REAL-TIME MONITORING OF INSULIN USING A GRAPHENE APTAMERIC NANOSENSOR

Zhuang Hao[1,2], Yibo Zhu[1], Xuejun Wang[1], Pavana Rotti[3], Christopher Dimarco[1], Shuyu Lin[1], Xuezeng Zhao[2], John Engelhardt[3], James Hone[1], and Qiao Lin[1]

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

[2] Department of Mechanical Engineering, Harbin Institute of Technology, Harbin, CHINA

[3]Department of Anatomy and Cell Biology, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA.

ABSTRACT

We present a new approach for real-time, specific and sensitive detection of insulin using a graphene aptameric nanosensor. The nanosensor is configured as a graphene field effect transistor, where the graphene conducting channel was functionalized with a guanine-rich aptamer IGA3 that would form an anti-parallel G-quadruplex upon insulin binding. The aptamer conformational changes altered the electrical conductance of graphene by varying the carrier density of the graphene. The nanosensor allowed us to perform real-time, label-free and specific detection of insulin concentrations as low as 1 nM.

INTRODUCTION

Insulin plays a very important role in the control of blood glucose concentration. Type-1 diabetes (T1D) is a metabolic disease, with which patients lack the ability to produce insulin and must be managed with insulin injections [1]. However, insulin levels of T1D patients are time-varying, hence, to develop real-time monitor insulin techniques is of great significance. Current insulin detection methods such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) are time-consuming offline techniques. As a result, they are not adequate enough to predict the administration of correct insulin doses in time. Recently, electrochemical and optical platforms have been attempted for insulin sensing [2-5], but are still hampered by limitations such as low sensitivity [5] and requirement of offline detection [4]. This paper presents a new approach that incorporates the G-quadruplex structure-switching signaling principle in a graphene-based field-effect transistor (FET) nanosensor for real-time monitoring of insulin.

Prediction of required insulin dose to alleviate symptoms of diabetes is critical and needs to rely on rapid and accurate measurement of the insulin level in blood. Therefore, real-time monitoring of insulin at physiologically relevant concentrations would be highly desirable. Unfortunately, this remains challenging for typical fluorescence or electrochemical methods, due to the low-molecular-mass and weak charges of insulin. The graphene conductance of the nanosensor, varies sensitively upon binding of insulin and aptamer IGA3, thereby allowing us to monitor changes of insulin concentrations. By using a graphene FET nanosensor to detect the aptamer conformational change (the formation of G-quardruplex), which is caused upon binding of insulin of aptamer IGA3 [6] and not significant enough to be observed in existing platforms, the approach can get distinguishable signals when insulin concentration is 1 nM which is 10 fold better than the LOD of existing inline methods and is capable of detecting physiologically relevant insulin levels.

The real-time measurements show that the graphene aptameric nanosensor can be used for real-time monitoring of insulin concentrations from 1 nM to 500 nM.

DESIGN AND PRINCIPLE

The graphene aptameric nanosensor is configured as an electrolyte-gated graphene field-effect transistor (Figure 1). The graphene, serving as the conducting channel between drain and source electrodes, is immobilized with 1-pyrenebutanoic acid succinimidyl ester (PASE) via π- π stacking between pyrene group and graphene. Then insulin-specific aptamer IGA3 is conjugated to PASE by forming amide bond.

