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# Rapid $\beta$ -human chorionic gonadotropin detection in urine with electric-double-layer gated field-effect transistor biosensors and a handheld device

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## ABSTRACT

In this experimental study, a portable biosensor was developed to detect  $\beta$ -human chorionic gonadotropin ( $\beta$ -hCG), which is extensively used in pregnancy tests and serves as a biomarker for ectopic pregnancy. The sensor used is an electric-double-layer field-effect transistor biosensor with the extended-gate design. Bias voltage is applied on the sensor to measure the resulting drain current signals. Gold electrode surface is functionally activated with an anti- $\beta$ -hCG antibody to capture  $\beta$ -hCG protein. Fluorescence imaging technique is utilized to confirm the surface functionalization. The biosensor demonstrates a dynamically wide range of molecules as detection targets at very low sample concentrations, which shows the potential to detect ectopic pregnancy in very early stages and easily keep track of its periodic changes. It can be produced en masse and does not use additional labels/reagents or pre-processing techniques for the sample. This biosensor can significantly reduce the manufacturing costs and is comparable with the currently available commercial  $\beta$ -hCG assays. It is suitable for early diagnosis of ectopic pregnancy with low cost and easy operation at home with urine samples.

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## I. INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by placenta starting from ten to twelve days after conception. It is composed of  $\alpha$  and  $\beta$  peptide subunits that are composed of 92 and 145 amino acids, respectively.<sup>1,2</sup> The structure of the  $\alpha$ -subunit is similar to the  $\alpha$ -subunit LH, FSH, and TSH, while the  $\beta$ -subunit has a unique structure that possesses hormonal activity.<sup>3</sup> Not only is it relevant for gynecological disease for females but also strongly related to diseases occurring in males, such as testicular cancer and germ cell tumor.<sup>4–6</sup>

$\beta$ -hCG is most commonly used in pregnancy tests for qualitative or quantitative inference. Protein levels are clinically tested in

urine, serum, plasma, and whole blood samples.  $\beta$ -hCG concentration level changes at different rates at different weeks of pregnancy. For example, the concentration of  $\beta$ -hCG is about 5–50 IU/l in the initial three weeks after woman's last menstrual period. It keeps on increasing till eighth to tenth week and then drops until it reaches to about 1000 IU/l.<sup>7,8</sup>

There are numerous kinds of pregnancy conditions, namely, normal pregnancy, ectopic pregnancy, fetal death, and hydatidiform mole, which develops into subsequent malignant sequelae.<sup>9,10</sup> Ectopic pregnancy is life-threatening since the embryo attaches outside the uterus.<sup>11</sup> There is a strong correlation between plasma hCG levels and gestational timeline with different

kinds of pregnancy. Gestational age can be defined as the period from woman's last menstrual period. It can be used to easily distinguish different kinds of pregnancy through the measurement of maternal plasma hCG slope during the early pregnancy. From the research of Constance and Moravek,<sup>9</sup> the slope of the diagnostic hCG levels at different times in pregnancy represents the rate of increase in concentration. Normal pregnancy has a steeper uphill slope than ectopic pregnancy.<sup>9,12</sup> Thus, ectopic pregnancy can be detected by quantitative detection of  $\beta$ -hCG. In normal pregnancy,  $\beta$ -hCG concentration increases by a minimum of 66% after every 48 h.<sup>13</sup> If this increase in the concentration level of  $\beta$ -hCG is less than 66% after every 48 h compared to the initial value, an ectopic pregnancy might occur and patient needs to be alerted. Using currently available devices in the market, users cannot monitor their  $\beta$ -hCG concentration levels daily at home.

Many research studies indicate that diagnosis in the early stage and immediate treatments can considerably reduce morbidity and mortality during ectopic pregnancy.<sup>14–16</sup> The usage of ultrasound irradiation supplemented with measurement of  $\beta$ -hCG could help doctors to diagnose ectopic pregnancy accurately.<sup>17</sup> However, even in emergency situations, patients still need to wait for at least one hour to obtain the test results.

Considering the rapid development of biosensors in recent years, construction of more sensitive and specific sensors for biological sample detection has become a major focus of various research teams dedicated to develop such devices. New techniques for manufacturing electronic devices are expected to integrate into the existing processes and reduce the size of resulting devices. Toward this goal, FET-based sensors gain more attention for the synthesis of various kinds of sensors such as gas sensors, chemical sensors, biosensors, and heavy metal sensors.<sup>18–24</sup> Due to the fact that  $\beta$ -hCG is the crucial biomarker for ectopic pregnancy, in this study, antigen–antibody interaction was used to develop a detection device.

Traditional FET biosensors are challenged by the charge screening effect.<sup>25</sup> The charge screening effect reduces target potential because of short Debye length in high ionic strength solutions such as urine, whole blood, and serum.<sup>26</sup> Although high ion strength of the sample can be diluted, or short aptamer and segment antibody can be used to conquer charge screening effect, it needs complicated pretreatment and cannot directly detect clinical samples.<sup>27–29</sup> This also limits the potential for detection and application fields for FET based biosensors.

