DIFFERENTIAL METHOD FOR UNDISTURBED DETECTION OF 17β-ESTRADIOL USING AN INTEGRATED APTAMERIC GRAPHENE NANOSENSOR

Yijun Li[1,2], Yibo Zhu[1], Zhuang Hao[1], Cheng Wang[3], Zhixing Zhang[1], Miao He[2], and Qiao Lin[1]

[1]Department of Mechanical Engineering, Columbia University, New York, USA

[2] Center for Sensor Technology of Environment and Health, State Key Joint Laboratory of ESPC, School of Environment, Tsinghua University, Beijing, CHINA

[3]College of Electronic and Communication Engineering, Tianjin Normal University, Tianjin, CHINA.

ABSTRACT

This paper presents a differential method based on the graphene field effect biosensor for the rapid and specific quantification of environmental pollution-related biochemical analyte. Selectively functionalizing one of the two identical graphene channels and calibrating the sensitivity difference of the two channels allowed us to extract analyte binding-induced signals by subtracting irregular and unpredictable background. Using this method we demonstrated real-time monitoring of 17β-estradiol in electrolytes with varying pH value. This work represents important progress of graphene field effect sensors toward use with complex real samples.

INTRODUCTION

17β-estradiol (E2), the most active estrogen classified as a typical environmental endocrine disrupting chemical, has been widely used in the medical treatment, and the livestock and poultry breeding. It also becomes a priority in the environmental estrogen pollution [1]. Numerous biosensors have been developed to complement the shortage in chemical analytical instruments for the rapid E2 detection [2-5]. However, most of these biosensors were only demonstrated in the buffer, an ideal stable testing environment, and few of them were tested in the complex sample condition due to the background disturbance. Signals, arising from the variation of pH value, ionic strength and even unpredictable in the complex sample, can overlay the binding-induced signal from E2, and prevent these sensors from the further application.

To eliminate the disturbance signal from the resultant signal in the multi-variable testing environment, the differential method, normally required for an extra reference detection, is a feasible and straight-forward way to achieve this goal. Although it has been applied in the ion and the DNA detection [6-8], however, the sensitivity difference between channels has not been considered yet. Thus, the reported method still has defect in the application.

Here, we present a differential method for the E2 detection using the graphene aptasensor. The detection is based on the platform of graphene field-effect transistor (FET), and the polydimethylsiloxane (PDMS) microfluidic channel was employed to package the sensor. We verified our method on the detection of E2 by comparing the differential results measured in the condition of one variable, a stable testing environment, and the condition of the dual variable. In contrast to the reported work, our differential method has introduced the sensitivity factor, based on the graphene transconductance, to calibrate the sensitivity difference. In addition, the real-time monitoring of E2 was taken in the dual variable sample, which is a more complex testing environment and much closer to the real sample detection condition.

Our results show that the disturbance from pH value variation can be effectively eliminated using our differential method, and that the similar limit of detection (LOD) in the E2 detection, as low as ~1 pM, can be achieved with and without the disturbance from pH. These results demonstrate the feasibility of this novel differential method in the undisturbed detection of E2.

DESIGN AND PRINCIPLE

The integrated FET sensor is configured as shown in Figure 1. Two groups of FETs were fabricated in one piece of the substrate, and modulated by the same integrated planar gate electrode. Graphene was used as the conductive and sensitive material. Packaging with PDMS microfluidic channels, these two groups of FETs were separated and defined as the testing channel and the reference channel, respectively. As Figure 1(b) shown, the tubing was used to deliver and drain liquid chemicals and samples.

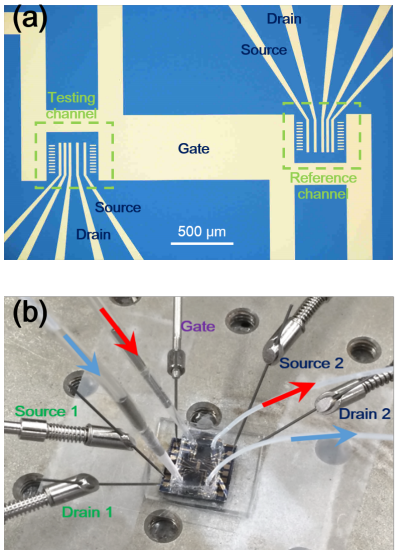

Figure 1: Fabricated FET sensor. (a) Optical micrograph of a fabricated FET sensor. (b) FET sensor packaged with PDMS microfluidic channels. Each microfluidic channel covered the corresponding sensitive area of one group FET.