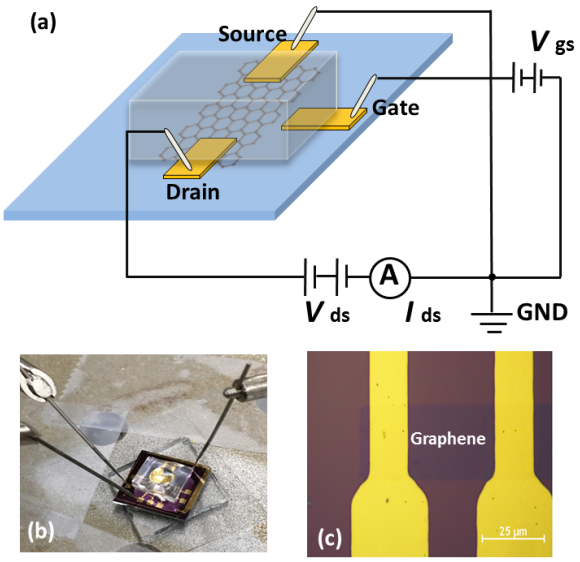

Figure 1: Schematic and fabrication of the graphene nanosensor. (a) Illustration of a solution-gated graphene field effect transistor. The electrical double layer at the solution-graphene interface is served as the gate capacitor. (b) Single nanosensor is packaged by a PDMS well for the liquid handling. (c) Micrograph of a fabricated sensor. The graphene conducting channel connects the source and drain electrodes.

In this sensor, an electrical double layer (EDL) at the interface of the graphene and the electrolyte serves as the gate dielectric layer. A source-drain bias (V_{ds}) applied between the drain and source electrodes generates current I_{ds} through the graphene channel, as a function of applied gate voltage (V_g). The functionalized guanine-rich aptamer IGA3 can form an anti-parallel G-quadruplex that binds to

insulin selectively [2, 4, 5]. The binding of insulin and aptamer IGA3 also promotes the G-quadruplex formation [2], a conformation change that alters the carrier concentration in the graphene, yielding a continuous detectable signal of I_{ds} (Figure 2).

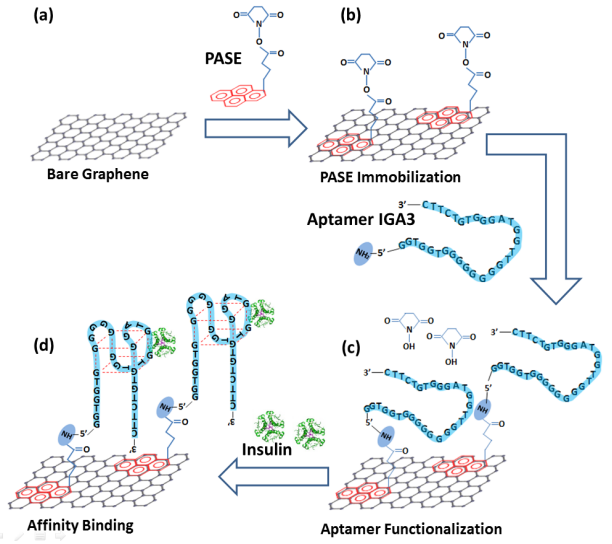

Figure 2: Principle of the graphene nanosensor for insulin detection. (a, b, c) The sensing surface is prepared through Shciff-base reaction between aptamer IGA3 and the graphene immobilized PASE linker. Sensing mechanism: (d) Aptamer IGA3 can specifically bind to target insulin molecules in sample solution. This specific binding promotes the structural conformation change of aptamers (the formation of G-quadruplex).

EXPERIMENTAL METHODS

Fabrication

Chemical vapor deposition (CVD) synthesized graphene was transferred onto the silicon oxide substrate. Gate, drain and source electrodes were subsequently fabricated using standard micro and nanofabrication technologies (5 nm thick chrome (Cr)/ 45 nm thick gold (Au) layers deposited on SiO_2) [7]. The graphene then was patterned to define the conducting channel [8].

Biofuctionalization

To immobilize the aptamer onto graphene, the sensor was firstly immersed in 5 mM PASE solution for 2 hours at room temperature, sequentially rinsed with dimethylformamide (DMF) and phosphate buffered saline (PBS) buffer to remove free PASE. The device was then rinsed with PBS followed by incubation with 100 nM aptamer IGA3 solution for overnight at room temperature. After rinsing with PBS, 100 mM ethanolamine was added onto the graphene channel for 1 h to deactivate and block the excess reactive groups remaining on graphene surface. A polydimethylsiloxane (PDMS)-based open well (~20 µL) used to hold sample solutions was lastly bonded to the device[9, 10].