The aim of this research is to develop electric-double-layer (EDL)-FET biosensors and a portable device for quantitatively monitoring  $\beta$ -hCG daily at home. This sensor has several advantages such as ease of operation, short detection time, low cost, and high sensitivity. The EDL-FET biosensor is designed and demonstrated in detecting proteins in high ionic strength solution directly, without any pretreatment. The operating procedure for using the device is quite simple. A drop of urine without any pretreatment will be dropped on the sensor and after five minutes, the test result can be obtained. A model of the sensing mechanism is also proposed in this article. Overall, this technology can play a significant role in homecare for early risk evaluation of ectopic pregnancy.

## II. EXPERIMENTAL

### A. Sensor chip fabrication

Our biosensor array chip is fabricated by the following steps. First, polydimethylsiloxane (PDMS) is poured on a PMMA mold made by laser cutting and put in oven to cure for 2 h at 65 °C. After that, epoxy resin was poured on the PDMS mold and cured at 125 °C for 1 h and 165 °C for 1.5 h. Next, an epoxy resin substrate was released from the template of PDMS, and photolithography is patterned for metal deposition. Following this, titanium and gold were deposited on the epoxy resin substrate using electron beam evaporator and lifted-off in acetone. Finally, photoresist was used to passivate the non-sensing region, and photolithography was used to open the sensing regions. A schematic diagram for the fabrication process is shown in Fig. 1. There are two gold electrodes for each sensor. One is used as the reference gate electrode connected to the  $V_g$  supply. The other is sensing electrode linked to the gate terminal of MOSFET. The open sensor area is 600 by 600  $\mu\text{m}$  and the distance between the two electrodes is 185  $\mu\text{m}$ .

### B. Surface functionalization

Immobilized thiol-polyethylene glycol-amine (Biochempeg Scientific Inc.) was placed on the gate electrode surface and incubated for 2 h at room temperature to form a thiol–gold (sulfur–Au) bond.<sup>30,31</sup> Monoclonal Anti-hCG antibody purchase from Hytest Inc. (2H8-28A4) and EDC/NHS (Thermo Fisher Scientific Inc.) were mixed at room temperature for 2 h and then 7 K MWCO was used in a micro-spin desalting column to remove unbound molecules. After that, the mixture was poured on the amine-terminated gate electrode for 12 h and washed with 1 $\times$  PBS on a shaker at 100 rpm for 15 min. This can confirm that the direction of antibody is upward facing (light chains facing away from the electrodes). After surface functionalization,  $\beta$ -hCG samples of different concentrations, prepared in 1 $\times$  PBS, 1 $\times$  PBS with 4% BSA, artificial urine (1700-0017, Pickering Laboratories, Inc.) were dropped on the sensor surface to conduct the experiment.

### C. Fluorescence confirmation

In order to confirm antibody immobilization level, a fluorescence dye was conjugated on the secondary antibody against the primary antibody.<sup>32</sup> Dylight 550 was used to conjugate a secondary antibody. After dying, the chip was soaked in 1 $\times$  PBS for 15 min to wash away any unbound Dylight 550. A fluorescence microscope (LEICA DM2500 LED) purchased from the LEICA company was used to capture fluorescent images and used Leica Application Suite X software to analyze the fluorescent image. Quantitative analysis of the results was done with the software image J.

### D. Sensor measurements

The sensor chip was inserted into the portable prototype device and 50  $\mu\text{l}$  solution was dropped on the sensing electrode surface for 5 min. Corresponding data points were then obtained. The portable prototype device supplemented with a N-Channel depletion-mode MOSFET was connected to a computer screen to display the measurement results as shown in Fig. 2(a). The MOSFET (Cat. No.

