Design of a Myelin Basic Protein Biosensor based on EnFET Technology

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Abstract: In this work, the design of a biosensor based on FET technology have been proposed, simulating the modification to the gate of an Ion Sensitive Field-Effect Transistor (ISFET) with a synthetic polymer to entrap the desired analyte which contains Myelin Basic Protein (MBP). This analyte is generally used in test to find out if someone is suffering a demyelinating disease, and is commonly detected by Enzyme Linked Immunosorbent Assays (ELISA). Based on this principle, we propose a simpler method, fundamented on Enzyme Field-Effect Transistor (EnFET) technology in order to develop a new device applied to the diagnosis of demyelinating diseases. Simulation examples are used in order to demonstrate the functionality for this type of biosensor to its exposure to MBP at concentrations of 10^{-4} to 10^{-1} mol/L, where the amount of analyte in the receptor located at the top of the gate will determine the level of voltage applied to create a channel and activate the device.

1 INTRODUCTION

The diagnosis of diseases is one of the principal concerns related to all aspects of life. One of the most alarming diseases is from the degenerative kind. These diseases are related to the motor and sensory function of the body, which are in charge of the nervous system, where the communication from cell to cell is conducted by neurons. If someone suffers from a demyelinating disease, the myelin sheath of the neuron loses myelin causing a bad conduction of nerve pulses.

Recent statistics carried out in 2013 on behalf of the Multiple Sclerosis International Federation (MSIF) reveal that 2.3 million people suffer from multiple sclerosis (Thompson et al., 2013). This has increased the interest from physicians and researchers in search of new methods of analysis.

Currently, demyelinating diseases are diagnosed by methods that get results after several tests. Performing a lumbar puncture to remove cerebrospinal fluid from the central nervous system and subsequently perform an Enzyme Linked Immunosorbent Assay (ELISA) test to obtain the levels of myelin is one of the most common tests because of its economy in comparison with other methods such as Magnetic Resonance Imaging (MRI) (Holland et al., 2007).

Recent investigations have developed immunosensors based on different receptors to detect myelin basic protein in order to have alternatives to the existing methods of diagnosis, reducing the consumption of time and costs (La Belle et al., 2007). Encouraging the development of new devices to be implemented both at the area of research and medical application of demyelinating diseases. However, the complexity of certain immunosensors makes them not suitable for mass production, but it is one of the main advantages of FET technology.

Although FETs can be aseptically manufactured and hermetically sealed, the biocompatibility of the materials with which they are made is the key so that they can be implemented for biomedical purposes; these devices are called biologically sensitive field-effect transistors (BioFETs). Some applications of BioFETs have been already studied, like the detection of DNA (Ozsoz, 2007), proteins (Park et al., 2005) and enzymes (Zayats et al., 2000). In principle, every charged molecule located in the solute that can be bound to the surface can be detected by a BioFET. In this work the objective was to design a biosensor that can detect myelin in

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order to help the research of demyelinating diseases, based on BioFET technology.

2 METHOD

A BioFET can be constructed from an ISFET, modifying the gate by a coupling of different biological recognition elements and processing the output signal. Basically, an EnFET is composed of an analyte, a receptor, a transducer and a signal acquisition system (Figure 1).

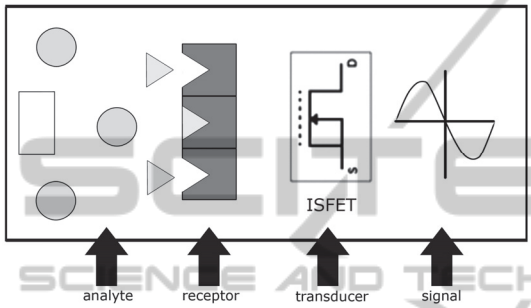

Figure 1: Schematic diagram of the EnFET operating principle.

The selected analyte was MBP (Myelin Basic Protein) which properties and interactions have been already studied (Boggs 2006). This analyte can be immobilized by polymer entrapment or covalent attachment. Based on the chemical structure of MBP (Figure 2), the chosen method for the analyte immobilization was polymer entrapment; making a hybrid gel conformed by polyvinyl alcohol (PVA), Tetraethyl orthosilicate (TEOS) and glutaraldehyde (GA) the receptor. Where the implementation of GA as a selective immobilizer to MBP has been used in related works (Burak et al., 2013).

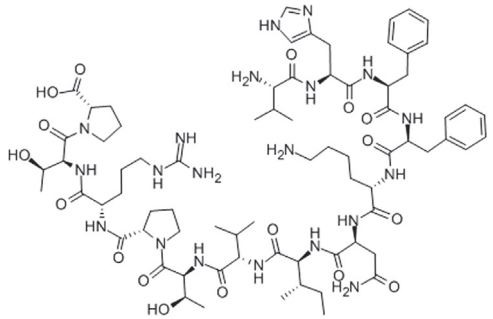

Figure 2: Schematic diagram of the EnFET operating principle.