Chemicals and samples were introduced into the channel using syringe pump (shown by red and blue arrow).

The sensing principle is designed as shown in Figure 2. An electrical double layer (EDL) is formed between the hydrophobic graphene surface and the electrolyte. The aptamer, of which one end is immobilized on the graphene, is negative charged, and electrostatically induces holes carrier in graphene through the EDL capacitance. The Debye length (λ_D) in our testing phosphate buffered saline buffer (PBS, pH 7.4) is around 7.5 nm, while the length of aptamers is around 11.9 nm. In the absence of E2, only part of charged nucleotides are within the Debye length, and can effectively induce holes in graphene due to the Debye screening effect. Thus, a slight *p*-type doping is observed. When binding with E2, the aptamer switches its secondary structure, and more charged nucleotides are pulled into the induction range, which results in the charge redistribution and the holes density increase in graphene. Therefore, the *p*-type doping is enhanced in the graphene, and the response could be measured by the drain-source current (I_{ds}).

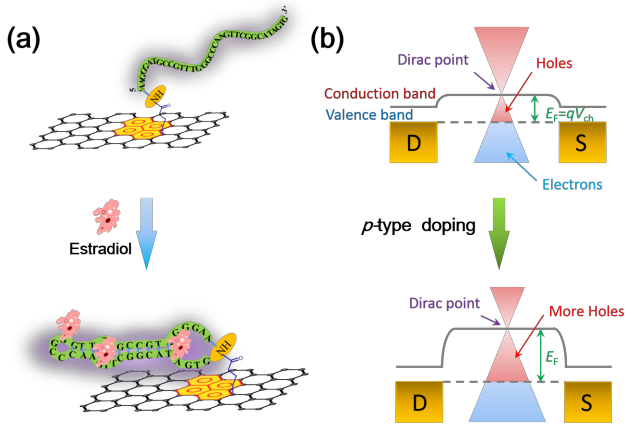

Figure 2: Sensing principle and electrical response mechanism. (a) The schematic diagram of the reaction process. The distance from the 3' end of the single-strain DNA aptamer to the graphene surface is changed with or without the existence of 17β-estradiol. The shadow represents the charge distribution on the aptamer. (b) The electrical response mechanism. The increase of holes density in the graphene can be understood as a p-type doping.

As our previous work reported [9], the sensitivity of graphene conducting channel is related to the total gating capacitance per unit area (C_{tot}) and the carrier mobility (μ) in graphene, and the transconductance parameter (g_m) can be used to evaluate the overall sensitivity performance, which can be described using

$$g_m = \frac{\partial I_{ds}}{\partial V_g} = \frac{W}{L} C_{tot} \mu V_{ds} \quad (1)$$

Where V_g is the gate voltage, V_{ds} is the drain-source voltage supplied in the FET sensor, W and L are the width and the length of graphene conducting channel,

Normally, there are two extremum points on the transconductance curve. The negative extremum (denoted g_{m_max}) on the hole branch was selected as the sensitivity indicator in our work due to the low operated V_g. To compare and calibrate the sensitivity between different channels, we defined the transconductance ratio of testing channel to reference channel on the hole branch as the sensitivity factor (denoted α).

EXPERIMENTAL METHODS

Fabrication

Standard MEMS fabrication procedure was taken to fabricate the sensor. Briefly, gate, drain and source electrodes were patterned onto the 285 nm SiO_2/Si substrate through photolithography and E-beam evaporation techniques with Cr/Au (3 nm/40 nm) layers. Monolayer graphene was transferred onto the electrodes using wet etching method [9, 10], and then patterned to the determined size.

SU-8 photoresist was used to fabricate the microfluidic mold on Si wafer. PDMS precursor and curing agent were mixed at 10:1, and poured into the mold. After the vacuum degrassing and baking on the hotplate (72℃ for 1 h), PDMS was peeled off. Finally, PDMS surface was treated using oxygen plasma (120 W, 500 mTorr, 20s), and then bonded with the sensor.

