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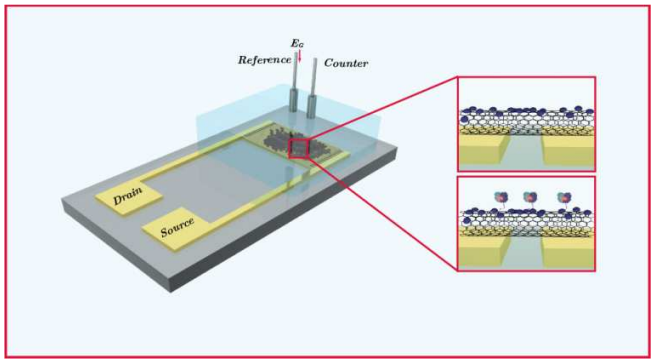

Fabrication of a liquid-gated enzyme field effect device for sensitive glucose detection

M. Fathollahzadeh,[a] *M. Hosseini,*[b] *B. Haghighi,*[c,a*] *M. Kolahdouz,*[b*] *M. Fathipour* [b]

[a] *College of Chemistry, Institute for Advanced Studies in Basic Sciences, P.O. Box 45195-1159, Gava Zang, Zanjan, Iran.*

[b] *Department of Electrical and Computer Engineering, University of Tehran, Tehran, Iran.*

[c] *Department of Chemistry, College of Sciences, Shiraz University, Shiraz 71454, Iran*

Abstract

This study presents fabrication of a liquid-gated enzyme field effect device and its implementation as a glucose biosensor. The device consisted of four electrodes on a glass substrate with a channel functionalized by carboxylated multi-walled carbon nanotubes-polyaniline nanocomposite (MWCNTCOOH/PAn) and glucose oxidase. The resistance of functionalized channel increased with increasing the concentration of glucose when an electric field was applied to the liquid gate. The most effective and stable performance was obtained at the applied electric field of 100 mV. The device resistance, R, exhibited a linear relationship with the logarithm of glucose concentration in the range between 0.005 and 500 mM glucose. The detection limit (S/N=3) for glucose was about

* Corresponding author. Tel: +98 24 3315 3126, Fax: +98 24 3315 3232
E-mail address: haghighi@iasbs.ac.ir (B. Haghighi)
*Corresponding author. Tel: +98 21 6111 9749, Fax: +98 21 6111 9749
E-mail address: Kolahdouz@ut.ac.ir (M. Kolahdouz)

0.5 μM. Large effective area and good conductivity properties of MWCNTCOOH/PAn nanocomposite were the key features of the fabricated sensitive and stable glucose biosensor.

Keywords:

Liquid-gated enzyme field effect device; glucose biosensor; MWCNTs-polyaniline nanocomposite; glucose; diabetes.

1. Introduction

Nowadays, diabetes is introduced as a worldwide public health crisis. Diagnosis of diabetes and its control require continuous and exact monitoring of blood glucose level. Accordingly, many devices with different designs, fabrication procedures and detection protocols have been proposed [1-4] for glucose determination. However, manufacturing of a cheap, easy to use and fully biocompatible device is still a challenge. Among the various devices which have been developed for glucose detection, glucose biosensors find a special place. In general, a biosensor is an analytical device in which a biological component has been combined with a physicochemical transducer for the detection of a specific analyte. The transducer transforms the arising signal from the interaction of the analyte with the biological element into a measurable signal (e.g. current, voltage, etc.) which can be quantified easily [5-7].

A field-effect transistor (FET) is a promising electronic device which can be employed as a physicochemical transducer for the fabrication of the miniaturized biosensors. The electric field in a field-effect transistor controls the conductivity of its channel. In the other word, the voltage applied between gate and source terminals of a FET (V_{GS}) influences the current between its drain and source terminals (I_{DS}) [8-11]. It seems one-dimensional (1D) nano-materials are attractive candidates not only for the formation of conductive channels but also for the providing high effective area for the immobilization of bio-molecules [12-17]. Based on the mentioned idea it is possible detect very low concentrations of various analytes using a FET constructed by 1D nano materials which its channel region has been functionalized with the biomolecule such as enzyme or antibody [18-20]. The binding of the analyte to the functionalized channel of the mentioned FET causes a change in the gate electric field and consequently change in I_{DS}.

In this work, the channel of the proposed field-effect device (FED) was functionalized by carboxylated multi-walled carbon nanotubes-polyaniline nanocomposite (MWCNTCOOH/PAn) and glucose oxidase (GOx) to fabricate a glucose biosensor. The proposed nanocomposite polymer provided a high effective surface area in the channel with a confined electric transport. In the proposed design, the applied electric field affects the device performance but not in the usual way which is seen in FETs.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical reagent grade and used without further purification. Multi-walled carbon nanotubes (MWCNTs, 95% purity, OD = 10-30 nm, ID = 5-10 nm and length = 0.5-500 nm) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aldrich (Steinheim, Germany). The aniline monomer, dimethyl sulfoxide (DMSO), HNO_3, H_3PO_4, KOH and KCl were obtained from Merck (Darmstadt, Germany). N-hydroxysuccinimide (NHS) and ethanol (96%) were obtained from Fluka (Buchs, Switzerland). Glucose oxidase (GOx, EC 1.1.3.4, type VII from Aspergillus Niger, 221 U mg^{-1}) and D (+)-glucose (97%) were obtained from Sigma (St. Louis, MO, USA). Aniline and DMSO were purified before use. Deionized water was used throughout.

0.01 M H_3PO_4 and 0.01 M KCl were used to prepare phosphate buffer solution (PBS, 0.01 M) as supporting electrolyte and its pH was adjusted to 7.3 using KOH solution. 0.8 mg of GOx was dissolved in 400 μL of PBS (0.01 M, pH=7.3) to prepare a 442 U mL^{-1} of GOx working solution. A

stock solution of glucose (1 M) was prepared using deionized water and stored at 4 °C when not in use. The glucose stock solution was allowed to mutarotate at room temperature for at least 24 h before use. Standard working solutions of glucose were prepared freshly by diluting its stock solution with PBS (0.01 M and pH=7.3).