Operation

Insulin and glucagon were dissolved in PBS buffer (pH 7.4) to obtain desired concentrations. These solutions were used in all of the subsequent experiments. During the transfer characterization experiment, the conducting IGA3 modified graphene channel was exposed to 20 µL different concentrations of insulin solution for 10 min respectively [11]. In the FET-based electrical measurement, both the drain and gate voltage were supplied by sourcemeters (Keithley 2400, Tektronix), and I_{ds} was simultaneously measured under control of a Labview program.

RESULTS AND DISCUSSION

Biofuctionalization Characterization

To verify the successful IGA3 functionalization, we conducted the transfer characterization on the graphene sample before and after functionalized by PASE and guanine-rich aptamer IGA3. As shown in Figure 3, the incubation of guanine-rich IGA3 solution obviously shifts the graphene transfer characteristic curve from 0.12 V to 0.2 V, suggesting the aptamer IGA3 functionalization is successful [9, 12] and can be used for insulin detection.

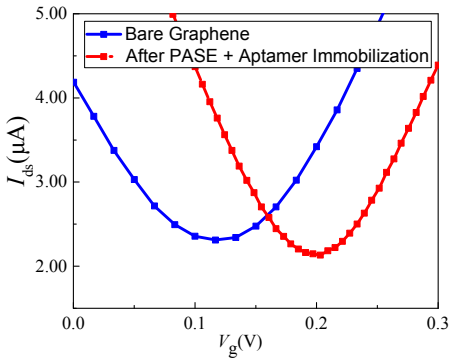

Figure 3: Transfer characteristics of graphene before and after PASE, aptamer IGA3 functionalization.

Sensor Performance

To verify the stability, the nanosensor was investigated using control experiments. First, insulin of 100 nM and 500 nM concentrations were introduced into the bare graphene without any chemical functionalization respectively. With the introduction of increasing concentrations of insulin, no significant shift of Dirac point voltage V_{Dirac} (the voltage at which the I_{ds} reaches its minimum) was observed as compared to the sensor experiments on introduction of target molecules. This suggested that insulin, at the selected concentrations, did not interact with graphene (Figure 4 a).

In order to test the insulin detecting performance of the nanosensor, the graphene transfer curve was plotted after functionalized with aptamer IGA3. After adding different concentrations of insulin (100 nM and 500 nM) while keeping V_{ds} constant at 0.01V, it was noticed that, a dynamic increase in current was observed in the n-conduction region of graphene which was the right side of the Dirac point. While a negative shift of the Dirac point from 200 mV to 145 mV was also observed on increasing the insulin concentrations (Figure 4 b). Graphene is known as a p-type semiconductor with holes as the majority of carrier, while, this shift in the Dirac point voltage was a result of n-type doping effect, suggesting the aptamer IGA3-insulin binding event lowered the carrier concentration in the bulk of graphene and hence reduced

the conductance [12]. We assumed that the conformational change of aptamer (the formation of anti-parallel G-quadruplex), which was caused upon the aptamer IGA3-insulin binding event, induced negative charge to the graphene. The hypothesis was consistent with previously reported works [2, 4, 5].