Functionalization

First, the 1-pyrenebutanoic acid succinimidyl ester (PASE) molecule dissolved in N,N-dimethylmethanamide (DMF) (2 mM) was introduced and retained in the chamber for 2 h to make the PASE adsorbed onto the graphene surface through π-π stacking. After rinsed by DMF and PBS sequentially, single-strained DNA (1 μM) was pumped into the microfluidic channels. Through the amino-group condensation with PASE, DNAs were immobilized onto the graphene surface. It is important to note that the DNA sequences were different in these two channels, and that only the testing channel was selectively functionalized with the aptamer that can bind with E2 [2, 11]. Therefore, testing channel should response to our target analyte while reference channel cannot, theoretically.

Operation

All detections were taken in the testing buffer (pH 7.4, 0.05 × PBS with 5% dimethyl sulfoxide (DMSO)). E2 was dissolved in DMSO (5 mM) as stocking solution and diluted with testing buffer for variable concentrations. During the detection, V_{gs}, V_{ds} and I_{ds} were supplied and measured using digital sourcemeters (Keithley 2400, Tektronix). All the measurement were controlled and recorded through a Labview program.

RESULTS AND DISCUSSION

Functionalization Characterization

The whole functionalization process was characterized using the electrical measurement method. Transfer characteristic curves at each step were measured as Figure 3 shown. After the PASE adsorption, Dirac point (V_{Dirac}) moved from 0.26 V to 0.29 V because of the *p*-type doping arising from PASE, and the V_{Dirac} moved back to 0.18 V due to the *n*-type doping caused by the negative-charged aptamer, which is in consistence with the previous work [10, 12, 13]. Therefore, the functionalization of the

sensor is verified and ready for the detection.

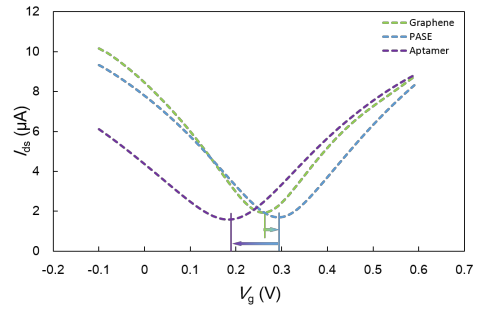

Figure 3: Transfer characteristic curves of graphene before and after PASE, aptamer functionalization.

E2 Detection

Before the detection of E2 in the dual variable condition, the sensing capability of this FET sensor was tested in the testing buffer, where the concentration of E2 was the only variable. The drain-source voltage was fixed at 10 mV.

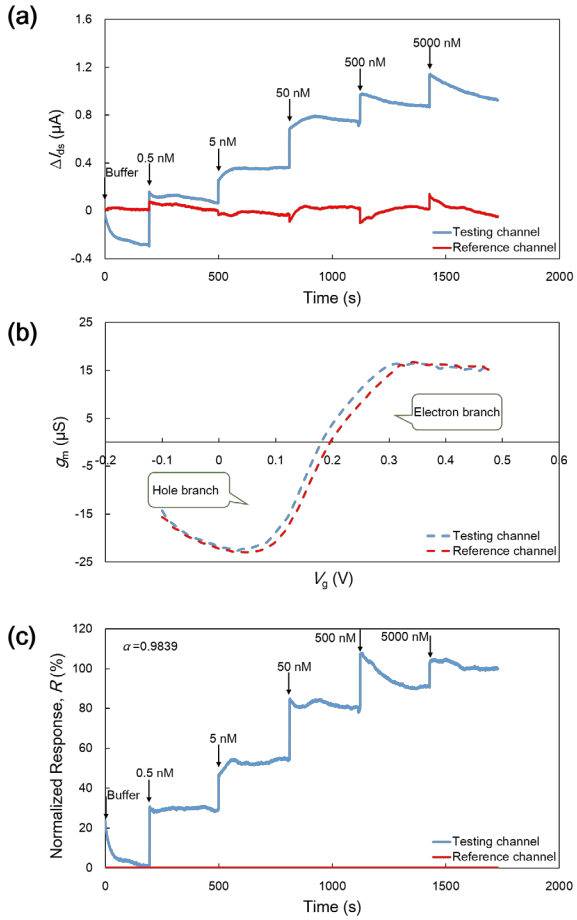

Figure 4: Detection results of the 17β-estradiol in testing buffer. (a) Original drain-source current variation response to the 17β-estradiol over time at various concentrations. The response in the testing channel (blue line) reflected the change from the concentration and the testing environment, while the response in the reference channel (red line) only reflected the change from the environment. (b) Transconductance curves. (c) Differential and normalized response extracted from (a) with α value.