2.2. Preparation of MWCNTCOOH/PAn nanocomposite

Carboxylated multi-walled carbon nanotubes (MWCNTCOOH) was prepared by refluxing of MWCNTs (30 mg) in HNO_3 (40 mL, 70%) for at least 16 h. The product was filtered, washed thoroughly to reach a pH of 7.0 for the filtrate and then dried in a vacuum oven. MWCNTCOOH/PAn nanocomposite was synthesized using the procedure reported previously by Hua et al. [21] without modification.

2.3. Preparation of MWCNTCOOH/PAn/GOx functionalized FED

Glass substrate (GS) was used as a cheap substrate to fabricate the proposed FED-based glucose biosensor. After cleaning with piranha solution (H_2SO_4 - H_2O_2, 3:1 V/V), Ti/Au layers with respective thicknesses of 100 nm and 200 nm were deposited on the glass substrates. A pair of gold interdigitated microelectrodes as shown in Fig. 1 with 50 fingers (finger dimensions: width 40 μm, length 4000 μm, interfinger spacing 40 μm) was patterned on Ti/Au layer via a photolithographic process to prepare S and D terminals of the proposed FED. Six mg of MWCNTCOOH and six mg of MWCNTCOOH/PAn were dispersed in 1 ml of PBS by ultrasonic agitation for 15 min. A solution containing of 20 μl of each suspension was prepared and drop cast on the surface of S and D

terminals and the solvent was evaporated at room temperature. The MWCNTCOOH/PAn functionalized FED was later treated with a mixture of 20 mM NHS and 10 mM EDC solution for 2 h and then 50 μL of PBS containing desired amount of GOx was placed on the surface of functionalized S and D terminals. The GOx-treated functionalized FED was left for about 1 h at room temperature to bind GOx and then rinsed with deionized water to remove unbounded GOx. The prepared FED with the functionalized channel was stored in a refrigerator at 4°C for further use. The whole reactions were carried out in PBS (0.01 M) at pH of 7.3 and 25 °C.

2.4. Apparatus and measurements

A Metrohm 691 pH meter was used for pH adjustment. Field emission scanning electron microscopy was performed on a HITACHI S-4160 (Hitachi, Japan). A Plexiglas chamber with a volume of 1 mL was fabricated for all solution-based measurements. As shown in Fig.1, a liquid-gated field effect device was configured using the prepared Plexiglas chamber. Electrical measurements were conducted using a Keithley 2361 parameter analyzer with a four-terminal setup. An Ag|AgCl|KCl_{sat} electrode and a platinum wire were immersed in electrolyte solution (PBS, 0.01 M, pH=7.3) and used as the reference (R) and auxiliary (C) electrodes, respectively. Two terminals of Keithley analyzer were connected to the R and C and the other two terminals were connected to the S and D terminals of the prepared functionalized FED. The distance between the R and S/D terminals was carefully controlled. The S terminal was connected to the ground and the gate voltage (V_{GS}) was applied between S and R. The counter electrode was used to minimize the current passed through the electrolyte solution between the gate and S/D. All measurements were performed at room temperature.

3. Results and Discussion

The SEM images of the prepared MWCNTCOOH, MWCNTCOOH/PAn and MWCNTCOOH/PAn/GOx are shown in Fig. 2. It can be seen that the modification of MWCNTCOOH by PAn and GOx produced a smooth surface with a slight increase in tube diameters of both PAn/MWCNTCOOH and MWCNTCOOH/PAn/GOx nanocomposites. Fig. 3 illustrates the current-voltage (I_{DS}-V_{DS}) characteristics of the MWCNTCOOH/PAn functionalized FED before and after immobilization of GOx in dry status i.e. without electrolyte. As shown in Fig. 3, both MWCNTCOOH/PAn functionalized FED and MWCNTCOOH/PAn/GOx functionalized FED showed ohmic behavior, linear I-V characteristic, with a constant dI/dV values (conductivity). The conductivity of conductive channel between S and D in MWCNTCOOH/PAn (0.0241 Ω^{-1}) was higher (for about 1.8 times) than that observed for MWCNTCOOH/PAn/GOx (0.0134 Ω^{-1}) functionalized FED. The decline of conductivity of the conductive channel between S and D in MWCNTCOOH/PAn/GOx functionalized FED can be explained by the fact that the covalent bond between the GOx and MWCNTCOOH/PAn nanocomposite accumulates positive charges on the nanocomposite surface because of the presence of net negative surface charge of GOx. This electrostatic interaction reduces the hole conductivity of the p-type polymer by decreasing the number of available holes for conductance. Similar phenomenon has been observed in previous study [13] in which the change of conductance in receptor-functionalized field effect transistors has been attributed to the charge accumulation in the channel.

In order to determine the lower and upper limits of the gate voltage (V_{GS}) in experimental measurements, I_{DS} was recorded while sweeping V_{GS} between -0.2 and +1.2 V in the absence of glucose. As shown in Fig. 4, the redox reaction of polymer and reduction of dissolved oxygen are

observed at the starting potentials about 0.5 and -0.1 V, respectively. Therefore, the applicable V_{GS} was in the range between -0.1 and 0.5 V.

In order to investigate the effects of PAn and GOx immobilization on the sensor response, the gate voltage, $V_{GS,}$ was fixed at 100 mV. Thereafter, current-voltage (I_{DS}-V_{DS}) characteristic curves obtained for MWCNTCOOH/GOx, MWCNTCOOH/PAn and MWCNTCOOH/PAn/GOx functionalized FED in the absence and presence of 10 mM glucose in PBS (0.01 M and pH=7.3). As shown in Fig. 5, the conductivity of conductive channel between S and D in MWCNTCOOH/GOx (0.0030 Ω^{-1}) was about 4.3 times of that observed for MWCNTCOOH/PAn/GOx (0.0007 Ω^{-1}) in the presence of 10 mM glucose. But, the conductivity of conductive channel in the absence of glucose for MWCNTCOOH/PAn/GOx and MWCNTCOOH/PAn was similar and about 0.0038 Ω^{-1}. Also, the conductivity of conductive channel for MWCNTCOOH/PAn in the absence and presence of 10 mM glucose was similar and about 0.0038 Ω^{-1}. The obtained results verified that the presence of net negative surface charge of GOx accumulated the positive charges on the surface of nanocomposite, efficiently. In the presence of a p-type polymer such as that used in this study, PAn, the number of available holes and consequently the hole conductivity decreased significantly by electrostatic interaction between net negative surface charge of GOx and the accumulated positive charges.