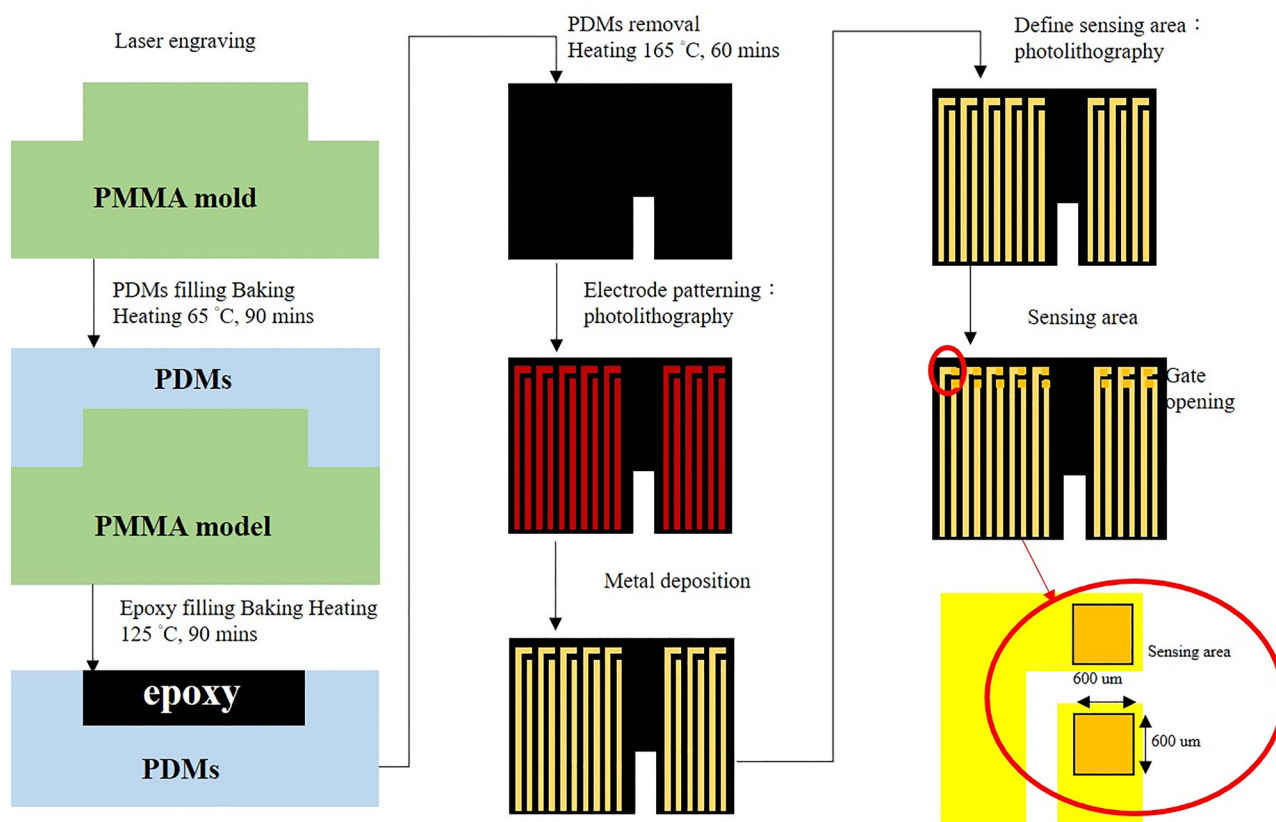


FIG. 1. Extended gate sensor array chip fabrication process.

LND150) was provided by Microchip Technology Inc. It steadily supplies 2 V DC bias as a drain–source voltage. Gate pulse amplitude was set to 1 V at a reference electrode. As shown in Fig. 2(b), during the measurement, gate pulse was maintained at 0 V for 100  $\mu$ s and 1 V for 1000  $\mu$ s subsequently. The characteristic figures  $I_D$ – $V_D$  and  $I_D$ – $V_g$  for LND150 are shown in Figs. 2(c) and 2(d), respectively.

Drain current shift may be observed due to many factors such as external impedance effects and thermal noise. Therefore, sensor signal is denoted by current change ( $\Delta I_D$ ) instead of the absolute drain current. As shown in Eq. (1), current change is the difference of drain current from gate voltage of 0 and 1 V,