Real-time current responses in both channels were achieved simultaneously, and plotted using the current variation (ΔI_{ds}) as Figure 4(a) shown. The current response in testing channel (blue curve) increased mainly arising from the change of E2 concentration, while the response in the reference channel (red curve) showed a little fluctuation, which might result from the pulse and the surface disturbance created by the solution change. To eliminate the pulse and the surface disturbance effect, the α value was obtained through the transconductance curve as shown in Figure 4 (b). Then, we amplified the response in the reference channel with α value to calibrate the sensitivity difference, and subtracted the amplified response from the response in the testing channel. The normalized result was shown in Figure 4 (c), and the result was fitted using Hill-Langmuir equation. The limit of detection (LOD) was estimated to be 0.22 pM using our previous method [10].

Next, we introduced another variable, the pH value, to the detection. The testing buffer was adjusted to different pH values (pH = 6.5, 7.4, 8.0), and other detection conditions were set the same as before. As Figure 5(a) shown, signals in both channels responded to the pH variation, which could be regarded as the disturbance response, and which could cover the response to the E2 concentration change during detection. To eliminate the disturbance, the same differential method was used. As Figure 5 (b) shown, the differential result indicated that the current response in the testing channel changed with the concentration variation, and that the disturbance from pH was almost eliminated. We also fitted the result using Hill-Langmuir equation, and achieved the LOD (1.48 pM), which was similar to the LOD value calculated in the one variable detection. As the pH value might fluctuate in the natural water and disturb the detection signal, our results demonstrate that this differential method has the potential to realize the detection in the complex testing condition.

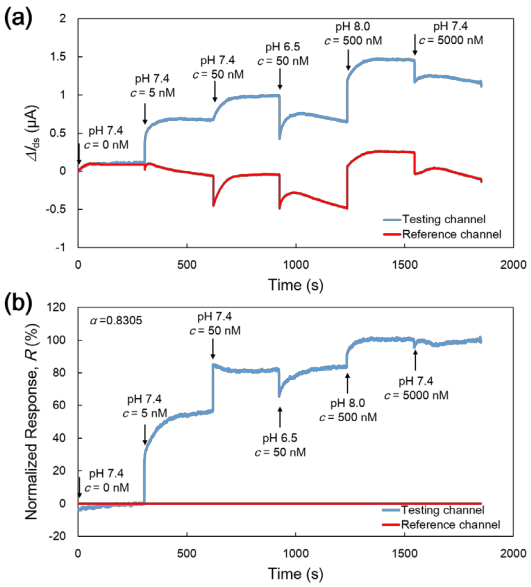

Figure 5: Detection results of the 17β-estradiol in the dual variable condition. (a) Original current variation response to the 17β-estradiol over time at various pH value and 17β-estradiol concentrations. (b) Differential and normalized response.

CONCLUSIONS

In this paper, we presents a differential method for the E2 detection using the differential graphene FET biosensor. Compared with the reported work, E2 was detected in the dual variable condition which is closer to the condition of the real complex sample. The sensitivity factor, based on the transconductance of graphene, was introduced for the first time to calibrate the sensitivity difference between channels. In the E2 detection, the detection result, measured in the dual variable testing environment, shows that the disturbance response, caused by the pH variation, can be eliminated using the differential method, and the LOD is similar to the result achieved in the one variable testing environment. Thus, this novel differential method can achieve the undisturbed detection of the E2 in the complex testing environment, and it can be reference for the undisturbed detection in other sensor platforms.

ACKNOWLEDGEMENTS

The authors would acknowledge the financial support from the National Institutes of Health (Grant No. 1DP3 DK101085-01 and 1R33CA196470-01A1) and the National Science Foundation (Grant No. ECCS-1509760). The author Y. Li also gratefully acknowledges a National Scholarship (award number 201606210346) from the China Scholarship Council.

REFERENCES

[1] N. Yildirim, F. Long, C. Gao, M. He, H. Shi, and A. Z. Gu, "Aptamer-Based Optical Biosensor For Rapid and Sensitive Detection of 17β-Estradiol In Water Samples," *Environmental Science & Technology,* vol. 46, pp. 3288-3294, 2012-03-20 2012.