The most important point for the device is the transferring of data from the part of biologic recognition to the transduction of the signal. To achieve this task, an ISFET is used as a transducer. Basically, ISFETs are implemented to measure the concentration of ions in an electrolytic solution, and are used in biosensor development due to their favourable characteristics such as sensitivity, miniaturization, fast response and low cost (Bergveld, 2003a). An ISFET threshold voltage can be calculated by the following equation (Bergveld, 2003b):

$$V_T = E_{ref} - \psi_0 + X^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{SS} + Q_{ox} + Q_B}{C_{ox}} + 2\emptyset_F \quad (1)$$

Where E_{ref} represents the reference electrode potential, ψ_0 the electrochemical potential at the dielectric-electrolyte interface, X^{sol} the surface potential of the solution, Φ_{Si} the work function of the semiconductor, Q_{SS} the density of accumulated charge in the oxide-semiconductor interface, Q_{ox} the density of accumulated charge in the oxide, Q_B the density of accumulated charge in the region of the interface close to the semiconductor, C_{ox} the capacitance of the oxide layer and $2\emptyset_F$ the difference between half of the band gap and the Fermi level.

From the expression given in (1) all potentials are constant, except the electrochemical potential (ψ_0) that depends on the ionic concentration of the solution, which can be calculated as follows:

$$V_T = V_{T0} - \psi_0 \quad (2)$$

Substituting (2) on (1) the expression can be reduced to

$$V_{T0} = E_{ref} + X^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{SS} + Q_{ox} + Q_B}{C_{ox}} + 2\emptyset_F \quad (3)$$

Since the threshold voltage is a function of ψ_0, the drain current will be influenced by the changes in the electrochemical potential ψ_0. Where the drain to source current of an ISFET in the linear region is given by the following expression (Lee et al., 2009):

$$I_{DS} = \mu_n C_{ox} \frac{W}{L} [(V_{GS} - V_{T0}) V_{DS} - \frac{{V_{DS}}^2}{2}] \quad (4)$$

Where μ_n represents the charge-carrier effective mobility in the channel, C_{ox} the capacitance of the oxide layer, W the channel width, L the channel length, V_{GS} the gate to source voltage, V_{T0} the threshold voltage for zero substrate bias and V_{DS} the drain to source voltage.

Once V_{T0} and I_D are theoretically calculated, all the parameters applied to the simulation of the ISFET can be defined.

3 SIMULATION

The transducer device was simulated using SILVACO a Technology Computer Aided Design (TCAD) tool, its software models semiconductor fabrication and device operation. In this work the ATHENA and ATLAS modules were used. The proposal device was designed to have a dense mesh in the regions of drain, source and channel, selecting a substrate material of Silicon (Si) and a dielectric compound layer of SiO_2/Si_3N_4 deposited over the channel region. This layer is commonly used in ISFET sensors as a sensitive membrane to H^+ and OH^- ions and it is implemented to approach the sensibility of the device to a Nernstian value of 59 mV/pH (Kühnhold and Ryssel, 2000). Finally the source, drain and gate regions were defined with its electrodes to be used as contacts, the final structure is shown in Figure 3.

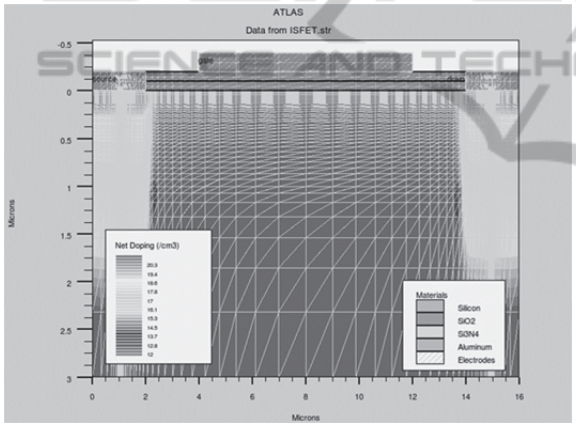

Figure 3: Resulting structure made on the ATHENA module and used as an input for ATLAS.

As a part of the design, an analytical model was developed based in the mechanism of diffusion in the interface between the hybrid gel (PVA/TEOS/GA) and Si_3N_4 layers located over the gate of the ISFET based on the Fick law of diffusion.

$$J(x) = -D * \frac{\partial c(x,t)}{\partial x} \quad (5)$$

Where D is the diffusion coefficient of the studied biochemical species in cm^2/s and c(x, t) is the concentration level of that species represented as follows:

$$\frac{\partial c(x,t)}{\partial t} = D\frac{\partial^2 c(x,t)}{\partial x^2} \quad (6)$$

The diffusion coefficients (D) in the solution of different biochemical species are calculated from the relation of Einstein-Stokes.

$$D = \frac{1}{6\pi\eta}\left(\frac{4\pi\rho N_A}{\sqrt[3]{3M}}kT\right) \quad (7)$$

A MATLAB program was developed to solve the Fick law of diffusion, and also to simulate the generation of biochemical species in the electrolyte-dielectric interface based on the Michaelis-Menten kinetics.