$$\Delta I_D = I_D(V_G = 1 \text{ V}) - I_D(V_G = 0 \text{ V}). \quad (1)$$

### III. RESULTS AND DISCUSSION

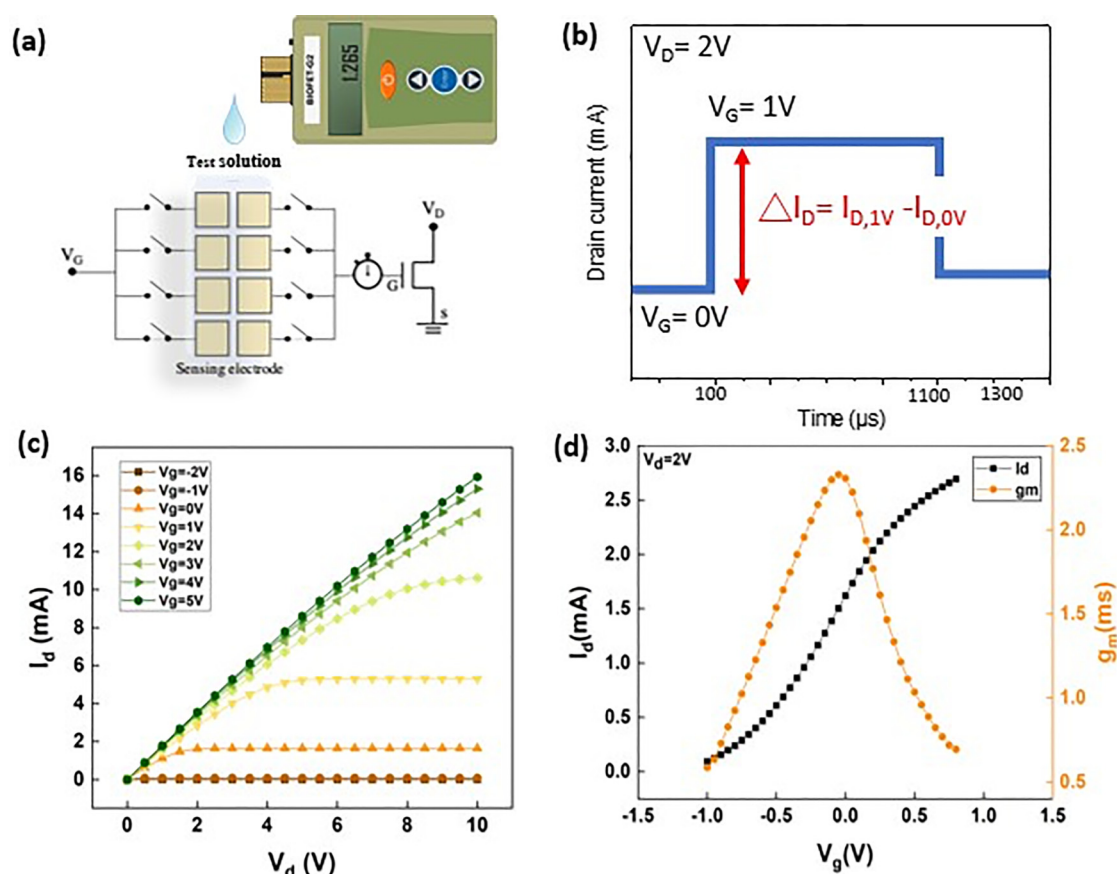
#### A. Electrical signal detection

Figure 3 shows the calibration curve of FET sensor current change with 0 (150 mM PBS buffer solution), 0.5, 5, 25, 50, and 100 ng/ml of  $\beta$ -hCG (same as 0.45, 4.5, 22.5, 45, and 90 IU/l) in different solutions. As shown in Fig. 3(a), the protein was measured

on bare gold without any immobilized antibody to evaluate non-specific binding signal for test buffer solution. This shows that when  $\beta$ -hCG protein concentration increases,  $\Delta I_D$  changes little if antibody is not immobilized on electrodes (less than 5  $\mu$ A).

After anti-hCG antibody captures the  $\beta$ -hCG protein, EDL on two electrodes is rearranged, causing an increase in potential. This indicates that the potential between two electrodes rises with the formation of more antibody–antigen complexes on the sensor surface. The increase in potential with the concentration increase of  $\beta$ -hCG leads to higher  $V_{g, \text{ext}}$  (effective gate voltage remained in the gate terminal of the MOSFET), which brings about  $\Delta I_D$  decrease. Figure 3(b) illustrates that  $\Delta I_D$  was changed to around 30  $\mu$ A in 1 $\times$  PBS buffer solution. The decrease in  $\Delta I_D$  actually represents the increase in drain current ( $I_D$ ). This is due to the definition of which is  $I_D$  at  $V_g = 1$  V subtracting  $I_D$  at  $V_g = 0$  V. Because the transconductance ( $g_m$ ) at  $V_g = 0$  V is much larger than that at  $V_g = 1$  V, the  $I_D$  change caused by  $\beta$ -hCG protein at  $V_g = 0$  V is larger than that at  $V_g = 1$  V. Therefore, the trend of  $\Delta I_D$  and drain current is always opposite.

After testing in 1 $\times$  PBS (high ionic strength solution), identical antigen concentrations were measured in 1 $\times$  PBS with 4% BSA, which is closer to human serum solutions. As shown in Fig. 3(c),



**FIG. 2.** (a) Schematic diagram of FET extended gate biosensor. (b) The definition of current change ( $\Delta I_D$ ). (c) The characteristic  $I_D$ - $V_D$  figure of LND150. (d) The characteristic  $I_D$ - $V_G$  figure of LND150.

as the concentration of antigen in  $1 \times$  PBS with 4% BSA is increased,  $\Delta I_D$  decreased by around  $30 \mu A$  and the error bar of signal was quite small.

It is very difficult to obtain serum samples in daily life. However, urine sample is much easier to be collected. In this study, in order to achieve the goal of easy point-of-care diagnosis, the detection experiments were performed in urine. Regular urine tests require that the patients should not drink too much water to avoid diluted target concentration and collect the first early morning urine on the next day. Researches have shown that the average sensitivity of the urine hCG test for ectopic pregnancy is 96%, which is significant enough to be used as a biomarker for risk assessment for ectopic pregnancy.

