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Gold extended gate field-effect transistor was applied as immunosensor for the label-free recognition of dengue virus nonstructural protein 1 (NS1).
Label-Free Electrical Recognition of Dengue Virus Protein Using the SEG-FET Simplified Measurement System

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Gold separative extended gate field-effect transistor (SEG-FET) was applied as an immunosensor for the label-free recognition of dengue virus nonstructural protein 1 (NS1). NS1 was detected in a concentration range from 0.25 to 5.0 µg.mL⁻¹, indicating that the system is promising for the early and simple diagnosis of dengue.

The control of the MOSFET (metal-oxide-semiconductor field-effect transistor) conductivity by chemical and biological species interactions 1 has opened the way for the construction of field-effect devices for many applications, 2 including affinity biosensors or immunosensors. 2, 3 Using the field-effect transistor (FET) concept, the detection of protein interactions can be achieved in a label-free manner, i.e., without the use of labelling molecules, such as fluorophores, enzymes or other redox agents. 2 The detection principle of a FET that acts as an immunosensor is the measure of the change in the charge distribution when a target protein has been recognized by immobilized receptors on the FET surface. 2, 4 As an example, antibodies can be immobilized on FET surfaces in order to recognize a specific antigen, acting as a disease biomarker. 2, 3, 5

Despite several advantages, some detection restrictions are imposed by FET-based immunosensors. Only the charge change due to antigen-antibody interactions that occur within the Debye length can be measured by a FET immunosensor. 2, 4 This is because the inorganic counter ions from the diffuse layer of the electrolyte solution generate screening effects in the immobilized antibodies. 2, 4 In particular, it has been proposed that only materials that exhibit a non-Nernstian behaviour (pH sensitivity < 59.15 mV.pH⁻¹) are able to detect antigen-antibody interactions as FET electrodes. 2, 4 Therefore, the well-known gold (Au) electrodes are excellent to be used in FET immunosensors since they exhibit non-Nernstian behaviour toward pH variations. 6, 7

This communication reports on the direct electrical recognition of dengue virus nonstructural protein 1 (NS1) by a commercial MOSFET in conjunction with an Au electrode modified with anti-NS1 antibodies. This apparatus, the so-called separative extended gate field-effect transistor (SEG-FET), represents a low-cost alternative to conventional ISFET (ion-sensitive field-effect transistor). Presently, the detection of NS1 antigens is the “gold” method for dengue diagnosis because NS1 is secreted by dengue virus in the first days of infection.8-10

Fig. 1a shows a schematic diagram of the proposed system for the label-free electrical recognition of NS1 protein. The device comprises an Au electrode (extended gate) connected to the gate terminal of the commercial MOSFET (CD4007UB, Texas Instruments) and a Ag/AgCl as the reference electrode. 11, 12 SEG-FET is an elegant alternative to isolate the FET from the chemical environment, and the robustness of the extended gate facilitates its manipulation for biomolecule immobilization.

For electrode fabrication, a 100 nm-thick Au film was evaporated on glass plates previously covered with a 30 nm-thick chromium layer. Self-assembled monolayers (SAMs) comprising cysteamine (Sigma-Aldrich) and glutaraldehyde (Sigma-Aldrich) were used for the immobilization of anti-NS1 antibodies (anti-dengue type 2 virus NS1 glycoprotein antibody, Abcam). The Au electrode remained immersed in a cysteamine ethanolic solution (10

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mM) at room temperature for 12 hours. To complete the activation of the Au surface, the electrodes were modified with glutaraldehyde by sequentially immersing the amino-terminated Au electrode in a glutaraldehyde water solution (2.5%) for 30 min. Finally, anti-NS1 antibodies were immobilized by immersing the electrodes in a 200 µg.mL\(^{-1}\) antibody solution in 1X PBS (phosphate buffered saline, 150 mM). In order to prevent non-specific reactions, the electrodes remained in an ethanolamine (Sigma-Aldrich) solution (50 mM) for 30 min. The schematic representation of the electrode preparation is summarized in Fig. 1b.

Electrochemical impedance spectroscopy (EIS) was carried out to investigate the immobilization of antibodies on the Au electrode. This technique is especially useful to reveal the changes occurring on the electrode surface based upon changes in the impedance caused molecules immobilization.\(^{13}\) EIS measurements were conducted in an Autolab PGSTAT12 Potentiostat/Galvanostat using the Au electrode as a working electrode, a platinum wire as counter electrode and a Ag/AgCl as reference electrode. The measurements were carried out in the presence of 1.0 mM \([\text{Fe(CN)}_6]^{3-}/4^-\) in a 0.1 M KCl solution at a potential of 220 mV. Fig. 2 shows the Nyquist plots corresponding to the immobilization steps.\(^{13}\) The black curve shows the Au electrode without modification. The following immobilization steps corresponding to the formation of the cysteamine, glutaraldehyde (Cys-GA) (red curve) and the attachment of antibodies (blue curve) exhibited the successive growth of the resistive arch, mostly due to the proteins, meaning that these molecules block the electron transfer between the Au electrode surface and the electrolyte.\(^{13}\) This is an evidence of the attachment of the antibody to the electrode surface, validating the immobilization protocol.

