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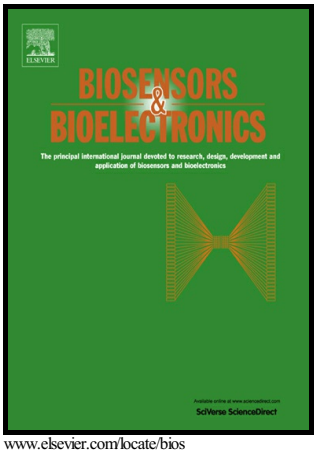

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Direct immune-detection of cortisol by chemiresistor graphene oxide sensor

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ABSTRACT

In this study, a biosensor to detect a stress biomarker of cortisol using cortisol monoclonal antibody (c-Mab) covalently immobilized on reduced graphene oxide (rGO) channel as electrical sensing element was demonstrated. Highly specific immune-recognition between the c-Mab and the cortisol was identified and characterized on a basis of resistance change at the rGO channel based chemiresistor sensor achieving the limit of detection of 10 pg/mL (27.6 pM). In addition, cortisol concentrations of real human salivary sample and buffer solution of rat adrenal gland acute slices, which could secret the cortisol induced by adrenocorticotropic hormone (ACTH), were directly measured by the chemiresistor corresponding to the specific sensing of the cortisol. The rGO chemiresistor could selectively measure the cortisol levels in spite of diverse neuroendocrine's existence. The potential perspective of this study can be a protocol of new cortisol sensor development, which will be applicable to point-of-care testing (POCT) targeted for salivary cortisol, *in vitro* psychobiological study on cortisol induction, and implantable sensor chip in the future.

Keywords

Graphene oxide, Cortisol, Electrical sensor, Chemiresistor sensor, Adrenal gland, Saliva

1. Introduction

Electrical biosensors at an intersection of diagnostic biology and electronics are expected to transform future lives of human beings by an introduction of new lab-on-a-chips for healthcare, point-of-care test (POCT), and implantable devices to the biological organisms (Tam et al., 2009). For these accomplishments, the interface between biological system and electronic signal is frequently a critical factor to facilitate its further developement. For the electronic signal, measurement of resistance change has been continuously adopted and developed for the detection of targeted biomolecules or viruses (So et al., 2005; Tlili et al., 2011; Liu et al., 2013).

Cortisol is one of the well-analyzed stress biomarker in its function and mechanims (Arya et al., 2010a). At the same time, the cortisol is one of the most potent disease evoking hormones when its level is kept continuously high. Therefore, the cortisol and its strong recognition molecules have been investigated to develop sensor formats to detect stress-related chronic diseases. Meanwhile, the clinical evaluation of the cortisol has been permitted to fluorescent enzyme-linked immunosorbent assays (ELISA) or radioimmunoassay (RIA) (Sun et al., 2008) and detections of cortisol levels could have been continuously reported using surface plasmon resonance (SPR) sensor, electrochemical sensor, impedimetric biosensor, chemiresistor, and so on (Arya et al., 2010b; Vasudev et al., 2013; Yamaguchi et al., 2013; Tlili et al., 2011). Here, current change measurement by resistance or conductance variations caused by cortisol binding on its corresponding antibody has been developed in the name of chemiresistor with the single wall carbon nanotube (SWCNT) channel (So et al., 2005 ; Tlili et al., 2011). In the CNT chemiresistor study, first layer of cortisol-N-hydroxysuccinimide (cortisol-NHS) was coupled on the CNT sensing region, and then cortisol antibody was immuno-bound to the anchored cortisol, and then sample cortisol was introduced to be sensed by immuno-adsorption, which mimicked

typical ELISA having sandwiched antibody layer (Tlili et al., 2011). However, the SWCNT chemiresistor was based on a competitive immunoassay requiring an indirect measurement of cortisol, which the amount of detected molecules could be limeted by the number of pre-bound cortisol-NHS or sandwiched cortisol antibody (Tlili et al., 2011). Therefore, it would be desirable to develop a cortisol chemiresistor available with a direct measurement accompanied by chemical conjugation of cortisol probe or cortisol specific antibody.

Frequently, the biomolecules directly conjugated to other electronic materials were preserved in the electronic device to show no severe electrical failures (Kim et al., 2014; Oh et al., 2013). It implicated that versatile biomolecules including hormone, peptides, nucleotides, and so on would be applicable for electrical sensors (Akkerman and de Boer, 2008).