Various concentrations of 0.5, 5, 25, 50, and 100 ng/ml  $\beta$ -hCG were prepared in an artificial urine. The result is shown in Fig. 3(d), where a decreasing trend of  $\Delta I_D$  is observed when  $\beta$ -hCG concentration increases. Furthermore, the sensor shows high sensitivity in urine, which is similar to that in  $1 \times$  PBS with 4% BSA. Because our sensor's signals are dominated by the EDL capacitance change, only ingredients appearing in this region can contribute to the signals. As

a certain gate bias is applied, the EDL forms mainly depending on the small ions, such as the salt ions in the solution. Therefore, only the fixed moiety, such as the captured antigen, can effectively change the EDL capacitance. For other free molecules, they are not able to compete with salt ions in the amount (concentration) and also will not be captured by the antibody. Therefore, in spite of the complexity of the urine samples, the sensor is not affected. Previously, we also demonstrated protein detection in human serum without significant interference.<sup>39</sup>

Selectivity is a very critical property for a protein sensor. There are various compounds along with protein such as salts in human body, which help stabilize the protein structure for metabolic processes.  $1 \times$  PBS buffer solution was used for the sensor. Luteinizing hormone (LH), that stimulates ovulation and controls the growth of the corpus luteum, was chosen to confirm the selectivity of the sensor. Depending on the menstrual cycle, which has different LH concentrations in different phases, five concentrations of LH (1.2, 2.8, 4.2, 8.4, 14.6 ng/ml same as 6, 14, 21, 42, 73 IU/l, respectively) were prepared. The measurement was taken following the same experimental process for  $\beta$ -hCG and LH. Figure 4(a)

