Materials Science and Engineering B 169 (2010) 182–185

Contents lists available at ScienceDirect

Materials Science and Engineering B

journal homepage: www.elsevier.com/locate/mseb

Nanosensors for label-free measurement of sodium ion fluxes of neuronal cells

Michael Gebinoga[a,*], Liele Silveira[a], Irina Cimalla[a], Andreea Dumitrescu[c], Mario Kittler[a], Benedikt Lübbers[a], Annette Becker[a], Vadim Lebedev[b], Andreas Schober[a]

[a] ZIK MacroNano Microfluidics and Biosensors, Technical University Ilmenau, P.O. Box 100565, D-98684 Ilmenau, Germany
[b] Fraunhofer Institute for Solid State Physics, Tullastr. 7, D-79108 Freiburg, Germany
[c] University of Pennsylvania – School of Engineering & Applied Science, Philadelphia, PA 19104-6391, USA

ARTICLE INFO

Article history:
Received 5 June 2009
Received in revised form
18 December 2009
Accepted 23 December 2009

Keywords:
Assay technology
AlGaN/GaN
Nanosensor
Non-destructive measurement
Label-free

ABSTRACT

Novel nanosensors based on aluminium gallium nitrides (AlGaN/GaN) high electron mobility transistors have been of high interest during the last years, especially for their electrical characteristics as open gate field effect transistors. These nanosensors provide a valuable tool for high content screening in drug discovery, cell monitoring and liquid analyses focusing on applications of electrochemical detection technology. Our own measurements with these sensors confirm their pH sensitivity and in addition the possibility of detection of other ions in aqueous media. These measurements deal with the reactions of NG 108-15 (mouse neuroblastoma × rat glioma hybrid) neuronal cells in response to different acetylcholinesterase inhibitors. Our experimental approach shows some advantages. The first advantage is the label-free measurement of ion fluxes, and another advantage is the possibility non-destructively to estimate cell signals.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

A disease or a metabolic disorder is defined by the fact that certain metabolic processes are no longer in the range of their equilibrium. Medicaments and treatments work in such a way that they help once again to reach the equilibrium ranges. For these treatments it is at all times necessary to estimate the different cellular and enzymatic activities. Ion channels are interesting because their properties can be modulated by many pharmacological active compounds and malfunctions of ion channels are involved in the molecular pathophysiology of many diseases [1]. Ion channels are, therefore, very important targets in the drug discovery process, and the monitoring of their behavior is an important task for pharmaceutical screening as well as for basic research. Ion channels are voltage gated or chemical gated. From the chemical gated ion channels, we focus on the acetylcholine-gated ion channels that allow a short influx of sodium after acetylcholine attached to the channel protein and can trigger a nerve impulse in the nerve cells that carry the provoked acetylcholine-gated ion channel.

Today thousands of assays exists for measurements of enzyme activities and metabolic processes. Most of them are assays that work with a fluorescence label in different stages of sophistication [2–5]. Aside from these optical methods to estimate cellular activities there are only few other principles of assays. The advantages of fluorescence assays are obvious. They are fast, sensitive and available for nearly all processes. In addition to advantages, there are also many fluorescence dyes and fluorescence readers commercially available.

Only a few processes are not easily representable with fluorescence label techniques. Ion channels are one form of cellular processes that are not so easy to catch and a more sophisticated method is necessary to make them transparent [6]. Patch-clamp measurements represent a very sophisticated biophysical evaluation of compound action. The ability to clamp either voltage or current across a cell membrane, and to manipulate the ionic composition on either side of the membrane, enables the possibility for a detailed characterization of ion-channel gating, permeability, and drug interactions. Additionally, this technique is sufficiently sensitive to study single ion channels [7]. This technique is well suited for a detailed investigation of ion-channel characteristics, however it is therefore an assay that works with one cell at a time and aside from the difficulty to automate it for high throughput screening (HTS), the cell is destroyed after the measurement. Two other techniques are based on distance dependent processes such as fluorescence resonance energy transfer (FRET) [8] and total internal reflection fluorescence microscopy (TIRFM) [9]. These techniques are also well suited to reveal many details of ion-channel activities.