Thereafter, the current-voltage (I_{DS}-V_{DS}) characteristic curves obtained for MWCNTCOOH/PAn/GOx functionalized FED in PBS (0.01 M and pH=7.3) and in the presence of glucose with the concentrations of 0.0, 0.005, 0.05, 0.5, 5, 50 and 500 mM at the gate voltage of 100 mV. As shown in Fig. 6 a linear behavior is observed for the proposed device in the presence of the glucose at different applied V_{DS}. Figure 7 demonstrates the calculated resistance values from the linear I-V curves shown in Fig. 6 at different glucose concentration. The decrease of conductance or

increase of resistance, with increasing the concentration of glucose can be explained considering enzymatic reaction of glucose oxidase with glucose and formation of the products [22]. Glucose is oxidized by the immobilized GOx on the surface of MWCNTCOOH/PAn/GOx functionalized FED and D-glucono-δ-lactone is produced as the product of enzymatic reaction. Subsequently, D-glucono-δ-lactone is hydrolyzed and the concentration of H^+ increases on surface of conductive channel of the functionalized FED. The positive field induced by H^+ at the surface of channel reduces the hole conductivity of the p-type MWCNTCOOH/PAn nanocomposite. So, an increasing trend is observed for the resistance with increasing the concentration of glucose. However, the resistance value reached to the plateau at the concentration about 500 mM glucose where the whole channel surface employed for the sensing process saturated with H^+ and adding more glucose does not change positive field induced by H^+, remarkably.

3.1. Analytical performance of MWCNTCOOH/PAn/GOx functionalized FED towards glucose

The parameter of "Glucose Coefficient of Resistance, (GCR)" shown in Eq. 1 was defined as the normalized change of the resistance with the change of glucose concentration:

$$GCR = \frac{1}{R_o}\frac{\partial R}{\partial [G]} \quad (1)$$

in which $R_\circ$ and G are the resistance of channel in the presence of electrolyte solution and glucose concentration, respectively. Using the proposed definition, an ideal sensor is a device which has a constant GCR at different concentration of analyte similar to that presented for the sensitivity of calibration curve. The log - log plot of GCR value vs. concentration of glucose obtained for MWCNTCOOH/PAn/GOx functionalized FED is shown in Fig. 8. As clear, the GCR value for low

concentration of glucose is much higher than that observed for high concentration. It means, with increasing the concentration of glucose the resistivity of conductive channel due to the presence of higher amounts of H^+ increases, the surface of conductive channel gets closer to saturation, ∂R and consequently GCR value decreases. Also, a straight line with a definite slope, S, is observed in the presented log - log plot that for an ideal sensor the S value is zero. So, the S value can be defined as a parameter for the comparison purposes.

In order to evaluate the effect of V_{GS} on the S value of the sensor, the gate voltage was swept from -200 to 200 mV and the S value was obtained from the I-V plot and GCR value at the applied V_{GS}. The minimum S value, S = 0.89, for MWCNTCOOH/PAn/GOx functionalized FED obtained at V_{GS} = 100 mV.

Under optimum experimental conditions, V_{GS} = 100 mV and V_{DS} = 10 mV, the plot of sensor response, R, versus logarithm of glucose concentration was linear in the concentration range between 0.005 and 500 mM glucose (Fig. 9). The detection limit (S/N=3) for glucose was calculated to be about 0.5 μM. The analytical features of some reported glucose biosensors based on the concept of field effect transistor (FET) are summarized in Table 1 and compared with those obtained in this study using MWCNTCOOH/PAn/GOx functionalized FED. As shown in Table1, the linear dynamic range of the proposed FED-based glucose biosensor was wider (2-3 orders of magnitude) than those reported previously for some glucose biosensors based on the concept of field effect transistor (FET) [11, 12, 23-26]. The response time of the proposed FED-based glucose biosensor (about 10 s) was more or less similar to those reported previously for some FET-based glucose biosensors [11, 12, 26]. But, the limit of detection of the reduced graphene oxide - carboxylated polypyrrole nanotube hybrids

(rGO/C-PPy NT) FET-based glucose biosensor [27] was better (about 2 orders of magnitude) than that obtained in this using the proposed FED-based glucose biosensor.

The operational stability of the fabricated biosensor was examined by monitoring of its response towards repetitive measurements of 0.01 mM glucose for ten times in each day over one week. The response of the proposed sensor gradually decreased to almost 90% of its initial value after one week.

3.2. Real sample analysis

The proposed FED glucose biosensor was employed for the determination of glucose in normal human serum, to examine its applicability for real sample analysis. 10 μL of human serum was mixed with 3 mL PBS and then analyzed using the proposed biosensor. The concentration of glucose in serum was determined and compared with that determined in local hospital. The concentration of glucose in human serum was determined to be 5.89 mM. The recovery of the analysis was about 97.3 %, considering the value determined by a local hospital (6.05 mM). Also, the reliability of the proposed glucose biosensor was evaluated using standard addition method with the spiking of specific amounts of glucose to the normal human serum. The recovery for each measurement was calculated by comparing the results obtained in the absence and presence of specific amounts of glucose. The obtained average recovery for the spiked samples was 94%.

3.3. Effect of interferences

The effect of the presence of some potent interfering compounds such as carbohydrates (galactose, maltose and fructose) and ions (K^+, Na^+, Mg^{2+}, NO_3^- ,SO_4^{2-}, Cl^-, Zn^{2+} and acetate) on the electric response, R, of the fabricated biosensor was investigated to evaluate the selectivity of

proposed glucose biosensor. A series of solution containing 0.01 mM glucose plus interfering compounds (1 mM) were prepared and their responses were compared with that obtained for standard glucose solution. A 5 % error criterion was adopted. The analysis of results showed that the tolerance of biosensor response for 0.01 µM glucose was less than 5% in the presence of 1 mM galactose, maltose, fructose, K^+, Na^+, Mg^{2+}, NO_3^- ,SO_4^{2-}, Cl^-, Zn^{2+} and acetate. The response of the sensor, R, increased for about more than 10% in the presence of 2 M galactose, maltose, fructose, K^+, Na^+, Mg^{2+}, NO_3^- ,SO_4^{2} and Zn^{2+}. But, the sensor response decreased 10% in the presence of 2 M Cl^- and acetate.