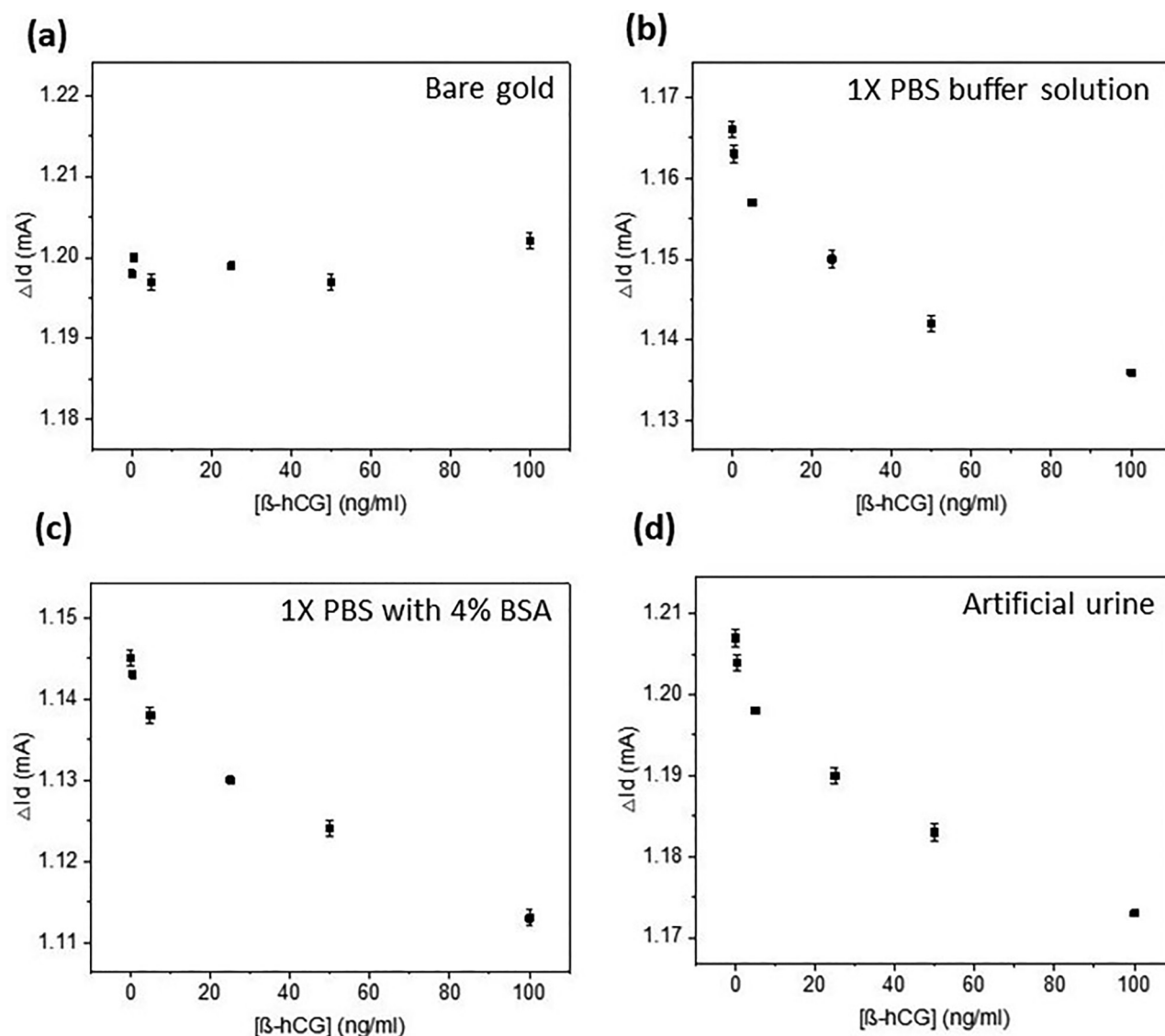


FIG. 3. Sensor calibration curve for testing  $\beta$ -hCG (a) on bare gold (b) in 1× PBS (c) in 1× PBS with 4% BSA (d) in artificial urine.

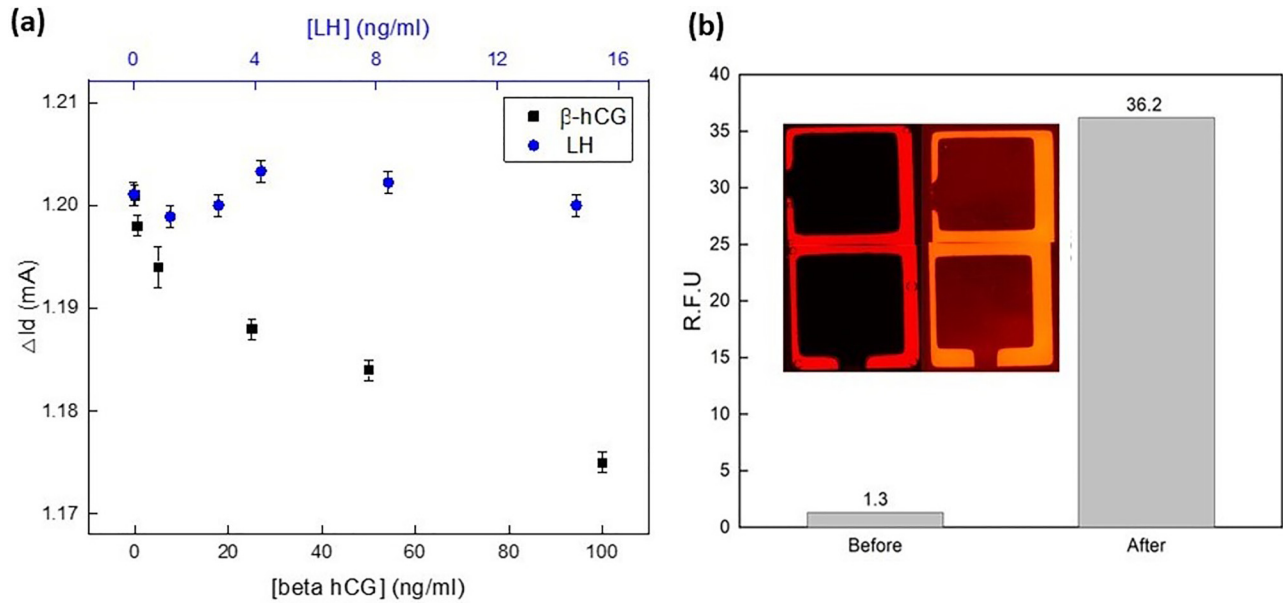
shows a large change in  $\Delta I_D$  for  $\beta$ -hCG protein and no significant change with LH increase. This indicates that the sensor is selectively binding to  $\beta$ -hCG. Thus, it can be inferred that the sensor response is truly resulted from the specific  $\beta$ -hCG binding.

The sensor demonstrates a wide dynamic range (0.5–100 ng/ml) and high sensitivity for  $\beta$ -hCG and is beneficial for clinical usage. Positive and negative pregnancy conditions (cut-off value 5–25 IU/l) can be distinguished easily due to the low detection limit (0.5 ng/ml same as 0.45 IU/l) of the sensor for  $\beta$ -hCG. In the case of positive pregnancy, the concentration changes between 25 and 100 ng/ml (same as 22.5–90 IU/l) with time can be used for early diagnosis of

ectopic pregnancy and could also be helpful for monitoring the health of embryo.<sup>33</sup> As the embryo develops with time,  $\beta$ -hCG will keep increasing beyond 100 ng/ml. In such a case, a simple dilution of urine with a standard buffer solution can easily solve the problem for the sensor to detect extremely high concentration of  $\beta$ -hCG.

## B. Fluorescence image confirmation

Figure 4(b) shows the fluorescence intensity before and after the primary antibody immobilized. The fluorescence probe is conjugated on the secondary antibody, which is against the primary antibody.



**FIG. 4.** (a) The selectivity measurement with LH and  $\beta$ -hCG. (b) Relative fluorescence intensity (R.F.U.) with the fluorescent image of the sensor before and after antibody immobilization.

