

Bioreactor with integrated nanosensor for the recording of extracellular potential of nerve cells: response to inhibitors record with AlGaN/GaN field – effect transistor

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ABSTRACT

Recently sensors based on the group III nitrides attract growing interest in the scientific community [1, 2, 3]. We report on the microscopic recording of the extracellular potential of NG108 – 15 (mouse neuroblastoma x rat glioma hybrid) nerve cells as response to different inhibitors using an open gate AlGaN/GaN electrolyte gate field effect transistor (EGFET). The biocompatibility study of our sensor materials with nerve cells shows a proliferation rate of about 95%. The inhibitors were added to the medium, and the source-drain current of the EGFET was recorded versus time. The sensor response was a fast decreasing of the drain current I_{DS} . This response is associated with cationic fluxes pumped through ionic channels present in the cellular membrane.

Keywords: AlGaN/GaN, biosensor, DFP, NG108–15 nerve cell, acetylcholinesterase

1 INTRODUCTION

It has been shown that sensors made of Aluminium-Gallium-Nitride/Gallium-Nitride (AlGaN/GaN) are suitable for recording action potential of muscle cells [4] or can serve as materials for biosensors [5] and show outstanding properties with respect to biocompatibility [6]. We report on the recording of the extra cellular potential of NG108-15 (mouse neuroblastoma x rat glioma hybrid) neuronal cells as response to different inhibitors as phenylmethanesulfonylfluoride (PMSF), diisopropylfluorophosphate (DFP) or amiloride using an open gate AlGaN/GaN electrolyte gate field effect transistor (EGFET) embedded in a bioreactor (Fig. 1). Nerve cells control actions and input/output processes of the brain to and from peripheral organs. In all these contexts, electrical signals are transformed to ion fluxes and conducted by members of the ion channel protein superfamily, a large set of structurally related pore-forming proteins. These proteins are integral parts of the cellular membrane and also responsible for maintaining the ion concentrations and gradients within the cell and in relation to the extracellular space. There are many types of cellular channels that allows passage of various ions (e.g. Na^+ , K^+ ,

Cl^- , Ca^{2+}) and are gated by different mechanisms (voltage or ligand gated [7]).

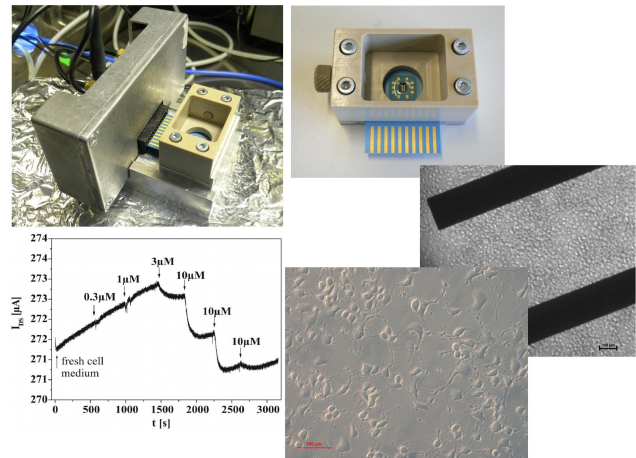


Figure 1: Bioreactor with sensor integrated in a LTCC frame (above). Right below: NG108 neuronal cells attached to the optical transparent sensor. Left below: I_{DS} versus t recording of medium with attached nerve cells, reaction to DFP inhibitor in different concentrations.

2 TECHNOLOGICAL SETUP

The advantage of an AlGaN/GaN heterostructure is the chemical stability [6] and the presence of a polarization induced two dimensional electron gas (2DEG), which responds on any change of charge on the free unpassivated gate surface [2], and realizes a low noise level [4]. Moreover, AlGaN/GaN sensors on sapphire are transparent for visible light, which allows implementation of additional optical techniques or observation by microscopy.

$\text{Al}_{0.28}\text{Ga}_{0.72}\text{N}/\text{GaN}$ heterostructures were grown by plasma induced molecular beam epitaxy on sapphire [8] (AlN nucleation layer, 250 nm GaN buffer layer, 13 nm AlGaN barrier and a 2 nm GaN cap layer). The transistor has an open gate with a channel dimension of $500 \times 2400\mu\text{m}$, respectively, and a polyimide passivation of the contacts. The sensor chip was mounted on a LTCC frame and is encapsulated in a special designed bioreactor with an

Ag/AgCl reference electrode incorporated into a conductive hydro gel containing 2 mM KCl. At usual operating conditions (drain-source voltage $V_{DS} = 0.5$ V) in pH 7 buffer solution the AlGaIn/GaN EGFET exhibited a maximum transconductance of 0.8 mS at $V_{GS} = -0.65$ V. At these conditions the leakage current from the source was found to be negligible (< 1 nA). For the medium with and without cells a pH value of 7.6 was determined using a classical glass electrode.

3 CELL BASED APPLICATIONS

3.1 Cell Culture

Undifferentiated NG108-15 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St.Louis, MO, USA) supplemented with 10% FCS + Penicillin and Streptomycin + L- Glutamine. After three days in incubator at 37°C, 5% CO₂ a very good proliferation was observed and the sensor surface is completely covered by the cells (Fig. 1, insert). Considering a mean cell diameter of 20 μ m, about 1×10^4 cells are grown on the sensor surface.

3.2 Biocompatibility of the AlGaIn/GaN sensor chip

Our previous biocompatibility studies of GaN surface with different types of living cells show a proliferation rate of 95% with a standard deviation of about 3% [6]. In this report we describe an AlGaIn/GaN electrolyte gate FET (EGFET), which was overgrown with nerve cells. There were no inhibiting effects on cell growth by the AlGaIn/GaN sensor surface.