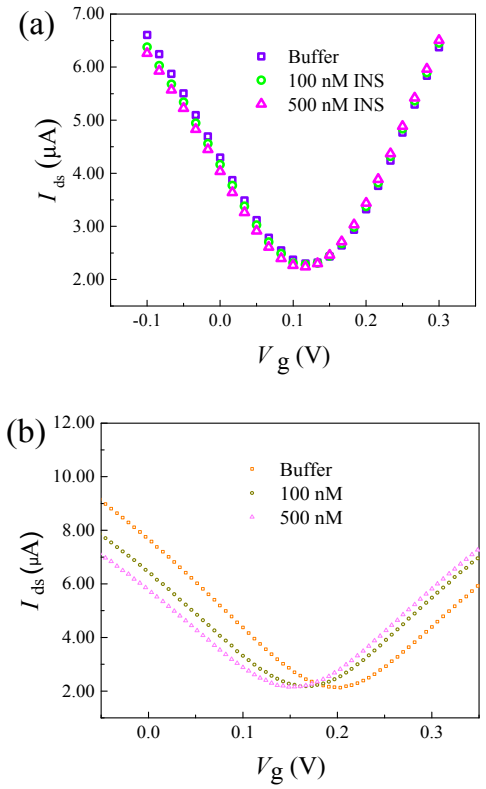

Figure 4: (a) Transfer characteristics of the pristine graphene exposed to insulin solutions (100 nM and 500 nM). (b) Transfer characteristics measured when the aptamer immobilized graphene is exposed to insulin solution (100 nM and 500 nM). The curve shifted to the left as a result of the increase of the insulin concentrations.

To identify the real-time monitoring performance of the IGA3 aptameric nanosensor, the I_{ds} was monitored in real time at constant V_{ds} = 50 mV (V_g = -0.05 V, a low operating voltage), upon the addition of various insulin concentrations. Figure 5 displayed the real-time responses of IGA3 aptameric nanosensor. The nanosensor showed a concentration-dependent decrease in I_{ds} when it was exposed to the target molecules insulin. The current decrease was a result of the accumulation of negative charge carriers induced by the aptamer IGA3-insulin binding event. Here, the change of I_{ds} before and after insulin solution incubation, denoted ΔI_{ds}, and the maximum ΔI_{ds}, the change before 1 nM insulin solution incubation and after 500 nM insulin solution incubation, denoted $\Delta I_{ds\text{-}max}$.

The selective response test toward insulin was characterized by including various concentrations (10 nM, 100 nM and 500 nM) of the following nontarget peptide molecules glucagon, which were also secreted by pancreatic islets cells. The results were shown in Figure 6. When the nanosensor was exposed with nontarget peptide molecules, no obvious comparable change was observed in I_{ds}, however, enough large changes in I_{ds} occurred upon addition of 39 nM and 80 nM of insulin, which was clearly meaningful. The ability to detect insulin with a high selectivity was achieved using the aptamer IGA3 with a sequence that was specific for insulin. Here, the change of I_{ds} before and after glucagon or insulin solution incubation, denoted ΔI_{ds}, and the maximum ΔI_{ds}, the change before 10 nM glucagon solution incubation and after 80 nM insulin solution incubation, denoted $\Delta I_{ds\text{-}max}$.

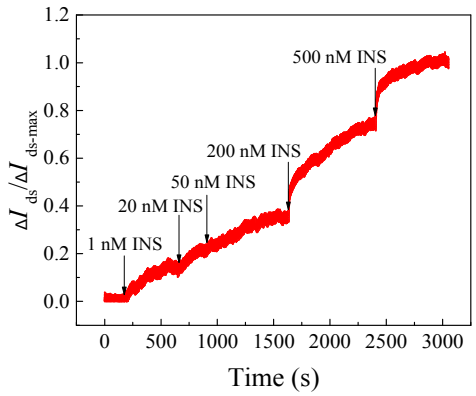

Figure 5: Real-time monitoring insulin concentrations change. The responses are demonstrated by the changes of the source-drain current Ids.

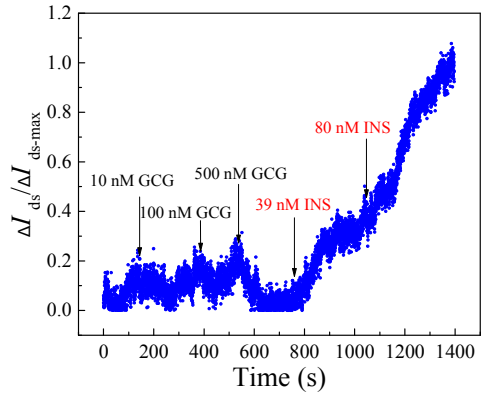

Figure 6: Selective real-time reponses of the IGA3 aptamer-based graphene nanosensor toward insulin (39 nM and 80 nM) and nontarget glucagon (10 nM, 100 nM and 500 nM).