Nearly all of these assay techniques have one or more weak points that are not easily overcome. They need a label for one of the compounds of the process and/or they destroy the sample during the measurement process. If an assay system should work with

* Corresponding author. Tel.: +49 3677 693382; fax: +49 3677 693379.
E-mail address: michael.gebinoga@tu-ilmenau.de (M. Gebinoga).

0921-5107/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.mseb.2009.12.053

the same sample over several days is necessary in order to estimate the activity over a time range and in the native state of the process is of interest then the list of available assays become quite short. We would like to introduce an assay system that has some promising characteristics. The basis of the system is an AlGaN/GaN nanosensor that has some advantages compared with other assay systems and techniques.

1. The sensor material itself is highly biocompatible, also without coating. Animal cells can attach and grow on the surface of the AlGaN/GaN nanosensor [10].
2. The nanosensor allows a non-destructive measurement of cells.
3. The AlGaN/GaN nanosensor enables label-free detection.
4. The sensor material is transparent and also allows for optical measurement methods and an observation of attached cells by inverse microscope techniques.
5. The sensor material is chemically resistant and can be sterilized by autoclave.

The AlGaN/GaN sensor is a nanosensor due to the dimensions of the electron gas which works as gate electrode. The two-dimensional electron gas is present at the lattice border of the GaN layer and the subsequently grown AlGaN layer. These layers have slightly different crystal lattice dimensions and the tension at the border enables the formation of a two-dimensional electron gas at a depth of 15 nm from the surface of the sensor. This two-dimensional electron gas exists only at this lattice border.

The pH sensitivity of the AlGaN/GaN nanosensor is well known and we also use the sensor for detection of pH changes [11]. Based on this knowledge, we have attempted to measure concentration changes of ions other than hydronium ions. We focused on sodium ions because of the importance of sodium for ion-channel activity and easier determination of changes of the sodium concentration outside the cells. For this purpose we prepared special buffer solutions to distinguish the reaction of the nanosensor in dependence on the presence of sodium ions.

2. Materials and methods

A sketch of the sensor and its dimensions is presented in Fig. 1. AlGaN/GaN nanosensors were grown by plasma induced molecular beam epitaxy on sapphire [12]. The sapphire was used as a transparent substrate and the epitaxial growth was initiated by an AlN nucleation layer followed by a 250 nm GaN buffer layer. Finally the growth was completed with 13 nm AlGaN barrier and a 2 nm GaN cap layer, which enhances the chemical stability of the nanosensor. The ohmic contacts consist of a Ti/Al/Ti/Au metalstack and were created by sputter deposition followed by rapid thermal annealing in nitrogen atmosphere at 750 °C for 60 s. Analysis of electron mobility and sheet carrier concentration were carried out by Hall measurements with results of 800 $cm^2/V s$ and 6.4×10^{12} cm^2. A mesa on the heterostructure was realized by chlorine-based inductively coupling plasma (ICP) etching to laterally confine the active area of the sensor [13].

The transistor has an open gate with a variable channel width and length (in general from 0.5 to 12 mm^2) and a polyimide layer was used for contact passivation (see Fig. 1). The sensor chip was encapsulated in a well plate with an Ag/AgCl reference electrode incorporated into a conductive hydro gel containing 2 mM KCl. At typical operating conditions (drain-source voltage V_{DS} = 0.5 V) in pH 7 buffer solution the AlGaN/GaN EGFET exhibited a maximum transconductance of 0.8 mS at V_{GS} = −0.5 V. The sensor sensitivity in pH buffer solution was found to be 53 mV/pH and 34 μA/pH. Under these conditions the leakage current from the source was found to be negligible (<1 nA). Regarding known pH sensitivity of the AlGaN/GaN nanosensor, the pH of the cell medium was adjusted to 7.5 and held constant using a 25 mM HEPES-TRIS buffer solution in 4 ml medium [11]. Electrical measurements were performed in constant voltage mode recording changes in the drain current I_{DS} since varying voltages are expected to affect cell reactions [14].