Meanwhile, carbon based electronic materials apart from the CNTs, such as graphene and gaphene oxide (GO) sheets, have been more actively introduced as a disparate platform for exploring biological sensings targeted to DNA, protein, and even antibiotics (Gu et al., 2003; Katz and Willner, 2004). So far, the GO sheet has been used in many applications including photoluminescent nodes, solar cell, gas sensor, and electronic component (Hung and Whang, 2005; Verbakel et al., 2006; Faber et al., 2009). Separately, reduced GO (rGO) sheet have been used as charging elements in capacitor format and channel layer of thin film transistors (TFTs) format (Kim et al., 2017) because of its two dimentional (2D) nature. Since the biofunctional molecules have been simply coupled to the 2D rGO, the rGO sheets have been frequently proposed as a strong candidate for a biosensing platform (Liu et al., 2013; Kim et al., 2013). In addtion, through hindrance of non-specific binding with denatured bovine serum albumin (BSA)'s π-stacking interactions on the rGO surface, rGO-based electrochemical impedance spectroscopy (EIS) sensors was successfully demonstrated (Kim et al., 2013). The EIS sensor

was based on selective detection of biological binding events of antigen–antibody interaction at a femtomolar range for the cortisol sensing (Kim et al., 2013, 2015). In the other hand, the functionalization method on the rGO nanosheet has been the key factor for a successful application of the rGO. The rGO sheet can be considered as one of oxide compounds, which can form hydroxylic groups easily at surface. Therefore, the chemical conjugations of biofunctionalities on the rGO sheet through strong covalent bonds have been accomplished without critical complications for the EIS or electrical detections (Liu et al., 2013; Kim et al., 2013, 2015). However, the electrochemical detection protocols have several restrictions due to its complexity of the system if they are to be miniaturized.

Typically, for the most of bio-fluids, label-free electrochemical or electrical sensor with a direct contact with liquid sample has overwhelmed other assays including many introductions of rinsing or functionalizing buffers. Even more, for some cases that have limitations in sampling with local concentration variation, the direct sensing is inevitable and promising. In addition, even though there have been plenty of cortisol sensors, their application for field use is not fully explored except for salivary cortisol (Pasha et al., 2014).

In this paper, a disparate approach of cortisol monoclonal antibody (c-Mab) conjugated rGO sheet was proposed as a direct sensing element to drive change of resistance or conductance between two coplanar parallel electrodes. The adoption of the rGO channel was advantageous for a chemiresistor sensing for a direct measurement of the cortisol. Current change derived from cortisol bindings between 2~4 μm short channels were obtained for the charged rGO sheet exhibiting resistance variations, which demonstrated the sensing fundamental. The rGO surface was covalently tethered with the c-Mab by way of chemical activator coupled to be a selective probe molecule targeted to the cortisol. Then, non-specific binding other than c-Mab was

controlled with ethanol amine (EA) passivation (Pasha et al., 2014). In addition, since the cortisol is the principal end product of hypothalamic-pituitary-adrenal (HPA) axis activation, it is very critical to measure the stimulated or induced cortisol level at an adrenal gland of mammal to correlate subseqeuntial physiological responses, or diseases. Here, the rGO chemiresistor was adopted to measure the cortisol level in the buffer solution of the rat adrenal gland slices, which will provide a novel *in vitro* study tool featured for label-free, direct detecting, and fast resposed sensor.

2. Material and Methods

2.1. Fabrication of rGO chemiresistor sensor

Firtst, indium-tin-oxide (ITO) glass (each of width and length was 5 cm, thickness was 0.7 mm, and sheet resistance was 10 Ω/sq.) was rinsed by acetone (J. T. Baker, cMOS), 2-propanol (IPA, J. T. Baker, cMOS), deionized water (DI water, Direct-Q, Millipore) with sonication for 15 min. Then, air gun blowing and drying was applied on the substrate and the galss was annealed on the hotplate at 80 ˚C for 5 min.

The ITO glass was spin coated with hexamethyldisilazane (HMDS, J. T. Baker) and AZ 1512 positive photoresist (AZ electronic materials). Then, the glass wafer was patterned by mask aligner (MDA-8000, Midas Co., Korea) and patterns of source/drain electrodes were devolped with AZ 300 MIF developer (AZ electronic materials). ITO electrode was etched by wet etching process with ITO etchant (LCE-12, Cyanteck Co.) at 37~38 ˚C for 4 min 30 s.

For activated ITO electrodes, patterned glass was treated by UV-ozone cleaner for 30 min. To modify the glass surface, the sample was functionalized with 3-aminopropyl-triethoxysilane (APTES) by dip-coating in 3 vol.% APTES stirred in anhydrous ethanol (Sigma-Aldrich) for 1 h.

Then, the substrate was immersed into graphene oxide solution at concentration of 0.1 mg / mL (GO, Graphene Supermarket, 500 mg/L, GO size: 0.5~5 μm) for dip-coating and subsequent reducing process was performed with hydrazine hydrate (H_2N_4, Sigma-Aldrich) at 200 ˚C for 2 min 30 sec.

