debye length and thereby sensor response. To alleviate this problem, we introduced the washing of the sensor with PB solution to reduce the ionic strength post antibody-antigen binding to recover the responses of MoS2-based biosensor. Figure 6c illustrates dynamic response of the biosensor at an applied source-drain voltage of 1 V (with no gate voltage) to $0-10^6$ ng/mL HIgG in AU. After PB priming, the injection of increasing concentrations of the target in AU produced large negative responses, which were opposite to the response obtained in PB (Figure 6a). After washing the test cell by 800 µL of PB, the responses became positive and showed agreement with the responses in PB. The calibration plot of the biosensor to HIgG in AU, shown in Figure 6d, had a linear relationship with the sensitivity (slope) of 0.0437 per Log₁₀ (ng/mL), which was in very good agreement with the sensitivity of 0.0529 per Log (ng/mL) in PB. These results present a simple approach for overcoming the effects from organics and salts present in AU and a high degree of accuracy even in a complex real-life sensing environment. Comparison with recent literature results on MoS2-based FET biosensors, presented in Table S1, clearly indicates the comparable performance of our MoS₂ biosensor based on simple chemiresistive principle using modulation of resistance instead of FET principle based on change in transfer characteristic of the device.

4. Conclusions

In conclusion, this work presents an extended deliberation for electrical measurement analysis of biosensors. Although the sensitivity aspect of a biosensor is intensively researched, a device's accuracy, specifically in an event of false positive/negative response, should be equally considered. CVD-grown MoS₂, due to the nature of its synthesis process, forms a great number of grain boundaries. As a result, a device using such materials is predisposed to undesirable delamination of the material from the substrate. A facile and effective strategy to mitigate this molecularly induced delamination process via physical passivation by atomic layer deposition of the aluminum oxide layer was demonstrated. In addition, the sensors passivated with such oxide layer benefit from a strong ndoping effect allowing another method of detection requiring lower source-drain bias and no gate. These metal oxide-passivated devices not only exhibit a higher physical and chemical stability but also demonstrate high sensitivity and selectivity in biosensing even in a complex real-life sensing environment. This fundamental finding paves the way to further expected improvement of the sensitivity of such biosensors using an optical gating as demonstrated in our previous work [38]. While electrical gating can damage the dielectric substrate by creating pin holes in the layer after repeating applied cycles and require a high range of tens of voltage, photogating is not only lower power consumption and compatible for portable on-chip designs but also has no detrimental effects on the physical device.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/bios11120514/s1, Figure S1: Raman spectra of MoS₂ before and after 30 min incubation in DI water. Figure S2: S1813-MoS₂-based device (a) schematic and (b) field-effect transistor characteristics after in DI water (pH = 7). Figure S3: SEM image of same location (a) before; (b) after 10 min incubation in 5 mM AuCl₃ solution. Figure S4: Schematic of MoS₂-based FETs with Al₂O₃ passive layer. Figure S5: Optical image of the sensor (a) before and (b) after Al₂O₃ deposition. (c) L1: SiO₂ and L2: Al₂O₃ thickness measured by ellipsometer. Table S1: Performance summary of MoS₂-based FET biosensors.

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