Extended Abstracts of the 2014 International Conference on Solid State Devices and Materials, Tsukuba, 2014, pp274-275

Detection of PCR Products by Micro- and Nanoscale Field-Effect Transistors

Miriam Schwartz[1,3], Thanh Chien Nguyen[1], Xuan Thang Vu[1,2], Patrick Wagner[3], and Sven Ingebrandt[1*]

[1] University of Applied Sciences Kaiserslautern, Department of Informatics and Microsystem Technology, Amerikastr. 1, 66482 Zweibruecken, Germany
Phone: +49-631-3724-5413 E-mail: sven.ingebrandt@fh-kl.de
[2] present address: RWTH Aachen University, Department of Physics, Otto-Blumenthal Street, 52074 Aachen, Germany
[3] Hasselt University, Institute for Materials Research, Wetenschapspark 1, B-3590 Diepenbeek, Belgium

Abstract

In this study, we demonstrate that micro- and nanoscale field-effect transistors (FET) are suitable for the detection of PCR products. We were able to detect PCR products by utilizing potentiometric and impedimetric readout modes for our FET devices. Furthermore, we compare the results of both devices in terms of the signal strength in different buffer solutions.

1. Introduction

Micro-and nanoscale field-effect devices can be applied for biomedical detection, like the detection of DNA [1-4][1–4] and antibodies [5] as well as for cellular recordings [6-9]. The advantage of those sensors is the label-free read-out. Compared to the microscale ion-sensitive field-effect transistors (ISFETs), nanoscale devices, like silicon nanowire field-effect transistors (SiNW FET) offer superior sensitivity. This is caused by the fact that the SiNW FET show a higher surface-to-volume ratio and that the size of the wires are typically in the range of the biomolecules under test. Therefore, SiNW FETs possess an ultrahigh sensitivity and – with an appropriate surface chemistry – a high selectivity as well [2,3]. A fast electronic read-out can be performed for both micro- and nanosystem. We previously demonstrated, that the detection can be performed by measuring in two different modes: The first one is the typical potentiometric method that is based on the change in surface potential, accompanied by a shift in threshold voltage (V_{TH}), which is caused by the binding of charged molecules [10,11]. The second one is an impedimetric method that is based on the change of the input impedance. The significances of both measurement modes are still under investigation in our group.

In this study, we report the detection of PCR products comparing both potentiometric and impedimetric readouts. For this purpose, we established a laboratory assay (EuroArray HLA B-27; ELISA Anti-Borrelia-plus-VIsE, Euroimmun AG, Germany) on the micro- as well as on the nanoscale FETs. The typification of the human leukocyte antigen allele (HLA B-27) is necessary to determine HLA-associated diseases, like autoimmune disease and for bone marrow, stem cell and organ transplantations. The higher the correlation between donor and recipient HLA, the higher is the chance of a tolerance of the donor material.

The potentiometric and impedimetric results gained by using the ISFETs are compared with those received by using the SiNW FETs. Since the impedimetric method is quite novel and the theory is still under investigation, we focused on the ac-method and did some detailed experiments.

2. Methods and results

Chip fabrication and surface treatment

All used chips were p-type transistors. For the fabrication of the ISFETs please refer to [6]. The SiNW FETs were top-down fabricated on 4'' silicon-on-insulator wafers (SOITEC, France). After having thinned-out the top silicon layer to 50 nm, nanoimprint lithography and wet anisotropic etching of silicon with tetramethylammonium (TMAH) were combined to define SiNWs and contact lines in the same step like reported in [4]. Prior to the experiments, the chip surface was cleaned and activated using Piranha solution (H_2SO_4:H_20_2 = 2:1). Afterwards, the sensors were silanized with 3-glycidoxypropyltrimethoxysilane (GOPS) to ensure a covalent binding of the amino-modified capture DNA sequences. The silanization process was performed under gas-phase conditions to create a homogeneous monolayer of silane and to avoid any damage of the encapsulation material.

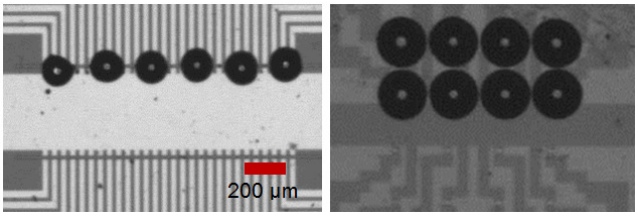

Fig. 1 Spotted capture DNA on the gates of an ISFET (right) and on the nanowire gates of a SiNW FET (left)

DNA immobilization and hybridization

Single-stranded capture DNA sequences (ssDNA) were covalently immobilized on the sensor surface. The capture DNA was diluted in 150 mM phosphate buffer with a pH value of 8.6. At this pH value, the epoxy ring of the silane opens and the amino-modified capture DNA can bind to the epoxy ring. For a site-specific immobilization of the capture molecules right on defined areas of the chips, a microspotting system was used (sciFLEXARRAYER S3, Sci-

enion AG, Germany). Microscopic images after a microspotting run on a SiNW array (Fig. 1 left) and an ISFET surface (Fig. 1 right) are shown. The DNA solution was spotted directly onto the gates of the sensor chips. In both cases no DNA sequences were immobilized on the lower gates so that they could serve as reference channels. After the immobilization, the surface was blocked to minimize unspecific binding events.