2.2. Probe functionalization on rGO surface with cortisol antibody (c-Mab)

The reduced gaphene oxide (rGO) surface was conjugated by the cortisol monoclonal antibody (c-Mab, Abcam Co., 10 μg/mL). The glass substrate having carboxylated rGO surface was immediately added in the c-Mab solution to couple the c-Mab to the carboxylic surface. The 2 μL of 0.1 mM N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC, Sigma-Aldrich) in DI water was treated by gentle incubation for 30 min, and then, 2 μL of 0.15 mM N-hydroxysuccinimide (NHS, Sigma-Aldrich) was also treated for 30 min for the conjugation chemistry. Then, the 2 μL of c-Mab solution was added to have a final concentration (5 μg/mL) for 30 min incubation process and its surface was rinsed by DI water for 5 min. Finally, for a passivation of non-specific protein binding, 2 μL of ethanol amine (EA, Sigma-Aldrich, 100 mM) was added and treated by incubation process for 30 min and rinsed by DI water for 5 min.

2.3. Measurement of standard cortisol solution and salivary cortisol

The rGO device's current-voltage (I-V) performance was measured by Agilent 4156C semiconductor parameter analyzer. For salivary cortisol detections, real salivary sample was collected by a human adut and it was centrifuged to remove other spiked materals, e.g., bulky enzyme or protein contaminants. Then, supernatant of the centrifuged salivary sample was diluted to 10 times and its supernatant was obtained. Separately, to detect the LOD, purchased

cortisol in powder form was dissolved in DI water and tested as standard cortisol solutions with different concentrations. Limit of detection (LOD) test was performed with DI water dilutions from 1 μg/mL to1 ng/mL (3.2 nM, cortisol MW: 362.46 g/mol).

2.4. Acute adrenal gland slice preparation.

Figure S4 shows the preparation process of the adrenal gland acute slice. First, Sprague Dawley rats (8~10-week-old males) were anesthetized by isoflurane and sacrificed by decapitation. The, adrenal glands were separated and surrounding adipose tissue and capsule were removed. Adrenal glands were cut in to half and 400 μm thick slices using a Mcllwain tissue chopper (Mickle Laboratory Engineering Co. Ltd.). For acute adrenal slice, dissected slices were allowed to stabilize in bicarbonate buffered saline (BBS) solution (125 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM glucose) for 2 h while constantly perfused in 95% O_2 and 5% CO_2 mixture. All BBS solutions were adjusted to pH 7.4 by bubbling with 95% O_2 and 5% CO_2.

2.5. Measurement of cortisol concentration of buffer solution of rat adrenal gland slice and proof by ELISA test

The BBS solution on rGO devices were examined by measurement of the I-V by Agilent 4156C semiconductor parameter analyzer, repeatedly. The concentrations of cortisol induced in the BBS solutions were unkown initially before further enzyme-linked immunosorbent assay (ELISA) measurement. Four BBS solutions containing acute rat adrenal gland slices were stimulated by adrenocorticotropic-hormone (ACTH) 1 nM for 1 hr, 1 nM for 2 hr, 10 nM for 1 hr, and 10 nM for 2 hr, respectively. For a control sample of cortisol incubation, powder of cortisol was dissolved into another BBS solution and incubated for 2 hr without ACTH induction. Then,

the control's solution was also measured by the rGO chemiresistor sensor and the ELISA identically with the ACTH induced BBS solutions. Table S1 shows the results of ELISA analysis. For the collection of data in Table S1, all ELISA samples were diluted by 20 times and used for accurate determination of cortisol concentration. And, sample standard ranges from 156 ~ 10,000 pg/mL. All the assays were performed by a single trail, n=1 and the concentrations were determined using the mean percent bound.

3. Results and Discussion

3.1. Fabrication of rGO chemiresistor sensor

Figure 1 shows a schematic illustration of processes to fabricate chemiresistor device. The process consists of simple lithography patterning, chemical binding, and reduction process. Detailed procedures are also explained in material and methods. The Fig. 1 also describes a representative reaction layout of the cortisol monoclonal antibody (c-Mab) conjugation on rGO via EDC/NHS coupling, which is a widely known chemical activator, with carboxyl group on the rGO's surface. The EDC/NHS conjugation has been reported to be successful to anchor the c-Mab on carbon material surfaces (Sun et al., 2008; Tlili et al., 2011). The coupling results from nucleophilic attack of carboxylate of the c-Mab with the EDC through formation of O-acylsourea having a by-product of urea. Even though the reduction from the hydrazine hydrate could reduce the oxygen contents on the rGO, there remained a certain amount of carboxylic group. In addition, the EDC conjugation was regarded to be adequate to anchor the c-Mab on rGO surface, since the chemical conjugation would strictly influence the current path of the rGO upon the c-Mab conjugation and subsequent bonding of cortisol rather than physical adsorption and π-

stacking. Also, the rGO can be regarded as one of oxide compounds like metal oxides (Kim et al., 2014).