3.4. FED vs. FET

It should be mentioned that the field in the proposed FED controls the resistance of conductive channel but not depletion and inversion of charges at the semiconductor/dielectric surface which usually observed in FET. So, the presented FED is not a transistor in contrary to the FET presented by Yoon et al. [12]. The manufactured FETs are benefitting from built-in amplification (V_{GS} to I_{DS}), however, FED has exhibited a very low price and short time of fabrication. The fabrication of FED is very simple compared to the transistor fabrication. The linear behavior of FED causes to have an easy measurement by the use of a multiplier. Moreover, FED sensor can work using a simple battery in contrary to the electrochemical sensors which may be need to the sophisticated sweeping voltage tools such as potentiostat.

4. Conclusions

Implementation of a liquid-gated FED with a four-electrode configuration for glucose detection was successfully demonstrated. The conductive channel of FED was functionalized by carboxylated multi-walled carbon nanotubes-polyaniline nanocomposite and glucose oxidase. In the proposed FED the resistance of conductive channel was monitored in contrary to that usually observed in FET i.e. the depletion and inversion of charges at the semiconductor/dielectric surface. The change of channel resistance was attributed to the change of electrostatic charges on the channel surface. The prepared biosensor showed high sensitivity towards glucose, wide range of linearity and low detection limit.

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Figures captions:

Fig. 1. Schematic diagram of the prepared four-electrode liquid-gated functionalized field effect device (FED) with the magnified view of the finger and the channel.

Fig. 2. SEM images of the prepared (a) MWCNTCOOH, b) MWCNTCOOH/PAn and c) MWCNTCOOH/PAn/GOx

Fig. 3. Current-voltage (I_{DS}-V_{DS}) characteristic curves for MWCNTCOOH/PAn functionalized FED before (broken line) and after (solid line) GOx immobilization in dry status.

Fig. 4. Current-voltage (I_{DS}-V_{GS}) characteristic curve for MWCNTCOOH/PAn/GOx functionalized FED in PBS (0.01 M and pH=7.3) and in the absence of glucose.

Fig. 5. The effect of (A) PAn and (B) GOx immobilization on current-voltage (I_{DS}-V_{DS}) characteristic curves of different functionalized FED. Conditions: V_{GS} = 100 mV; supporting electrolyte; PBS (0.01 M and pH=7.3); A) MWCNTCOOH/GOx (broken line) and MWCNTCOOH/PAn/GOx (solid line) in the presence of 10 mM glucose and MWCNTCOOH/PAn/GOx (dotted line) in the absence of glucose; B) MWCNTCOOH/PAn (broken line) and MWCNTCOOH/PAn/GOx (solid line) in the presence of 10 mM glucose and MWCNTCOOH/PAn/GOx (dotted line) in the absence of glucose (dotted line).

Fig. 6. Current-voltage (I_{DS}-V_{DS}) characteristic curve for MWCNTCOOH/PAn/GOx functionalized FED in PBS (0.01 M and pH=7.3) and in the presence of 0.0, 0.005, 0.05, 0.5, 5, 50 and 500 mM of glucose. V_{GS} = 100 mV. Inset: magnified view of a part of I_{DS}-V_{DS} curve.

Fig. 7. Plot of resistance value, R, calculated from the slope of I_{DS}-V_{DS} curve shown in Figure 6 at different concentration of glucose.

Fig. 8. Logarithmic plot of GCR value at different concentration of glucose obtained for MWCNTCOOH/PAn/GOx functionalized FED. V_{GS} = 100 mV.

Fig. 9. Calibration plot of sensor response, R, versus logarithm of glucose concentration. Conditions: supporting electrolyte; PBS (0.01 M and pH=7.3), V_{GS} = 100 mV, V_{DS} = 10 mV.

Table 1

Analytical features of some reported glucose biosensors based on the concept of field effect transistor (FET) and fabricated MWCNTCOOH/PAn/GOx functionalized FED.

Modified surface	sensitivity	LDR	DL	Response time (s)	pH	Reference
CPNTs	nr	0.5-50 mM	nr	5-10	7.0	[12]
PEDOT-PSS	1.6 μA/mM	1.1-16.5 mM	nr	10-20	6.5	[11]
ZnO	nr	1-100 μM	~ nM	<0.1	7.4	[23]
CVD-grown graphene	nr	3.3-10.9 mM	3.3 mM	nr	7.4	[24]
rGO/C-PPy NT	nr	nr	1 nM	<1	7.4	[27]
EGFET	7 mV/mM	2-8 mM	nr	nr	5.5	[25]
ZnO	2.7 A/mM	10-30 μM	nr	20	7.4	[26]
CNTCOOH/PAN	nr	0.005-50 mM	0.5 μM	10	7.4	This work

LDR, linear dynamic range; DL, detection limit; CPNTs, carboxylated polypyrrole nanotubes; PEDOT-PSS, poly (3, 4-ethylenedioxythiophene-poly(styrene-sulfonate); ZnO, Zinc oxide; CVD, chemical vapor deposition; rGO/C-PPy NT, reduced graphene oxide/carboxylated polypyrrole nanotube; EGFET, extended-gate field-effect Transistor; nr, not reported.

