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Field-Effect Biosensors Modified with *Tobacco Mosaic Virus* Nanotubes as Enzyme Nanocarrier †

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Abstract: A new concept for the development of semiconductor field-effect biosensors by modification of a gate surface with *tobacco mosaic virus* (TMV) nanotubes, serving as enzyme nanocarrier, is presented. TMV nanotubes enable an immobilization of a high amount of enzymes without substantial loss of their activity, resulting in an enhanced biosensor performance. This approach has been experimentally demonstrated by realizing a capacitive field-effect penicillin biosensor using TMV nanotubes functionalized with the enzyme penicillinase as model system.

Keywords: field-effect sensor; penicillin biosensor; enzyme penicillinase; *tobacco mosaic virus*; enzyme nanocarrier

1. Introduction

Although plant viruses are primarily known for their multifaceted interactions with plant hosts, their properties make them attractive as building blocks for nano- and biotechnology applications as well as for biosensing [1–4]. The *tobacco mosaic virus* (TMV) is one of the most studied plant viruses and is non-pathogenic for mammals. The surface of TMV nanotubes exhibits thousands of sites that can be used for selective binding of molecules, thus, opening new opportunities for biosensing.

Recently, we reported on a TMV-based amperometric sensor for the detection of glucose [5]. In this work, we present field-effect enzyme biosensor based on a capacitive Al-p-Si-SiO_2-Ta_2O_5 EIS (electrolyte-insulator-semiconductor) structure modified with TMV nanotubes as enzyme nanocarrier. The enzyme penicillinase/penicillin system was chosen as model system, because our group has long-time experience with field-effect penicillin sensors [6–10]. For the details of functioning of field-effect biosensors, see e.g., [11–13].

2. Experimental

2.1. Preparation of EIS Sensors

Figure 1 shows the schematic layer structure of the field-effect penicillin biosensor modified with TMV nanotubes. Capacitive field-effect EIS structures were fabricated using a p-Si substrate with a specific resistivity of 1–5 Ωcm. A ~30 nm thick SiO_2 insulating layer was formed via thermal oxidation of Si. Afterwards, a pH-sensitive Ta_2O_5 film with a thickness of ~60 nm was prepared by electron-beam evaporation of 30 nm Ta, followed by thermal oxidation in oxygen atmosphere.

The SiO_2 layer on the backside of the wafer has been removed with hydrofluoric acid. Then, a ~300 nm thick aluminium film has been deposited as rear-side contact by electron-beam evaporation. Finally, the wafer was cut into 10 mm × 10 mm sized single chips.

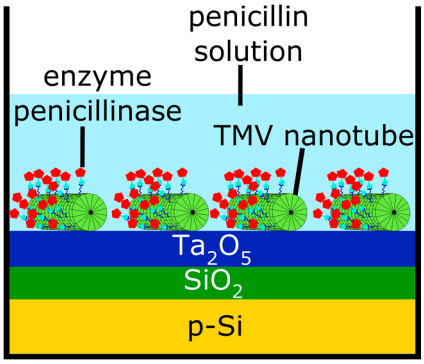

Figure 1. Schematic layer structure of the field-effect penicillin biosensor modified with TMV nanotubes as enzyme (penicillinase) nanocarrier.

2.2. Loading of TMV Nanotubes and Enzyme Immobilization

For the modification of EIS sensors, biotinylated TMV particles were dissolved in 10 mM sodium-potassium phosphate buffer with a concentration of 0.1 µg TMV/µL and incubated on the EIS sensors for 1 h at room temperature. Then, the streptavidin-conjugated enzyme penicillinase ([SA]-penicillinase) was immobilized on TMV nanotubes via biotin-streptavidin bioaffinity binding. The enzyme penicillinase (*Bacillus cereus*, specific activity: 2924 U/mg protein) was purchased from Sigma-Aldrich (Taufkirchen, Germany).