3.3 Inhibitors

For inhibition experiments we used PMSF, DFP and amiloride (Sigma-Aldrich, Taufkirchen, Germany). PMSF and DFP are both acetylcholinesterase (AChE) inhibitors. The loss of AChE activity in neuronal cells is of outstanding interest in Alzheimer's disease [9], which emphasizes the importance of the local and time resolved, long-time monitoring for medical research. In contrast, amiloride is a Na⁺ and Ca²⁺ channel blocker. The inhibitors were diluted in buffer and added to the cells. DFP dilution was prepared each time fresh, because of possible hydrolysis.

4 RESULTS AND DISCUSSION

The used inhibitors exhibits strong effects to the sensor attached neuronal cells, that we explain as change of the electrical surface charge by ion fluxes measured by the two dimensional electron gas (2DEG).

Fig. 2 shows the recorded I_{DS} during 4 measurement cycles (I-IV) as a reaction of the dosing of different neuro-inhibitors and the reaction of the pure EGFET without cultivated cells for comparison (Fig. 2, b, V). Obviously a drop of I_{DS} as reaction of the cells on the dosed inhibitors is monitored and the signal is much higher than the response of the uncultivated sensor on the inhibitors (Fig. 2, b, V) or on a dosing of medium (data not shown). The noise in the signals was around 100 nA, which is more than an order of magnitude lower than the signal.

These experiments clearly demonstrate the ability of the AlGaIn/GaN-EGFETs for quantitative analysis of cell reactions on different inhibitors.

The AlGaIn/GaN-EGFETs show stable operation under physiological conditions, exhibit a very good signal resolution and are suitable for long-time measurements. Currently the system and the experiments are compared with automated patch clamp systems like the commercially available "port-a-patch" of nanion.

For the validation of these effects, the used inhibitors were applied on the sensor surface without cells (see Fig. 2, b, V). The signal shape that we detect was clearly different to the signals with the cell covered surface and the signal size was significantly smaller. Further experiments with cell attached sensors and a medium substitute without sodium, potassium and chloride ions show no signal at all (data not shown).

For an interpretation of the results it is assumed that the sensor signal drop after titration is caused by a decreased concentration of positive ions. Usually, the Na⁺ concentration in the electrolyte is high while inside the cell the K⁺ concentration is high. Thus, the possible mechanism could be that after AChE inhibitors (e.g. DFP, PMSF) dosing ion channels are opened and Na⁺ ions are transported into the cell. The 2DEG of the sensor chip detect a short time drop of positive charge that coincides with the influx of sodium ions into the cell and the absence of such a signal in a medium lacking sodium ions.

These experiments demonstrate the ability of the AlGaIn/GaN sensor for quantitative analysis of cell reactions on different inhibitors. The sensor show stable operation under physiological conditions, exhibit a very good signal resolution and are suitable for long-time measurements. They enable easy measurement procedures, which can be used in every laboratory using small quantities of biological material, for pharmaceutical screening, neurotoxin detection or tumors analyzing.

5 ACKNOWLEDGEMENTS

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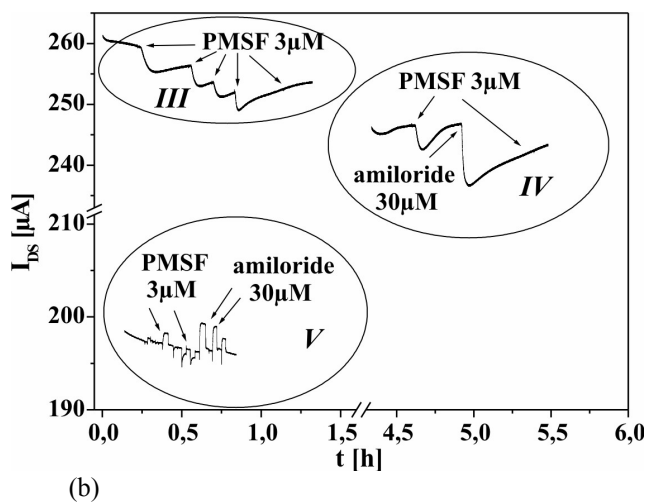
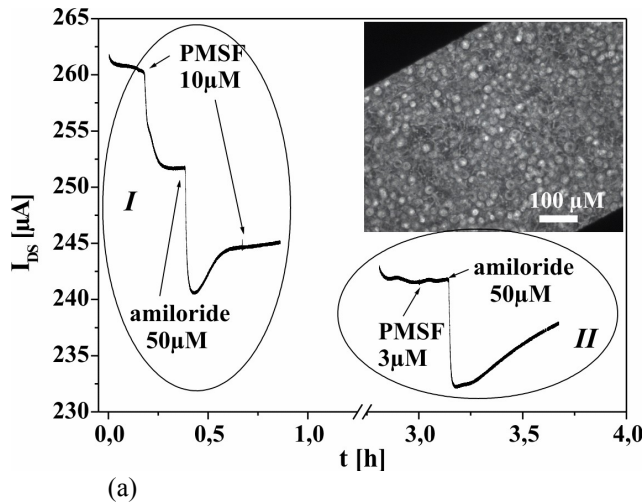


Figure 2. I_{DS} versus t as reaction of the cells in medium to different inhibitors (I- IV) at (a) the first day with a break of two hours and (b) at the second day with a break of three hours, and the reaction of the sensor on the inhibitors without cells for comparison (V). Insert (a): Inverse microscopy image of NG 108-15 cells cultivated on the device surface.

The sensors can be cleaned and sterilized even with hazardous procedures such as autoclaving or etching in hot alkali based solutions [6] and are reusable without further conditioning procedures for in vivo measurements. Group III materials allow for simultaneously optical and electrophysiological recording [10].