And, the EDC coupling agent has been widely adopted as zero-length linker between rGO and protein or peptide, where the linking or immobilization can be randomly achieved (Liu et al., 2013; Kim et al., 2014). Therefore, no severe obstacle in current conduction is anticipated due to the chemical activators for the electrical performance of the biosensor device (Akkerman and de Boer, 2008).

Moreover, very recently, the GO sheet have been increasingly reported as charging nodes for non-volatile memory device (Kim et al., 2017). Therefore, the conjugation of probe molecule like c-Mab and subsequent binding of target molecule such as cortisol could act as charging hode, which will hinder current pathway of the rGO or decrease its current between two electrodes.

Figure S1(a) shows atomic force microscopy (AFM) image of the rGO sensing channel layer. As shown in Fig. S1(a), layers of rGO were deposited in two different thickness (15 nm and 53 nm) in the sensing area depending on dip-coating time and concentrations of the GO solution.

3.2. Measurement characteristics of cortisol standard solutions

Figure 2 shows the I-V results of the chemiresistor device, which shows current change of device under 1 MHz frequency. Fig. 2(a) and Fig. 2(b) correspond to the sensing curves with 15 nm thick sensor. Here, the current levels were obtained by voltage sweep from 0 V to 3 V to survey the I-V measurement characteristics. Figure S2 also shows (a) I-V results of the 53 nm thick rGO chemiresistor sensor and (b) corresponding resistance calibration plot. Especially in Fig. S2(a), the results of continuous 10 times measurements for cortisol solutions (50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, and 500 μg/mL, twice respectively) were provided. It provides that the rGO chemiresistor could be stable under multiple sensing operations within

some variations between two identical cortisol concentration measurements. Separately, when the rGO layer was multiply stacked, the detecting current level of 53 nm rGO sensor was 3 ~ 4 order lower than 10^{-2} Amp current level of the 15 nm rGO sensor. As shown in Fig. 2(a) and Fig. S2(a), the resistance of the chemiresistor sensor increase or the currrent of the sensor decrease in a sequence with a pristine rGO sensor, c-Mab conjugated sensor, and cortisol bound rGO sensor, respectively. At the same time, both sensors showed differently sloped linear I-V curves depending on the correspondingly different cortisol concentrations of detected samples.

Therefore, chemiresistance effects on the rGO sheet were successfully demonstrated as shown in Fig. 2(a) and Fig. S2(a). Current level decreases or resistance increases sequentially upon conjugation of probe c-Mab and specific binding of cortisol on the c-Mab. At the same time, the higher level of cortisol was tested, the more resistances were shown with reduced current levels in Fig. 2(a) and Fig. S2(a). Disparate current levels were indentified upon test solutions of cortisol at the concentrations at 1 μg/mL (2.76 μM), 0.1 μg/mL (276 nM), 0.01 μg/mL (27.6 nM), 0.005 μg/mL (13.8 nM), and 1 ng/mL (2.76 nM). The I-V measurement could be repeated more than 10 times. Under low temperature preservation at 4 ^{o}C, the device in this study could be operable for several months in terms of stability (Oh et al., 2013).

When Fig. 2(a) and Fig. S2(a) are compared to each other, the I-V realtions of the Fig. 2(a) were straightly linear, while the I-V realations of the Fig. S2(a) showed non-straight linear or S-shaped depending on the applied voltage. At the same time, the sensing current level for 15 nm rGO sensor of Fig. 2(a) was upto ~10^{-2} Amp, which would have low fluctuations in measurement compared to ~10^{-6} Amp level of Fig. S2(a). Therefore, in this study, the 15 nm thick rGO sensor were mainly chosen for further sensing experiments for salivary sample and acute rat adrenal gland slices.

In addition, the magnitude of sensing current (10^{-2} Amp) in Fig. 2(a) was much higher than previously reported chemiresistor's source-darin current with single wall carbon nanotubes (SWCNTs) sensor for cortisol detection (Tlili, et al. 2011). Therefore, an direct detection for the cortisol could be more sensitively or efficiently accomplished with the rGO chemiresistor sensor unlike an indirect measurement strategy adopting pre-adsorbed c-Mab analogue layer.