The inset shows the fluorescent image of the two electrodes before and after the primary antibody immobilized on the left and right, respectively. It is obvious that the antibody well-immobilized surface shows much brighter image than before. Additionally, the relative fluorescent intensity (R.F.U.) was calculated and the difference was found to be more than 40. Hence, the primary (capture) antibody binding on our sensor surface was confirmed.

### C. Mechanism of our EDL-FET biosensors

Here, we introduce the conventional EDL theorem, the Gouy–Chapman–Stern model<sup>34</sup> into a part of the principle for our EDL FET biosensors. The model depicts that the double-layer consists of a Stern layer and a diffusive layer. When the EDL forms, the surface capacitance is generated. Hence, the total EDL capacitance is composed of a series network of the Stern layer ( $C_{stern}$ ) and a diffusive layer ( $C_{diff}$ ) in Eq. (2).<sup>35</sup> The electrical double layer capacitance ( $C_{dl}$ ) is approximately about two times of the capacitance of the solution ( $C_s$ ),<sup>34</sup>

$$\frac{1}{C_{dl}} = \frac{1}{C_{stern}} + \frac{1}{C_{diff}}. \quad (2)$$

The Stern layer capacitance can be expressed as

$$C_{stern} = \frac{\epsilon_{r,stern} \epsilon_0 A}{l_{stern}}, \quad (3)$$

where  $\epsilon_0$  is the vacuum permittivity,  $\epsilon_{r,stern}$  is the relative permittivity,  $l_{stern}$  is the Stern layer thickness, and  $A$  is the surface area of the

electrode. Also, the capacitance of the diffusive layer can be written as

$$C_{diff} = \frac{\epsilon_r \epsilon_0 A}{l_{diff}}. \quad (4)$$

Here,  $\epsilon_r$  is the relative permittivity in the diffusive layer and  $l_{diff}$  is approximately equal to the Debye length ( $k^{-1}$ ).

According to Bergveld's research, the electrical double layer capacitance ( $C_{dl}$ ) would be altered due to the changes in surface charge ( $\sigma_0$ ) as shown in Eq. (5).<sup>36</sup> This establishes a correlation among surface charge ( $\sigma_0$ ), double layer charge ( $\sigma_{dl}$ ), surface potential ( $\phi_0$ ), and the electric-double-layer capacitance ( $C_{dl}$ ),

$$\sigma_{dl} = -\sigma_0 = -C_{dl} \cdot \phi_0. \quad (5)$$

The surface potential ( $\phi_0$ ) is the difference between potentials of the electrode surface and the bulk solution. The Grahame equation is used to demonstrate the relationship between surface charge density and surface potential, as shown in Eq. (6),<sup>37</sup>

$$\sigma_0 = \sqrt{8c_0 \epsilon \epsilon_0 k_B T} \cdot \sinh\left(\frac{e \phi_0}{2k_B T}\right), \quad (6)$$

where  $\sigma$  represents the surface charge density;  $c_0$  represents the bulk concentration;  $\epsilon$  represents the relative permittivity of solvents;  $\epsilon_0$  represents the electrical permittivity of free space;  $k_B$  represents the Boltzmann constant;  $T$  represents the absolute temperature;  $e$  represents the elementary electric charge, and  $\psi_0$  represents the surface potential. By combining Eqs. (4) and (5),

and a known double layer capacitance, the surface potential (or the voltage drop from the solid surface to the solution) can be obtained.

However, Bergveld only described the theoretical mechanism for FET pH sensors, but not for the whole biosensor. We will adopt the similar way to explain the voltage drop in solution for our EDL FET biosensors. Currently, most protein biosensors cannot detect proteins present outside the diffusive layer because of short Debye length. Average size of IgG antibody is about 5 to 10 nm (depending on pre-processing of the antibody), which is much larger than the Debye length in 1× PBS (0.7 nm).<sup>38</sup> This limitation was overcome by using EDL-high electron mobility transistor (HEMT)-based biosensors.<sup>39</sup> In this study, we have demonstrated that our extended gate EDL FET biosensor design can also solve this issue.

In this research, the carboxyl group of antibodies was attached on the gold surface that resulted in formation of an immobile site, facing away from their electrode. The immobilized antibody makes the electrode surface no longer a smooth surface, and the antibody exceeds the idea Stern layer and the diffusive layer. The charge density may not be exactly following the Grahame equation, which is derived from an ideal smooth surface. However, the principle of the EDL capacitance and the relationship between surface charge density and the surface potential can still be used to explain the voltage drop across the EDL, leading to the change of the effective gate voltage ( $V_{g, \text{ext}}$ ) of the MOSFET. The functional diagram for this sensor is shown in Fig. 5(a). One electrode was connected to the voltage supply and the other electrode was connected to the extended gated terminal of MOSFET. When  $V_g$  is supplied to the

sensor, the potential drops through the test solution and remains  $V_{g, \text{ext}}$  on the extended gate electrode. The given gate voltage ( $V_g$ ) is the sum of the voltage drop in solution ( $\Delta V_s$ ) and the remaining voltage on the extended electrode ( $V_{g, \text{ext}}$ ) of the MOSFET as shown in Eq. (7),

$$V_G = \Delta V_s + V_{G, \text{ext}}. \quad (7)$$

We take the similar idea to explain the voltage drop in solution as Bergveld's. Therefore, the voltage drop in solution is determined by double capacitance and the double layer charge density, expressed as  $\left(\frac{C_{dl}}{2} \times \Delta V_s = \sigma_{dl}\right)$ .