[2] O. A. Alsager, S. Kumar, B. Zhu, J. Travas-Sejdic, K. P. McNatty, and J. M. Hodgkiss, "Ultrasensitive Colorimetric Detection of 17β-Estradiol: The Effect of Shortening DNA Aptamer Sequences," *Analytical Chemistry,* vol. 87, pp. 4201-4209, 2015-04-21 2015.

[3] Q. Han, X. Shen, W. Zhu, C. Zhu, X. Zhou, and H. Jiang, "Magnetic sensing film based on Fe 3 O 4 @Au-GSH molecularly imprinted polymers for the electrochemical detection of estradiol," *Biosensors and Bioelectronics,* vol. 79, pp. 180-186, 2016.

[4] H. Y. Zheng, O. A. Alsager, C. S. Wood, J. M. Hodgkiss, and N. O. V. Plank, "Carbon nanotube field effect transistor aptasensors for estrogen detection in liquids," *Journal of Vacuum Science & Technology B, Nanotechnology and Microelectronics: Materials, Processing, Measurement, and Phenomena,* vol. 33, pp. 06F904, 2015.

[5] O. A. Alsager, S. Kumar, G. R. Willmott, K. P. McNatty, and J. M. Hodgkiss, "Small molecule detection in solution via the size contraction response of aptamer functionalized nanoparticles," *Biosensors and Bioelectronics,* vol. 57, pp. 262-268, 2014.

[6] R. Stoop, M. Wipf, S. Müller, K. Bedner, I. Wright, C. Martin, E. Constable, A. Fanget, C. Schönenberger, and M. Calame, "Implementing Silicon Nanoribbon Field-Effect Transistors as Arrays for Multiple Ion Detection," *Biosensors,* vol. 6, p. 21, 2016-05-06 2016.

[7] J. Fritz, E. B. Cooper, S. Gaudet, P. K. Sorger, and S. R. Manalis, "Electronic detection of DNA by its intrinsic molecular charge," *Proceedings of the National Academy of sciences,* vol. 99, pp. 14142-14146, 2002.

[8] M. Wipf, R. L. Stoop, A. Tarasov, K. Bedner, W. Fu, I. A. Wright, C. J. Martin, E. C. Constable, M. Calame, and C. Schönenberger, "Selective Sodium Sensing with Gold-Coated Silicon Nanowire Field-Effect Transistors in a Differential Setup," *ACS Nano,* vol. 7, pp. 5978-5983, 2013-07-23 2013.

[9] C. Wang, Y. Li, Y. Zhu, X. Zhou, Q. Lin, and M. He, "High-κ Solid-Gate Transistor Configured Graphene Biosensor with Fully Integrated Structure and Enhanced Sensitivity," *Advanced Functional Materials,* vol. 26, pp. 7668-7678, 2016.

[10] Y. Li, C. Wang, Y. Zhu, X. Zhou, Y. Xiang, M. He, and S. Zeng, "Fully integrated graphene electronic biosensor for label-free detection of lead (II) ion based on G-quadruplex structure-switching," *Biosensors and Bioelectronics,* vol. 89, pp. 758-763, 2017.

[11] M. Svobodová, V. Skouridou, M. L. Botero, M. Jauset-Rubio, T. Schubert, A. S. Bashammakh, M. S. El-Shahawi, A. O. Alyoubi, and C. K. O Sullivan, "The characterization and validation of 17β-estradiol binding aptamers," *The Journal of Steroid Biochemistry and Molecular Biology,* vol. 167, pp. 14-22, 2017.

[12] Y. Zhu, Y. Hao, E. A. Adogla, J. Yan, D. Li, K. Xu, Q. Wang, J. Hone, and Q. Lin, "A graphene-based affinity nanosensor for detection of low-charge and low-molecular-weight molecules," *Nanoscale,* vol. 8, pp. 5815-5819, 2016.

[13] Z. Hao, Y. Zhu, X. Wang, P. G. Rotti, C. DiMarco, S. R. Tyler, X. Zhao, J. F. Engelhardt, J. Hone, and Q. Lin, "Real-Time Monitoring of Insulin Using a Graphene Field-Effect Transistor Aptameric Nanosensor," *ACS Applied Materials & Interfaces,* vol. 9, pp. 27504-27511, 2017-08-23 2017.

CONTACT

*Y. Li, tel: +1-917-361-1480;
lyjthu@gmail.com, yl3692@columbia.edu