2.3. Measurement Setup

The prepared biosensor chips were mounted into a home-made measuring cell and sealed by an O-ring. The contact area of the sensors surface with analyte solution was about 0.5 cm^2. The sensors were characterized by means of constant-capacitance (ConCap) mode using an impedance analyser Zennium (Zahner Elektrik, Kronach, Germany). For measurements, a DC (direct current) bias voltage and a small AC (alternating current) voltage of 20 mV were applied to the EIS structure via a conventional Ag/AgCl reference electrode (Metrohm, Filderstadt, Germany). Penicillin solutions with different concentrations ranging from 0.1 to 10 mM were prepared by dissolving penicillin G (Sigma-Aldrich) in a 0.25 mM polymix buffer solution, pH 8, containing 100 mM KCl.

3. Results and Discussion

The surface morphology of TMV-loaded sensors was characterized by scanning electron microscopy (SEM). Figure 2 demonstrates an example of a SEM image of the EIS sensor surface covered with TMV-[SA]-penicillinase nanotubes. Individual virus particles are clearly visible in the SEM image.

Figure 3 shows a ConCap response of the field-effect biosensor recorded in polymix buffer (pH 8) and in penicillin solutions of different concentrations of 100 µM, 1 mM and 10 mM. With increasing penicillin concentration, the concentration of the H^+ ions resulting from the penicillin hydrolysis by the enzyme penicillinase is increased, too [8,10]. As a result, the sensor signal is shifted in the direction of more negative voltages. The recorded potential steps correlate directly with the particular penicillin concentration in the solution. The penicillin sensitivity evaluated from the ConCap curves was about 90 mV/dec.

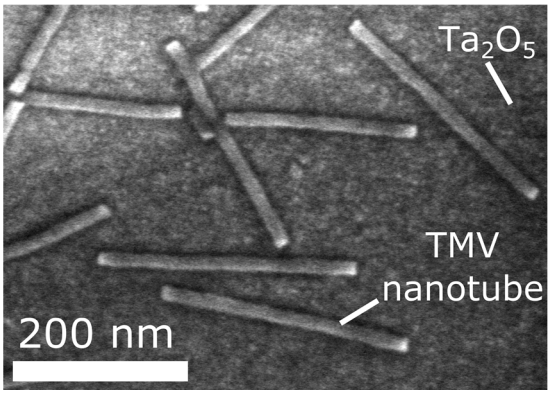

Figure 2. SEM image of the EIS sensor surface modified with TMV nanotubes with a typical length of about 300 nm and an outer diameter of 18 nm.

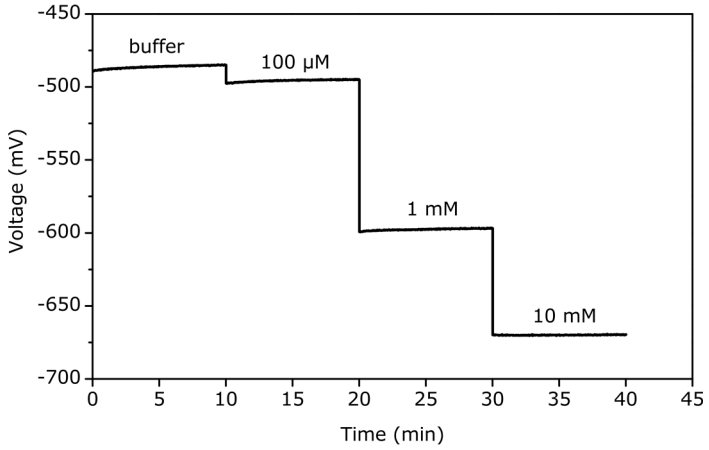

Figure 3. ConCap response of the TMV-based field-effect biosensor recorded in solutions with different penicillin concentrations.

4. Conclusions

The achieved results demonstrate the potential of TMV-modified field-effect structures as a new universal platform for the creation of enzyme biosensors.

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Conflicts of Interest: The authors declare no conflict of interest.

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