Here, in Fig. 2(b) and Fig. S2(b), replottings or calibrations were represented based on the Fig. 2(a) and Fig. S2(a) curves, which were obtained by the calculation of ratios, e.g., resistance of sensor having cortisol sample (R_{cort}) – resistance of c-Mab conjugated rGO sensor ($R_{c\text{-}Mab}$) / resistance of pristine rGO (R_{rGO}) – resistance of c-Mab conjugated rGO sensor ($R_{c\text{-}Mab}$). The resistance or current values were chosen and taken at 2 V in Fig. 2(a) and Fig. S2(a) for all $R_{rGO,}$ R_{cort}, and $R_{c\text{-}Mab}$ values, in a goal of low voltage operation for the sensor measurement.

The calibration plots of the $R_{cort} - R_{c\text{-}Mab}$ / $R_{rGO} - R_{c\text{-}Mab}$ in Fig. 2(b) and Fig. S2(b) were obtained by least square method and they could provide the normalized resistance changes of sensor, which could minimize variations of sensor performances depending on the fabrication batch processes or detection protocols. In addition, the normalized calibration would be more an accurately designed parameter to pursue a real time measurement of the sensor in the further applications (Liu, et al. 2013). The magnitude of $R_{cort} - R_{c\text{-}Mab}$ / $R_{rGO} - R_{c\text{-}Mab}$ values roughly linearly increase with the cortisol concentration of samples as shown in Fig. S2(b).

However, unlike the Fig. S2(b), an inlet graph of Fig. 2(b) did not show linear relationship between the resistance calibaration and the standard solution's cortisol concentrations. As indentified in Fig. 2(b), a linear relation could be kept only within the range of 1 ~ 10 ng/mL. High concentrations such as 100 ng/mL or 1,000 ng/mL cortisol were severly deviated from the linear relation with the resistance calibration. Therefore, for the detection of real samples such as

human salivary cortisol, it was concluded that dilution of samples would be advantageous. Those non-linearities having a saturated detection performances like the Fig. 2(b) are believed from the saturation of available probe molecules of the c-Mab at the surface of the rGO.

3.3. Measurement characteristics of real salivary cortisol and stimulated or induced cortisol by ACTH from acute rat adrenal gland slice

Figure 3 shows resistance calibration plot of I-V measurement for detection of human salivary samples at different ratios of 1×, 10×, 100×, and 200× dilutions as shown in Figure S3 having an extrapolated cortisol concentration plot calculated from the linear fitting region between the resistance calibration and cortisol standard soluions in Fig. 2(b) (1 ~ 10 ng/mL).

Typically, the level of salivary cortisol has been known to range between 1 ng/mL and 8 ng/mL in healthy adults (2.78 ~ 22.2 nM) (Arya et al., 2010a). Therefore, the ability to detect 200× diluted sample as shown in Fig. 3 proves that the rGO chemiresistor could cover the typical salivary sample. By comparison of Fig. 2 obtained by test solutions, the level of 1× salivary cortisol was measured as 2 ng/mL (5.55 nM) as calculated in $R_{cort} - R_{c\text{-}Mab}$ / $R_{rGO} - R_{c\text{-}Mab}$ ratio. Therefore, the 200× diluted sample in Fig. 3 corresponds to 10 pg/mL (27.75 pM) detection, which is quantified as the LOD. Further dilution after 200× sample did not produce any futher resistance change.

Figure 4 shows a calibration plot for the induced or sitmulated cortisol measurement originated from cortisol detection I-V realations in the buffer solution of rat adrenal gland slices induced by ACTH as shown in Figure S5. Meanwhile, a level of salivary cortisol could remark a mild mind-body inter-relationship, the cortisol level of cell incubation buffer will be severely correlated with chronic diseases. (Sun et al., 2008). Actually, it has been known that the level of salivary cortisol is strongly correlated with that of serum (Pasha et al., 2014). However, the

cortisol level in the adrenal gland cell has not been matched with other physiological fluids. By the sensing with the rGO chemiresistor, it was measured that the concentration of the cortisol was the highest with the cortisol-induced solution stimulated by 10 nM ACTH for 2 hours and the lowest with the buffer solution by 1 nM and one hour. For a cross-validation of the chemiresistor`s performance, cortisol ELISA test were executed for the identical samples. Fig. 4 and Table S1 shows, the results of the ELISA, which proves that the sensing ability of the chemiresistor exactly corresponds to the ELISA results.

The buffer solutions of the rat adrenal gland slices were stimulated by ACTH, which would provide not only cortisol but also various meuroendocrines, or cortisol-like hormones, in chemical structure, and biological funtions. Therefore, the rGO chemiresistor sensor could show somewhat interruptions by other hormones to sense the cortisol perfectly and selectively, when compared with the control sample having cortisol incubation for 2hr, as shown in Fig. 4 and Table S1. However, as shown in Fig. 4, the proof measurement of the ELISA had a wide standard deviation (SD) with the control sample and high concentrated cortisol sample. In addition, it is believed that there can be some amount of corticosteroid-binding globlin (CBG) in the induction or incubation buffers of the acute slices, which could bind cortisol molecules in blood upto 90 % of the cortisol (Arya et al., 2010b). Therefore, Fig. 4 and Table S1 confirmed that the rGO chemiresistor could work successfully with neuro-cell culture solution, which has abundant extra neuroendocrines, neuro-transmitters, proteins, and so on. Since the rGO chemiresistor was validated by label-free, direct quantifying, and point-of-use principle, its utlilization on the cortisol detection in the cell slice buffer solution was very adequate for the *in vitro* research of cortisol physiology.