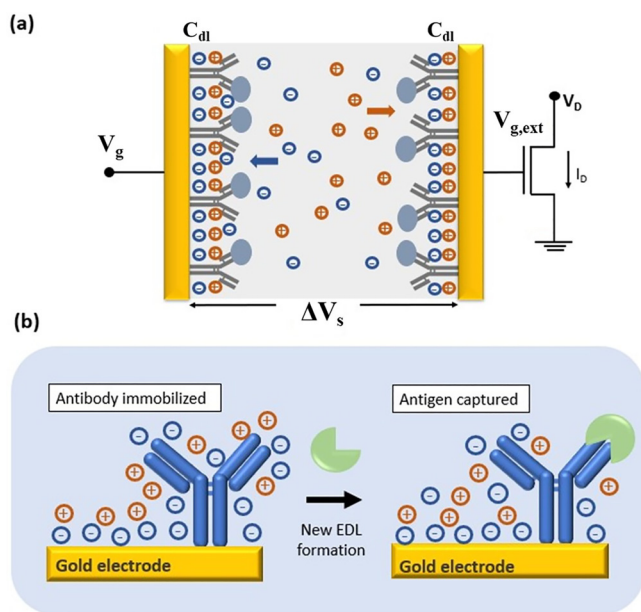
The reason of the formation of antibody-antigen complex beyond Debye length can be sensed by the sensor is explained as that a new EDL forms due to the surrounding antibody-antigen binding complexes as shown in Fig. 5(b). When the test solution was added to the sensors, the functional groups of antibodies attracted opposite charge in the test solution and lead to the formation of a new EDL. Not only the double layer charge density could change, but also the double layer capacitance may change, too. The antibody and the complex of antibody-antigen binding could contribute the change of the permittivity, leading to the change of  $C_{\text{Stern}}$  and  $C_{\text{diff}}$ . As the antibody and the antigen is binding on the electrode surface, it is not appropriate to simply use the permittivity of water molecules only. The permittivity of proteins should be also included into the overall surface capacitance. Hence, from the detected results in high ionic strength solutions, we believe that the permittivity of proteins (antibody and antigen) and the altered surface charge distribution both need to be included in the explanation of the results and the construction of the EDL FET models. Finally, the voltage drop caused by proteins in solution, will be amplified through the transconductance of the MOSFET and shown as in drain current change.

The sensor was tested after the solution of each concentration was incubated for 5 min on the sensor. It was measured with a short pulse bias with a duration of 100  $\mu\text{s}$ . Among each concentration, the sensor was not continuously measured to avoid the heating effect of the FET channel. The heating effect usually causes the baseline drift. The sensor can be used several times by eluting the antigen from the antibody, before the antibody degrades. We previously demonstrated several protein detection in blood and serum.<sup>40-42</sup>

## IV. CONCLUSION

A portable EDL FET biosensor with an extended gate design was developed for the detection of  $\beta\text{-hCG}$  and the mechanism of action of the biosensor was mentioned. Using this tool, females can easily distinguish between positive and negative pregnancies, monitor ectopic pregnancy, and track the embryo's health. On the other hand, males can also benefit for certain physiological conditions such as testicular cancer by early screening.

Moreover, the lower detection limit for our biosensor was calculated to be about 0.5 ng/ml, which is lower than commercially available kits. This biosensor has a wide dynamic range from 0.5 to 100 ng/ml of antigen concentration, which makes it possible for ectopic pregnancies to be discovered in early stage. The biosensor



**FIG. 5.** (a) Electrical double layer formed on the gold electrode surface governing the voltage drop across the solution. (b) The schematics of EDL formation when antibody or antigen is binding.

demonstrates excellent readings in 1× PBS solution, 1× PBS with 4% BSA, and untreated artificial urine without any pre-treatment. This research is expected to be valuable in optimizing the extended gate EDL FET biosensor. The portability of the device and ease of use even by untrained professionals could achieve the milestone of home-care diagnosis of  $\beta$ -hCG protein and improvement of public health by detection and monitoring of various conditions both in females and males. The model of the EDL FET biosensors is also discussed and suggested.

## ACKNOWLEDGMENTS

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## DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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