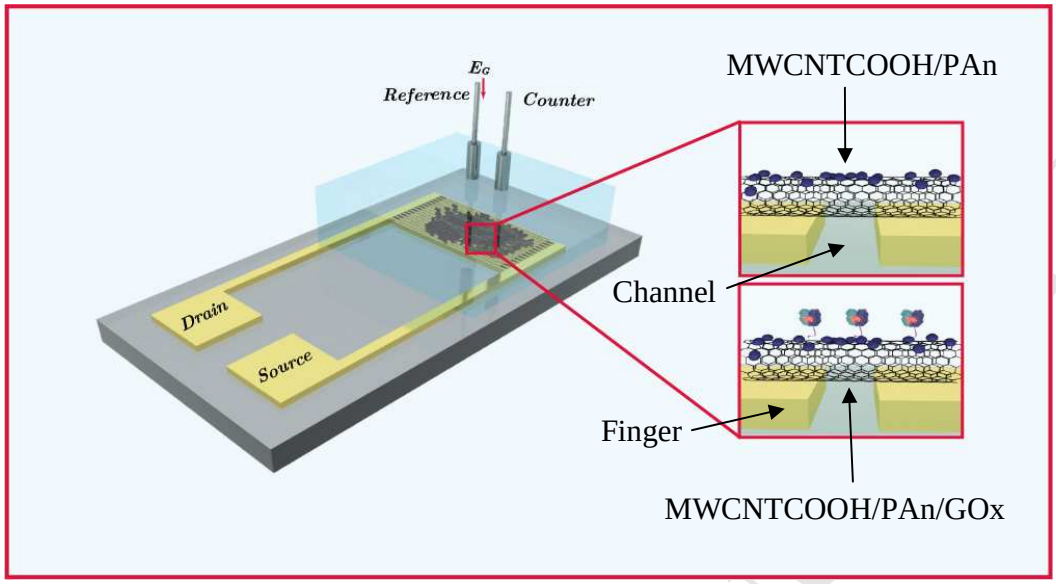

Fig. 1

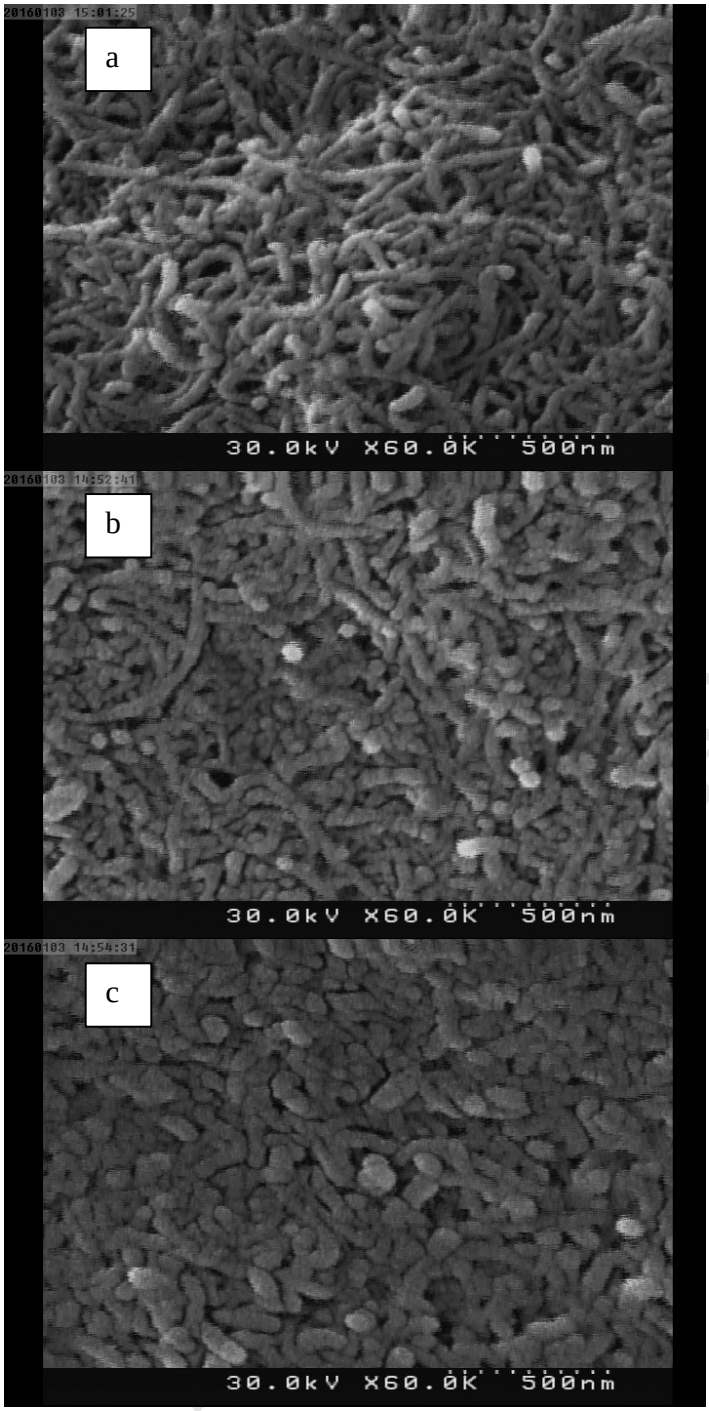

Fig. 2

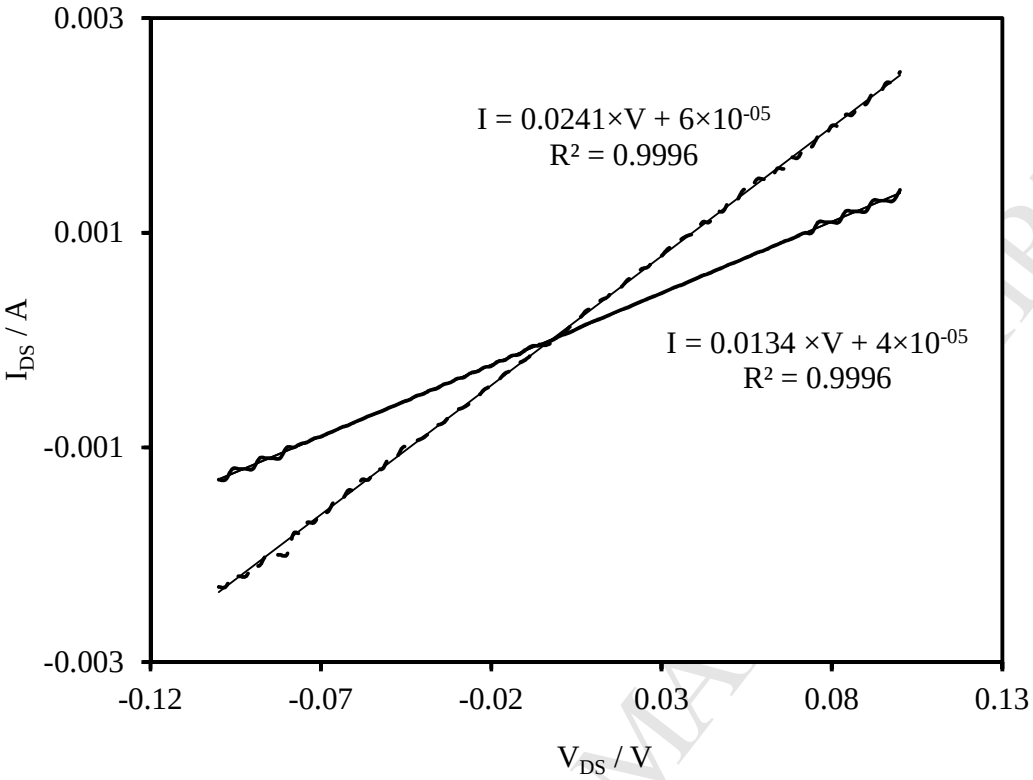

Fig. 3

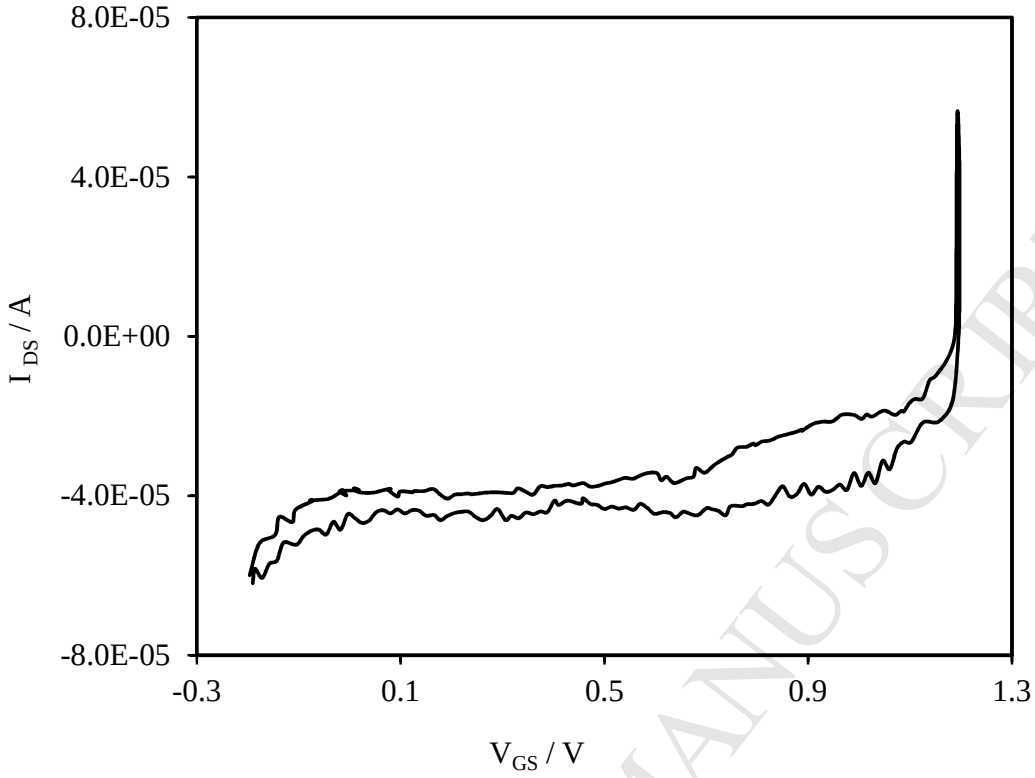