4. CONCLUSION

In conclusion, the fabrication of cortisol chemiresistor sensor using rGO sheet has been demonstrated in a simple format of 2 ~ 4 μm short channel between two coplanar electrodes as a proof-of-concept. The I-V characteristics of the rGO chemisresistor sensor exhibited the LOD of 10 pg/mL cortisol by immune-sensing of c-Mab. In addition, effective detections were confirmed with cortisol samples of adult human saliva and buffer solutions of acute adrenal gland slice of rat, which were induced by ACTH stimulus, respectively. The results in this study can extend a perspective on the development of a direct electrical sensor for salivary cortisol and *in vitro* study of cortisol-related psychobiology related organs.

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Figure 1. Schematic diagram of process to fabricate rGO chemisresistor sensor

Figure 2. (a) I-V curves of cortisol solutions on 15 nm thick rGO chemiresistive sensor, and (b) resistance calibration plot of various concentrations of cortisol solutions on the 15 nm thick sensor with an inlet graph of logarithmic concentration scale

Figure 3. Resistance calibration plot of I-V measurement with salivary cortisol sample on 15 nm rGO chemiresistive sensor

Figure 4. Resistance calibaration plot vs. corresponding ELISA measurement of cortisol induced or stimulated by ACTH from acute adrenal gland slice of rat

Figure 1. Biosens. Bioelect., Kim, et al.