CONCLUSIONS

We presented a new approach that employed the G-quardruplex structure-switching signaling principle in a graphene aptameric nanosensor for real-time monitoring of insulin. The insulin specifically bound to the surface-immobilized aptamer IGA3, promoting the formation of G-quadruplex and subsequently changing the carrier density in the graphene. The resulting change in the conductance of graphene was measured to determine the insulin concentration. The graphene nanosensor used by the approach demonstrated great performance in monitoring insulin levels ranging from 1 nM to 500 nM, hence, this approach held great potential to predict correct insulin doses for T1D patients.

ACKNOWLEDGEMENTS

The authors would acknowledge the financial support from the National Institutes of Health (Grant No. 1DP3 DK101085-01) and the National Science Foundation (Grant No. ECCS-1509760). Z.H. also gratefully acknowledges a National Scholarship (award number 201506120133) from the China Scholarship Council.

REFERENCES

[1] R. Hillson, *Diabetes: The Complete Guide-The Essential Introduction to Managing Diabetes*: Random House, 2012.

[2] J. Y. Gerasimov, C. S. Schaefer, W. Yang, R. L. Grout, and R. Y. Lai, "Development of an electrochemical insulin sensor based on the insulin-linked polymorphicregion," *Biosensors and Bioelectronics,* vol. 42, pp. 62-68, 2013.

[3] Y. Pu, Z. Zhu, D. Han, H. Liu, J. Liu, J. Liao*, et al.*, "Insulin-binding aptamer-conjugated graphene oxide for insulin detection," *Analyst,* vol. 136, pp. 4138-4140, 2011.

[4] I. Kubo and T. Eguchi, "Study on Electrochemical Insulin Sensing Utilizing a DNA Aptamer-Immobilized Gold Electrode," *Materials,* vol. 8, pp. 4710-4719, 2015.

[5] T.-G. Cha, B. A. Baker, M. D. Sauffer, J. Salgado, D. Jaroch, J. L. Rickus*, et al.*, "Optical nanosensor architecture for cell-signaling molecules using DNA aptamer-coated carbon nanotubes," *Acs Nano,* vol. 5, pp. 4236-4244, 2011.

[6] W. Yoshida, E. Mochizuki, M. Takase, H. Hasegawa, Y. Morita, H. Yamazaki*, et al.*, "Selection of DNA aptamers against insulin and construction of an aptameric enzyme subunit for insulin sensing," *Biosensors and Bioelectronics,* vol. 24, pp. 1116-1120, 2009.

[7] Y. Jia, H. Cai, and Q. Lin, "Thick-film MEMS thermoelectric sensor fabricated using a thermally assisted lift-off process," *Journal of Micro/Nanolithography, MEMS, and MOEMS,* vol. 15, pp. 024501-024501, 2016.

[8] Y. Zhu, C. Wang, N. Petrone, J. Yu, C. Nuckolls, J. Hone*, et al.*, "A solid dielectric gated graphene nanosensor in electrolyte solutions," *Applied Physics Letters,* vol. 106, p. 123503, 2015.

[9] C. Wang, J. Kim, Y. Zhu, J. Yang, G.-H. Lee, S. Lee*, et al.*, "An aptameric graphene nanosensor for label-free detection of small-molecule biomarkers," *Biosensors and Bioelectronics,* vol. 71, pp. 222-229, 2015.

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

[11] 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,* 2016.

[12] Y. Li, C. Wang, Y. Zhu, X. Zhou, Y. Xiang, M. He*, et al.*, "Fully integrated graphene electronic biosensor for label-free detection of lead (II) ion based on G-quadruplex structure-switching," *Biosensors and Bioelectronics,* 2016.

CONTACT

*Z. Hao, tel: +1-917-4032047; zh2266@columbia.edu