Fig. 4

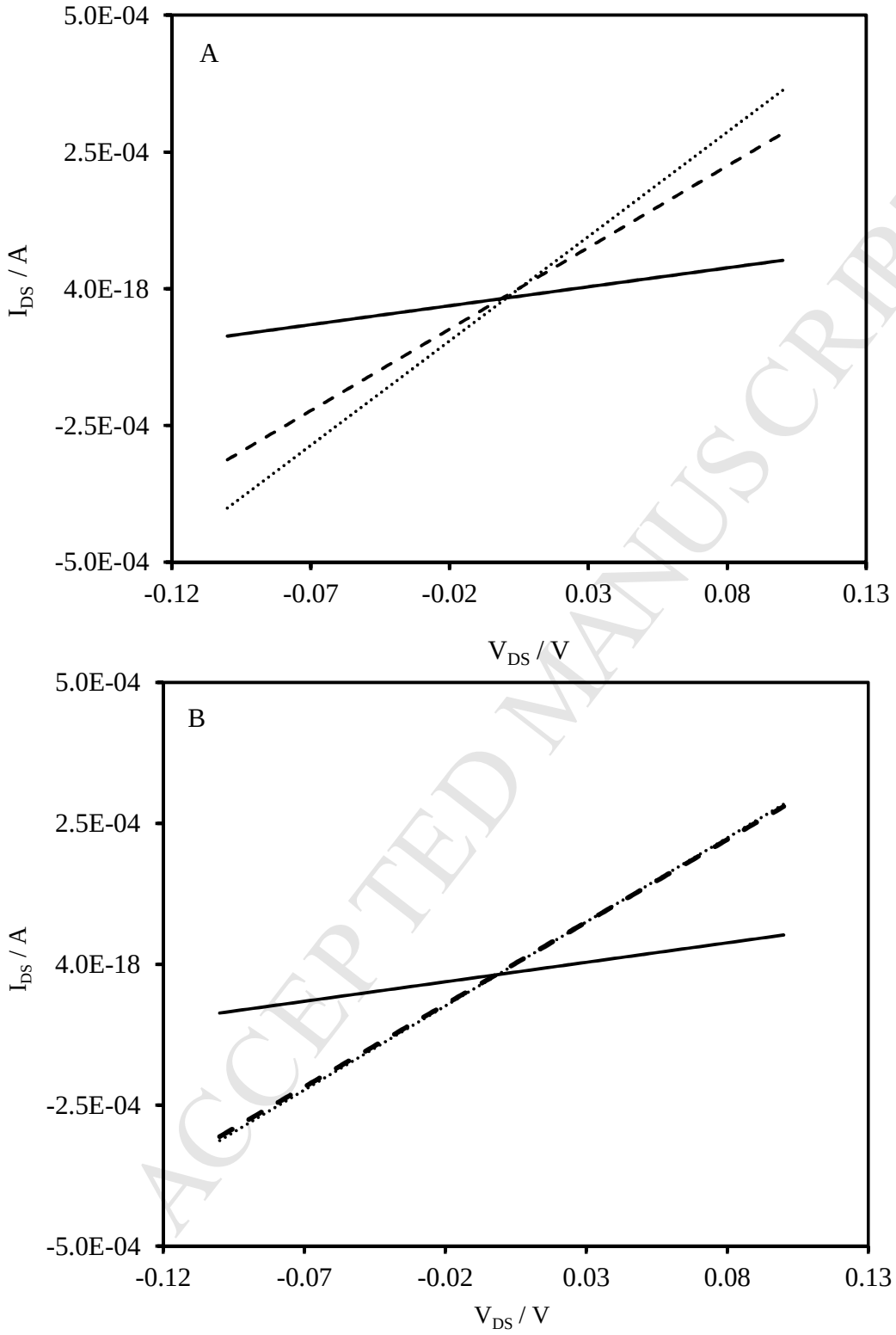

Fig. 5

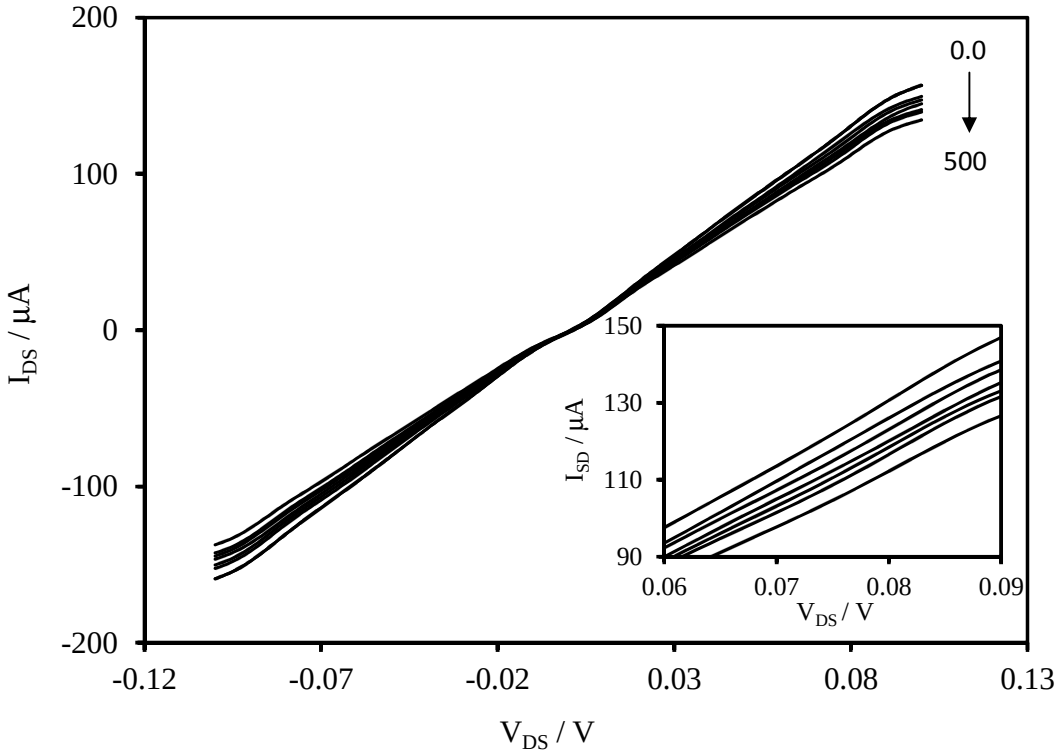

Fig. 6

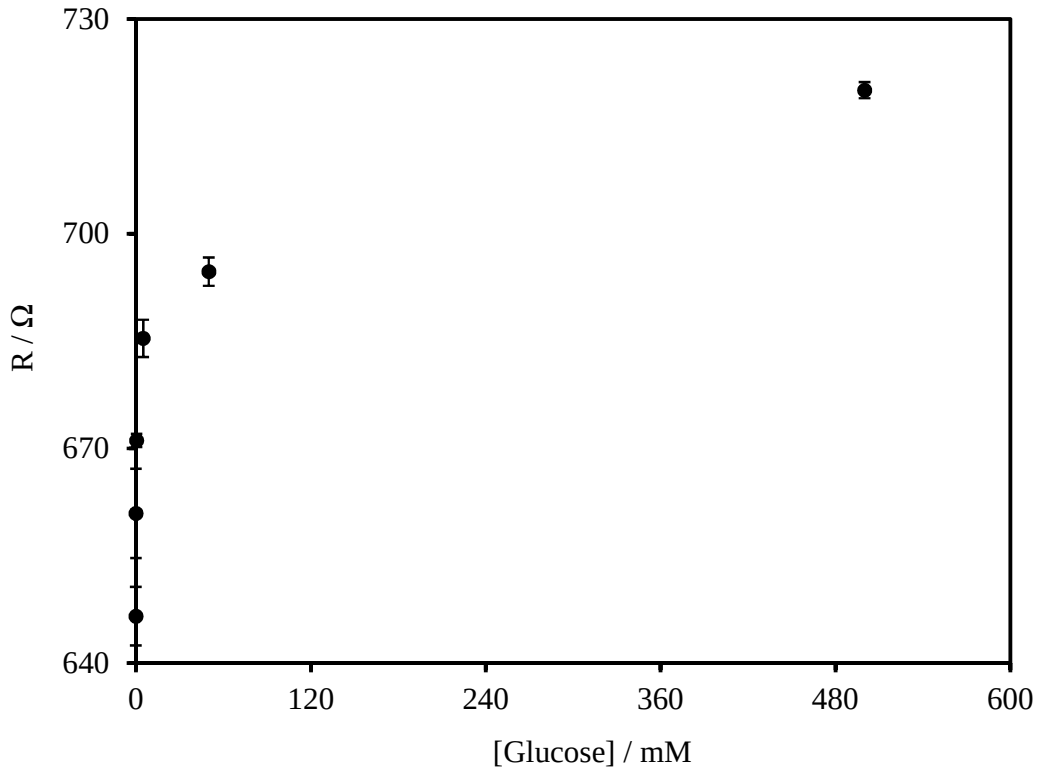

Fig. 7

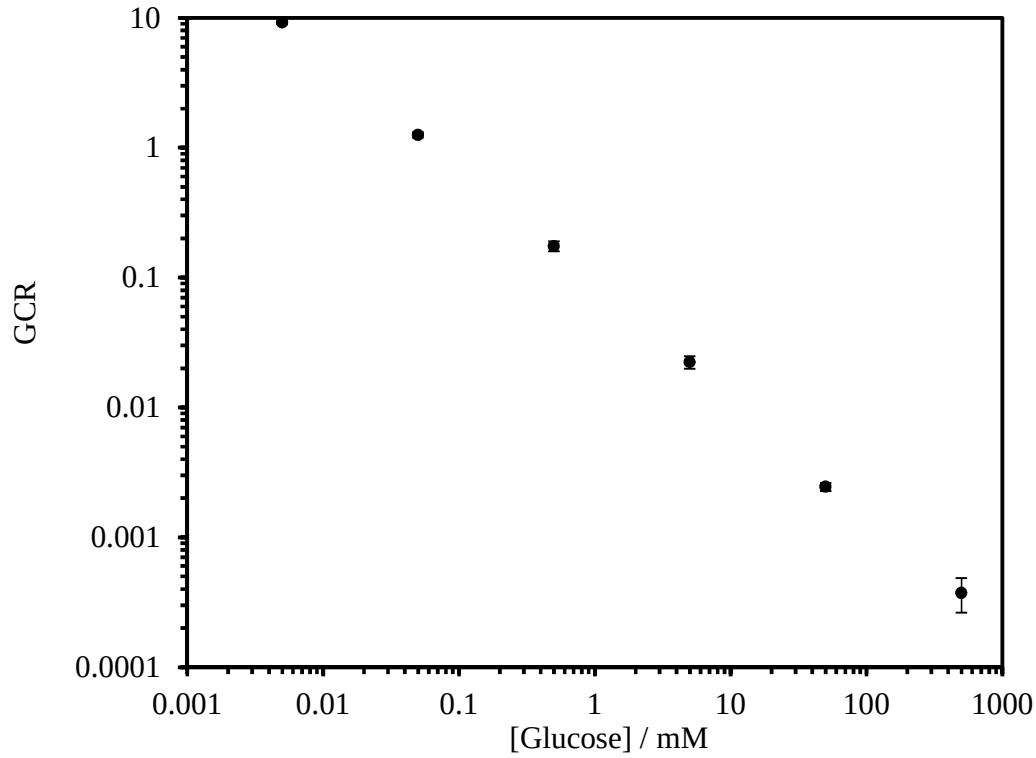