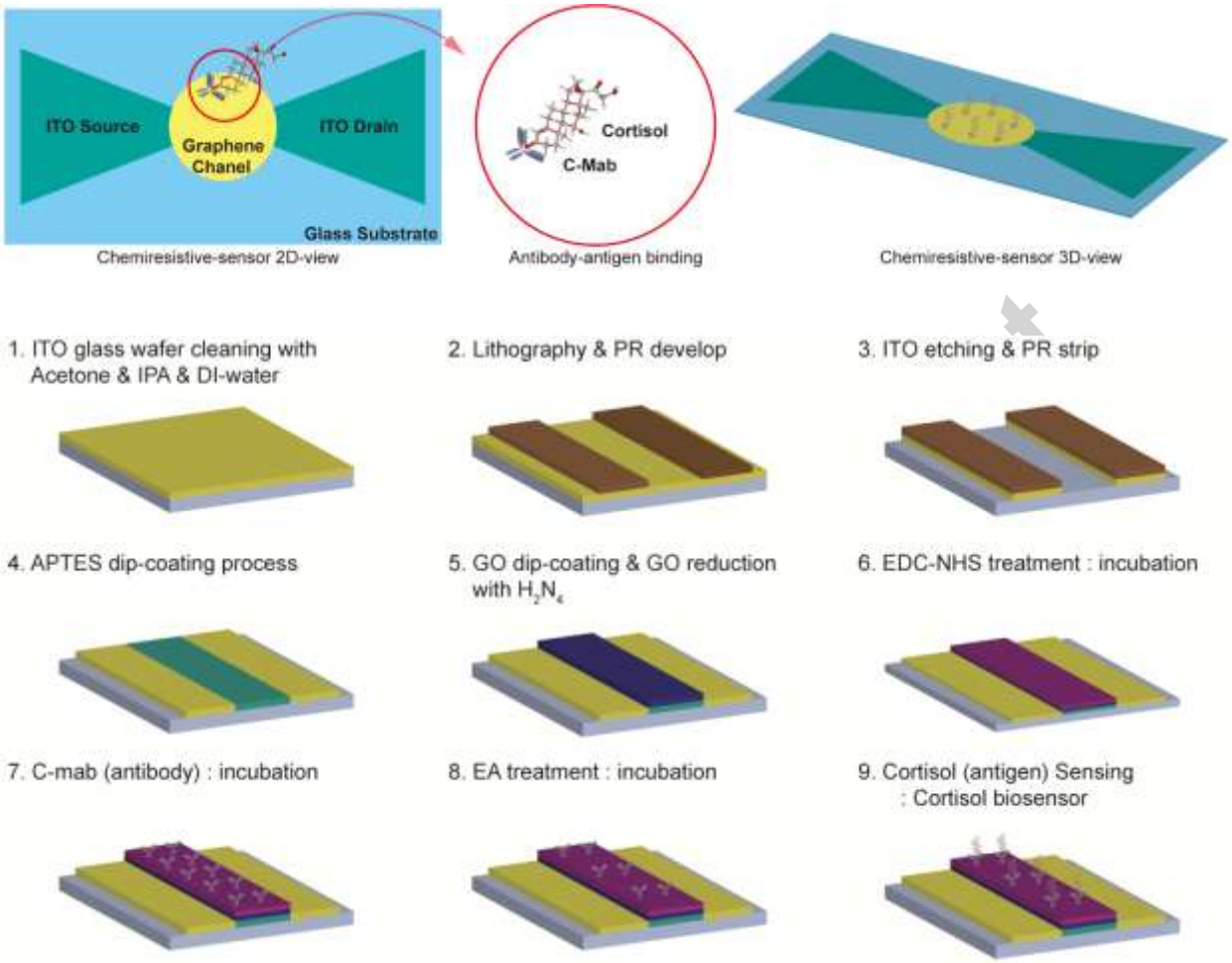
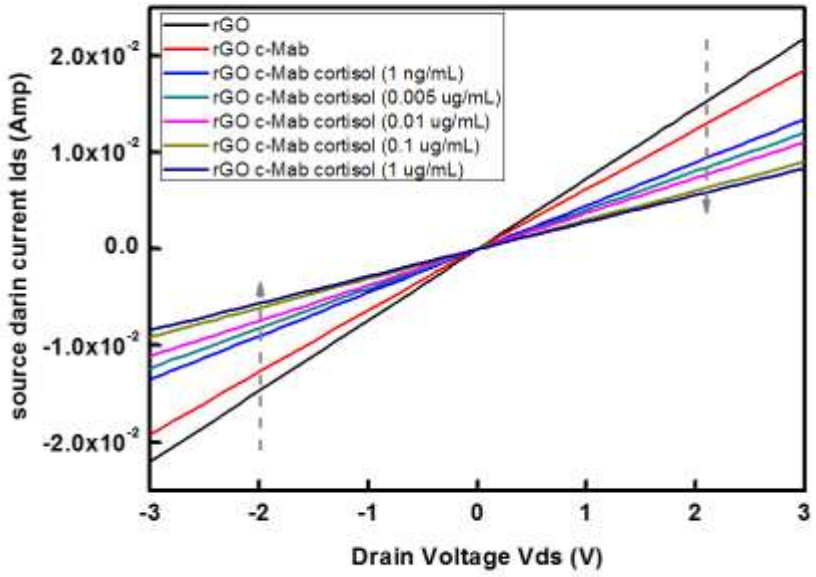
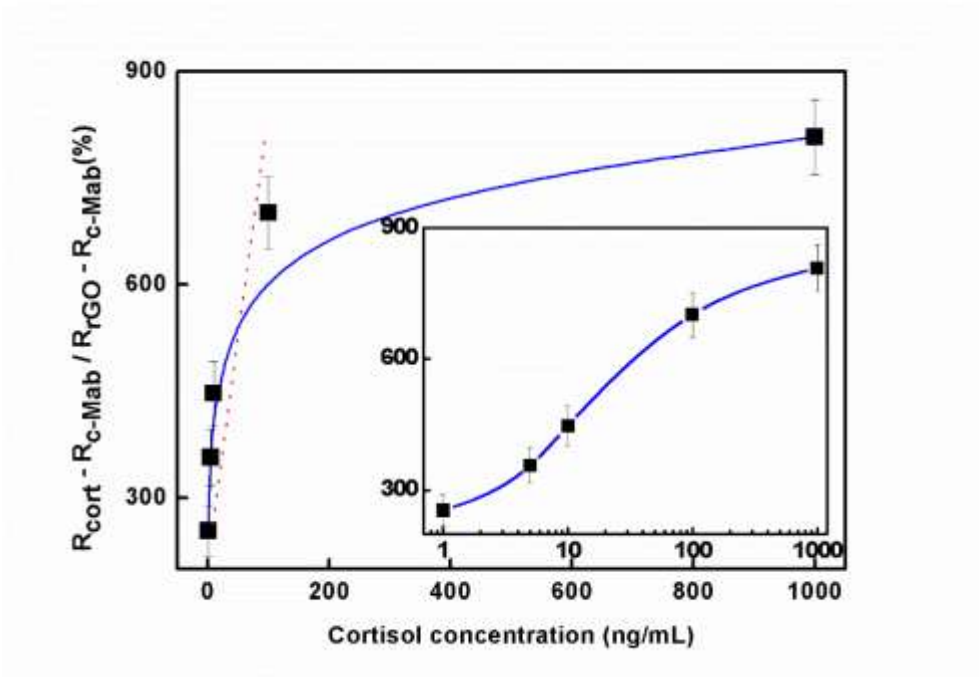


Figure 2. Biosens. Bioelect., Kim, et al.