4 RESULTS

Figure 4 and 5 represents the simulation results of the MATLAB program for three different enzyme concentrations of the layer in moles/dm^3. Figure 4 shows an increase in the output voltage depending on the analyte concentration in the surface of the device in M. As can be seen, the concentration of the sample containing MBP located in the surface between the values of 10^{-4} to 10^{-1} M have a voltage response from 0 to 1 V, which shows an increase from 10^{-3} to 10^{-2} M and reaching a constant value at 10^{-1} M.

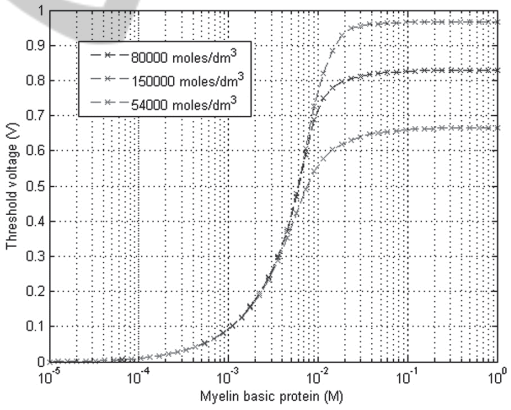

Figure 4: Output curves representing the changes in threshold voltage of the EnFET for different concentrations of MBP depending of concentration of enzyme.

Figure 5 represents the increase of reaction velocity, depending on the solution concentration based on the Michaelis Menten enzyme kinetics, where the solution concentration is gradually increased from 0 to 0.5 mol/L and the reaction velocity increases until it reaches a maximum where it keeps a steady value.

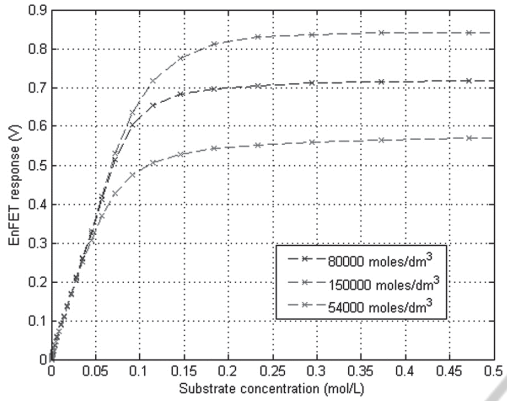

Figure 5: Output curves representing the velocity of reaction rate in the EnFET for different values of solution concentration depending of concentration of enzyme.

Based on the results obtained at the MATLAB simulation and the structure made in SILVACO, a voltage level was applied as gate bias in the module of ATLAS in order to check if the threshold voltage of the device is in range of the output voltage generated as EnFET response (Figure 6).

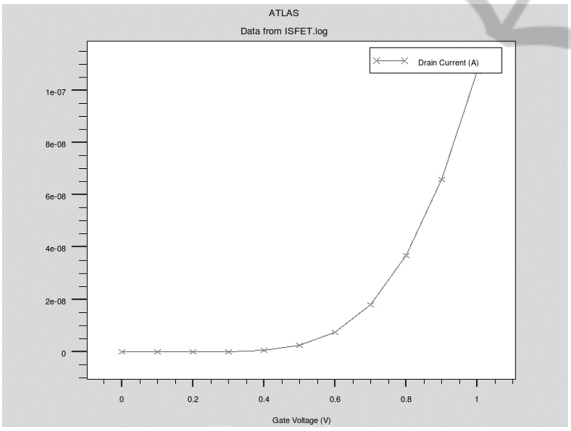

Figure 6: Characteristic curve obtained from ATLAS.

Figure 6 shows that the device turns on at a gate voltage between 0.5 and 0.7 V, matching the threshold voltage level generated in Figure 4, which corroborates that the EnFET biosensor switches on at those levels.

5 CONCLUSIONS

The main objective of this work was to design a biosensor based on the operation of an EnFET in order to detect MBP, using the output electrical characteristics due to the changes in concentration of the analyte. The threshold voltage was calculated and related to the electrical characteristics and the fabrication process of the transducer. Based on simulations, the device haves a voltage range from 0 to 1 Volt, having a concentration level between the values of 10^{-4} to 10^{-1} M of MBP, which is compatible with the FET technology for this application.

In comparison to other EnFET designs, the structure is also based on silicon technology, modifying the gate material by other ion sensitive membranes depending on the implementation. They also express results as the ones described in Figures 4 and 5. But the comparison of threshold voltage or output voltage of EnFET sensors to the design is always based on experimental basis. In this work, the use of a TCAD tool was used to simulate the response of the transducer, to ensure it activates in the range of the voltage generated from the diffusion of MBP in the (PVA/TEOS/GA) membrane and the concentration of substrate generated.

Although the presented work is only based in theoretical grounds, it provides a scheme of how to elaborate a biosensor based on techniques applied to microelectronics, also taking its advantages. For future investigations the characterization of the materials and the development of the EnFET based on flexible electronics can be applied. Also the results obtained should be verified by practical experiments in the future, in order to be applied on in vitro tests.

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