In order to study the pH sensitivity of the Au electrode, an experiment was performed by subsequent additions of NaOH (0.1 M) at a time interval of 5 min and using the Au electrode without modification. A pH meter was placed in contact with the solution, and the changes in pH the value were recorded along with the changes in the drain current (I\(_{DS}\)). Both OH\(^-\) ions (from NaOH addition) and NS1 protein were detected via electrical measurements by monitoring the drain-source current (I\(_{DS}\)) of the commercial MOSFET. An N-type MOSFET of the CD40007UB chip was chosen. A drain-source voltage (V\(_{DS}\) = 1.5 V) and a gate-source voltage (V\(_{GS}\) = 1.5 V) were applied to the MOSFET. In fact, V\(_{GS}\) is the voltage applied to the reference electrode. Under this condition, the MOSFET operated in saturation mode. All measurements were taken in low ionic strength using PBS diluted 100 times (0.01X PBS, pH 7.4) to achieve a Debye length (\(\lambda_D\)) of ca. 7.3 nm calculated according to the following equation:\(^{13}\)

\[
\lambda_D = \left( \frac{\varepsilon_0 k T}{2 q I} \right)^{1/2}
\]

where \(\varepsilon_0\) is the permittivity of vacuum, \(\varepsilon\) is the dielectric constant of electrolyte, k is the Boltzmann constant, T is the absolute temperature, q is the elementary charge and I is theionic strength.

Fig. 4 shows the dynamic response of the proposed SEGFET system for the detection of OH\(^-\) ions, revealing that the addition of NaOH decreases the I\(_{DS}\) values. The presence of a negative charge in the solution, and consequently, the decrease of positive charges on the Au surface induce the repulsion of electrons in the N-MOSFET channel. Since the FET-based sensor represents a surface-charge measuring device, the target biomarker must necessarily be diluted in the same buffer used in the measurement environment.

Confocal microscopy was used as technique for the verification of the presence of anti-NS1 antibodies on the Au electrode. In this case, the antibodies were labeled with FITC (fluorescein 5(6)-isothiocyanate, Sigma-Aldrich) according to the manufacturer’s protocol. The FITC-labelled antibodies were then immobilized on the Au electrode as previously described. The images were taken by a Zeiss LSM 780 confocal microscope. Fig. 2a illustrates the fluorescence confocal image of the Au electrode surface covered by FITC-labelled antibodies after the functionalization steps. The surface obtained has a wide antibody coverage, which confirmed the success of the immobilization process. Although some antibody aggregates were present, Fig. 3a shows that it has enough spacing between adjacent antibodies. Fig. 3b shows a fluorescence intensity graph (obtained from a transversal cut in the image of Fig. 2a), where we can see that there is a spacing between adjacent peaks (ca. few micrometers) indicating that there is no steric hindrance for the occurrence of antigen-antibody interactions.
Fig. 5a shows the response of the proposed SEGFET acting as an immunosensor for the detection of NS1. Before NS1 detection, a 0.01X PBS buffer aliquot was added to the measuring cell in order to investigate its effect. In this case, there was no change in the signal. On the other hand, when a low concentration of NS1 (0.5 μg.mL⁻¹) diluted in the same 0.01X PBS was added, an accentuated decrease was observed in $I_{DS}$. The $I_{DS}$ decrease can be attributed to a charge change in the active layer of the immunosensor (Au containing immobilized anti-NS1 antibodies) with no influence of the ions present in the solution. The isoelectric point (IP) of NS1 is approximately 5.7. NS1 is negatively charged at pH 7.4 and induces negative charges in the immobilized antibody layer when the antigen-antibody coupling occurs. This effect causes a decrease in $V_{GS}$, which implies a decrease of carriers (electrons) in the MOSFET channel, as observed upon NaOH addition.

The drift in the output signal, as observed in the baseline of Fig. 4 and Fig 5a, is an intrinsic characteristic of ISFET/SEGFT systems, in which the electrodes participate in redox reactions, especially those composed of metal oxides. In our case, however, the drift is very small and can be neglected in comparison to the detection of OH⁻ ions (Fig. 4) and NS1 protein (Fig. 5a).

Fig. 5b shows the analytic curve obtained from measurements of $I_{DS}$ variations for various NS1 concentrations. A good linearity is observed for concentrations lower than 1 μg.mL⁻¹. However, the immunosensor must indicate the presence of NS1 as low as 0.25 μg.mL⁻¹ up to 5 μg.mL⁻¹. Since NS1 circulates in high levels in the blood of patients infected with DF, the proposed immunosensor can provide a positive result even in mild cases of infection.

Conclusions

In summary, Au electrodes were functionalized with anti-Dengue NS1 antibodies and connected to the gate of a commercial MOSFET forming a SEGFET configuration used to detect the dengue virus NS1 protein. Besides being a low-cost alternative to ISFET, the proposed immunosensor can also isolate the FET from the chemical environment and improve the bound charge detection due to antigen-antibody interactions. Associating an MOSFET concept with biological recognition species, dengue biomarkers can be detected early in a simple and low-cost way.

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Notes and references


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