(a)



(b)

Figure 3. Biosens. Bioelect., Kim, et al.

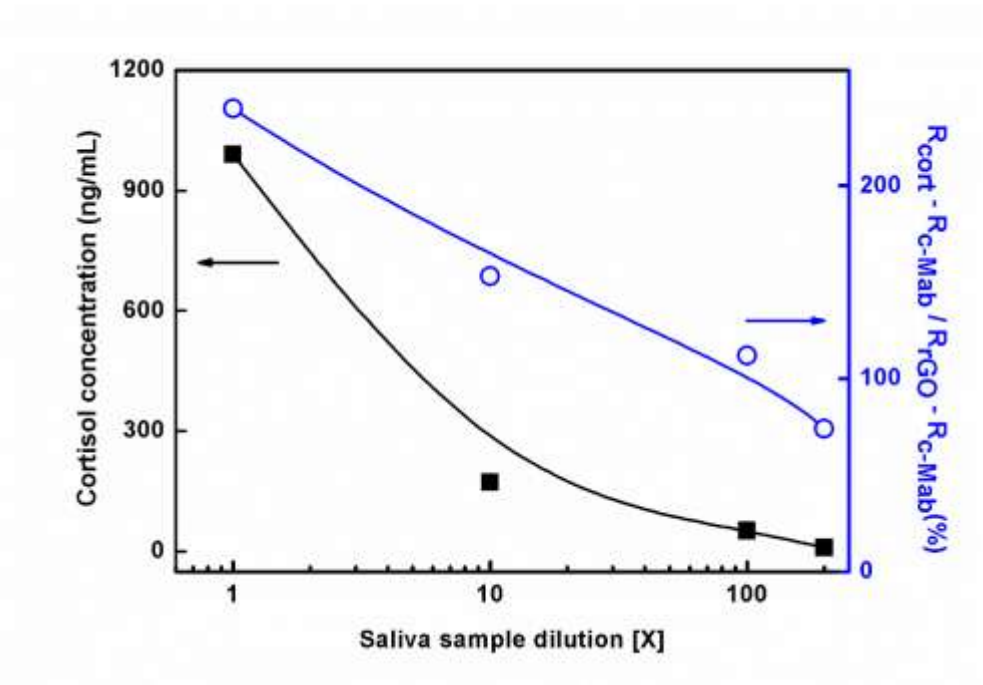
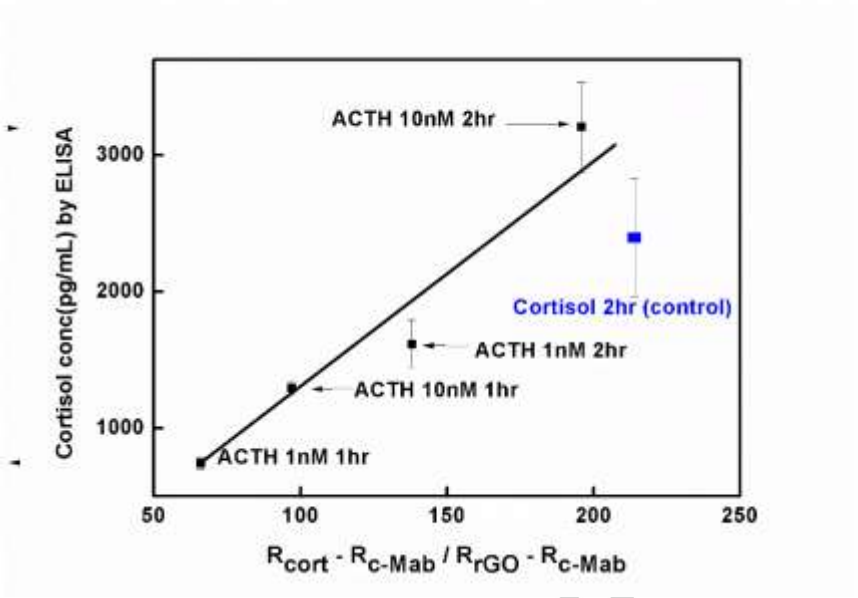


Figure 4. Biosens. Bioelect., Kim, et al.



Highlights

- Electronic chemiresistive sensor was demonstrated on rGO channel for cortisol detection using c-Mab in a direct measurement protocol.
- The rGO chemiresistor could measure cortisol's concentrations in human saliva and induced from adrenal gland slices by ACTH.
- Measurement profile of cortisol detection was correlated and confirmed by ELISA analysis for future *in vitro* physiological study about stress hormone.