The hybridization was performed by adding single-stranded, complementary target DNA (cDNA). The cDNA was produced by a previous PCR run with standard reagents of the company Euroimmun AG. The electronic detection was done after each experimental step in 0.15 mM, 1.5 mM, 15 mM and 150 mM phosphate buffer.

Electronic read-out

The electronic detection of the biomolecule binding was done by using a custom-made amplifier system, which was already described in previous reports [4,12]. It is possible to measure 16 channels simultaneously with this system. Moreover, a differential read-out with reference channel can be performed. Firstly, the transfer characteristic was measured. After having chosen the working point at maximum conductance, the transfer function was measured.

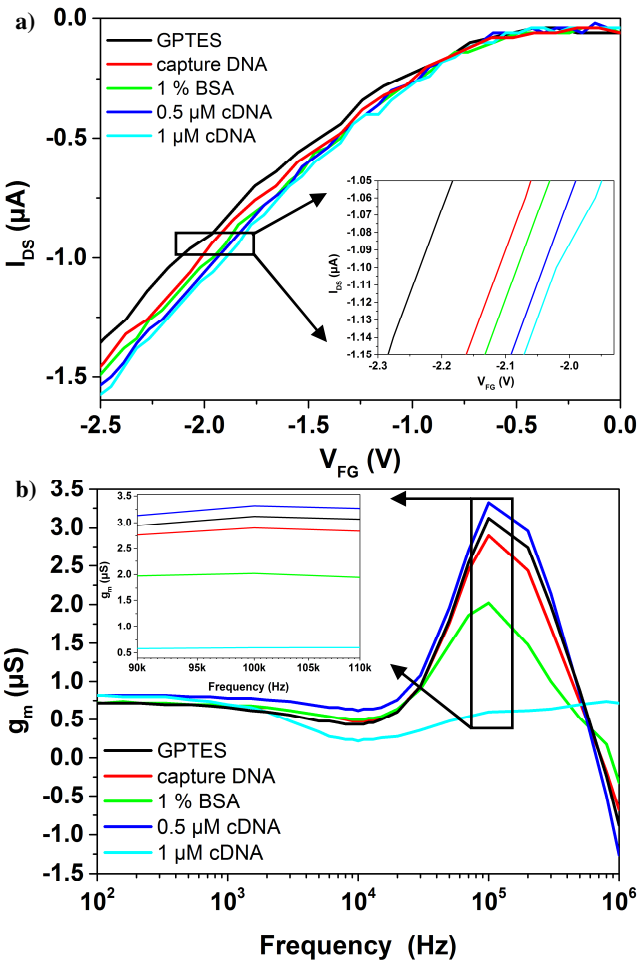

Fig. 2 Exemplary dc- (Fig. 2 a) and ac-measurement (Fig. 2 b)

In Figure 2, exemplary measurements of the transfer characteristic (Fig. 2 a) and the transfer function (Fig. 2 b) are shown. The dc- and ac-results of a SiNW FET after silanization (black curve), immobilization (red curve), blocking (dark blue curve) and after hybridization process are presented. The molecules are directly immobilized on the nanowire that serves as gate. Then the cDNA was added so that a hybridization process could be detected.

3. Conclusions

We successfully established an HLA-B27 laboratory assay on our ISFET and SiNW FET sensors. We were able to detect PCR products with both platforms. This was possible by either measuring the transfer characteristic or by measuring the transfer function. In terms of sensitivity, it seems that the nanoscale devices are more sensitive than the microscale transistors. However, further investigations and better statistical evaluations will be required. Nevertheless, our first results reveal that SiNW FETs may be suitable for future applications such as a point-of-care biomedical detection due to its higher sensitivity for detecting low concentrations of target analyte.

Acknowledgements

This work was funded by BMBF in framework of the project "Nanodrahtsensoren als markerfreie, voll-elektronische Immunosensoren". We thank the Euroimmun AG for generous support of this project by providing the professional assays. Furthermore, the authors would like to thank the group of Prof. A. Offenhäusser, Peter Grünberg Institute 8, Research Center Jülich, Germany, for the fabrication of the SiNW FETs.

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