Fig. 8

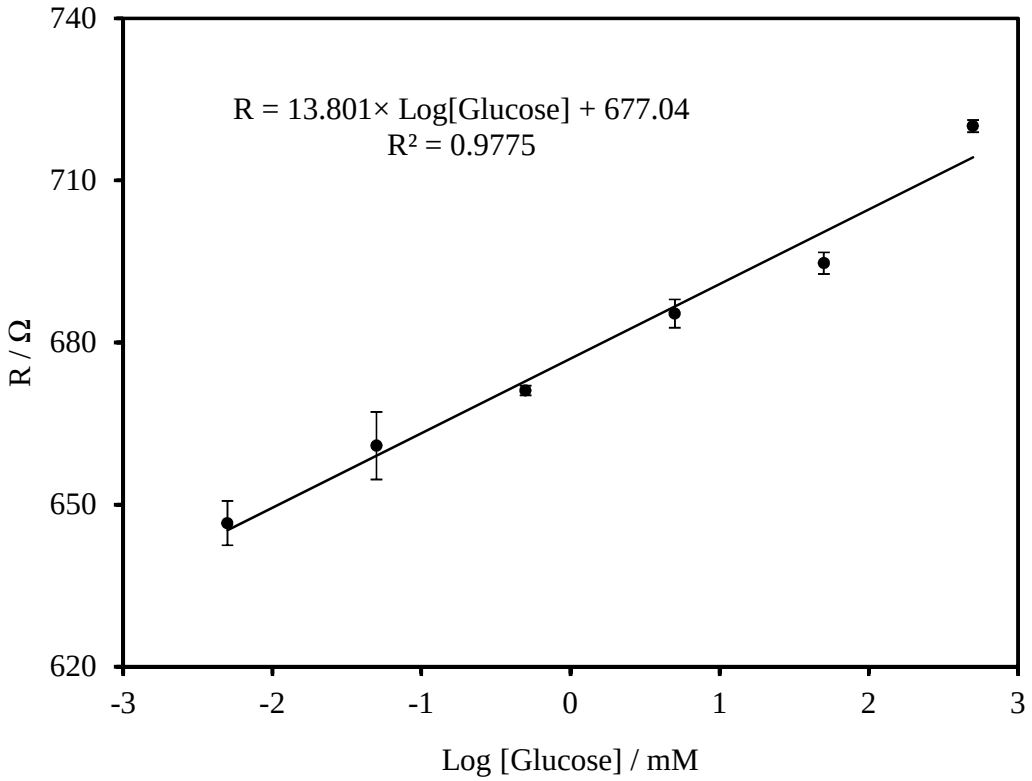

Fig. 9

A new type of liquid-gated enzyme field effect device (FED) was introduced.

The proposed FED consisted of 4 electrodes with a functionalized channel.

The fabricated FED was used as a glucose biosensor for glucose detection.

The FED response, resistance, increased with increasing glucose concentration.