For the experiments we used half adherent NG 108-15 cells (somatic cell hybrid; mouse neuroblastoma and rat glioma were used). For the attachment and proliferation we used an amount of cells that enable over three days a confluent cover with cells. Excessive seeding of cells at the beginning is not recommended. The hybrid cells are cultivated in Dulbecco's modified Eagle's medium-high glucose (DMEM) (Sigma, Taufkirchen, Germany) supplemented with 10% fetal calf serum (FCS) and 100 U/ml penicillin, 100 μg/ml streptomycin according to standards set by the American tissue-type culture collection (ATCC). After three days in a CO_2 incubator at 37 °C, 5% CO_2 a very good proliferation was observed and the sensor surface was completely covered by the cells (see Fig. 2). Proliferation of the cells on the nanosensor surface happens without inhibition and exhibits the excellent biocompatibility of the sensor material. The spreading of different animal cells on various surfaces revealed the advantage of GaN surfaces in com-

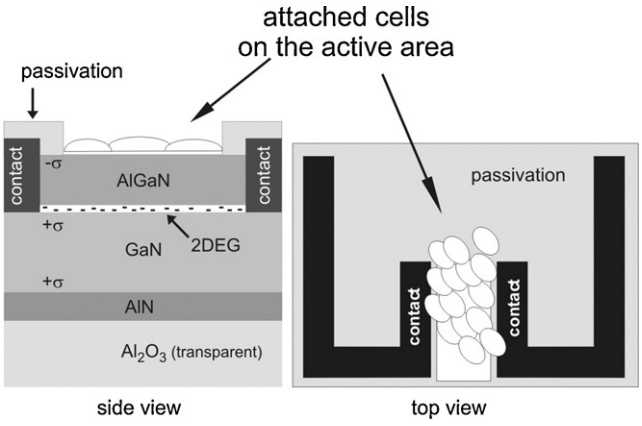

Fig. 1. Schematic of an AlGaN/GaN nanosensor in lateral view (left) and top view (right). The sensor dimensions are in the range of 5 mm × 5 mm. The open gate structure varies in size. Open gate structures of 2400 μm × 500 μm were used here. The nanoscopic dimension of the sensor is the two-dimensional electron gas that exists in a depth of 15 nm and shows nearly no vertical extension but laterally flows over the entire area of the open gate structure.

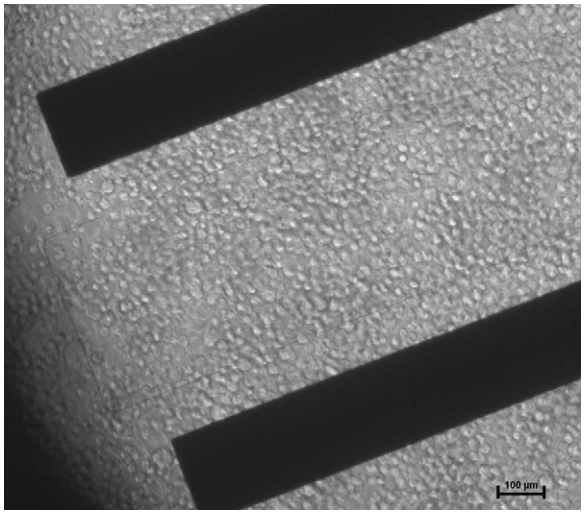

Fig. 2. Attachment and growth of NG 108-15 cells on the AlGaN/GaN nanosensor. The active area is visible as the cover slip like structure between the contacts (black bars). Conditions of cells were checked before and after the experiment.

Download English Version:

https://daneshyari.com/en/article/1529906

Download Persian Version:

https://daneshyari.com/article/1529906

Daneshyari.com