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Antibody Immobilization in ZnO-Thin Film Transistors for Low-Cost Biosensors Applications †

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Abstract: The antibody immobilization with low-cost materials and label-free methods are a challenge for the fabrication of biosensor devices. In this work, it was developed a strategy for antibody immobilization on ZnO TFTs over polyethylene terephthalate (PET) as a recyclable plastic substrate. Antibodies were biofunctionalized using a label-free strategy for *E. coli* detection. The use of a recyclable plastic substrate PET enables the compatibility with flexible electronics that could contribute for a low-cost biosensor useful in rural communities that do not have the necessary infrastructure and trained personnel for pathogenic bacterial detection in food or water.

Keywords: antibodies; ZnO TFTs; biosensors

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1. Introduction

Foodborne illnesses caused by pathogenic bacteria represent a worldwide concern of public health in many countries. Therefore, there is a growing interest for detection of pathogens. Conventional methods for detection and identification of microbial pathogenic agents mainly rely on specific microbiological, biochemical and molecular identification [1]. However, alternative detection techniques that allow the reduction of analysis times, reagents and specialized infrastructure are of special attention for several research teams, that have led to the development of new technologies such as the biosensor devices. A biosensor is an analytical device that has a biological receptor that can specifically recognize and detect a target analyte, a physical or chemical transducer that converts the biological and/or chemical reaction into a quantifiable signal [2–4]. The use of antibodies as the element for biological recognition is a common practice mainly due to their high affinity and selectivity for the antigen-antibody reaction [5]. In this way, immunosensing devices are a developing technology with a versatile application in the early detection of diseases, toxins or analytes of biological interest [6,7]. One of the most important aspects for the immunosensors development is the orientation of active sites during the antibody immobilization. However, there is not only one adequate orientation, because the orientation of the antibody could depend on the method to determinate the analyte. Label-free strategies and recyclable materials are viable alternative for the generation of low-cost devices. On the other hand, the use of plastic substrates such as polyethylene terephthalate allow a high compatibility with flexible electronics. In the present work, polyclonal antibodies were immobilized on thin-film transistors (TFTs) processed at low-temperature, with a perspective to be used for detection of foodborne pathogens.

2. Materials and Methods

2.1. Fabrication and Characterization of ZnO Thin Films Transistors

Polyethylene terephthalate (PET) was used as substrate for all devices covered with Indium tin oxide (ITO). 85 nm-thick Spin on glass (SOG-SiO_2) was used as the dielectric film. The thickness of aluminum contacts was 100 nm, deposited by e-gun and wet etching patterned. The ZnO active layer (90–100 nm) were deposited at 200 °C by high-frequency ultrasonic spray pyrolysis technique on a hot plate under atmospheric pressure [8]. The electrical characterization was made using a Keithley-4200 equipment.

2.2. ZnO Thin Film Transistors Functionalization

The biofunctionalization strategy has been reported in a previous work [8], with some modifications for compatibility with TFTs. 3-aminopropyltrimetoxysilane (APTMS) was employed for silanization, in order to obtain functional groups available for antibody binding on the active layer of transistor (ZnO). An initial stock of polyclonal antibodies (0.1 mg/mL) against *E. coli* EPEC were used for bacterial detection. The incubation time for antibody immobilization was 60 min.

2.3. Bacterial Detection

E. coli EPEC was used as our testing model, it was reactivated by streaking in Luria-Bertani (LB) plates. Posteriorly, an individual colony was propagated in 100 mL LB broth until a concentration of 1×10^8 CFU/mL was reached. Since the used contact time between our biosensor and the bacterial solution was just of few seconds, it was considered real-time detection.

3. Results and Discussion

Antibody Immobilization on ZnO TFTs

The authors have previously reported some works focusing on low-cost techniques for the fabrication of ZnO TFTs [9]. Some important characteristics that have been considered are the use of deposition techniques like spray pyrolysis and spin coating that are inexpensive, quite simple and can be operated at atmospheric pressure without a vacuum system. Otherwise, the use of plastic substrates requires the use of low temperatures that do not affect the material. Hence, the TFTs processing was carried out at 200 °C. For the fabrication of the ZnO TFTs, inverted coplanar structure was used. The Figure 1 shows a schematic representation of the device before the biological treatment with antibodies.

Figure 1. Schematic view on ZnO TFTs before the immobilization process.

The ZnO active layer was employed as a matrix for immobilization of the biological recognition element (antibody). The advantage when using the ZnO as an immobilization platform on TFTs is that it is an oxide; hence, there is a higher generation of OH^- groups. Therefore, the use of bifunctional molecules like silanes allow to have a higher amount of available binding sites for antibodies. The Figure 2 shows a representation of the immobilization process on ZnO TFTs.

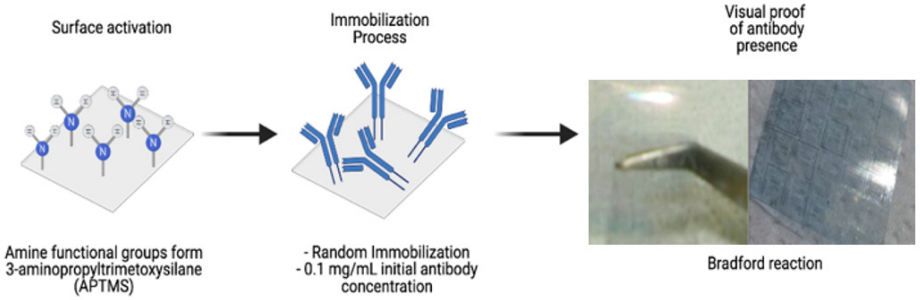

Figure 2. Methodological representation of the immobilization strategy (Biorender, 2021).

Previously, there have been reported the characteristic signals corresponding to the antibodies using the Fourier Transformed Infrared spectroscopy (FTIR) [8] to observe changes during the surface activation and immobilization. These signals were related to the amide I, II and disulfide bridges. The label-free immobilization strategy followed in this work contribute to the use of less reagents; however, a verification of antibodies bound on the surface is necessary. Since the antibodies are proteins, the Bradford method is a visual validation that allow to detect the blue coloration change after contact, as observed in Figure 2. This validates the presence of antibodies immobilized on ZnO TFTs. In this way, the electrical characterization allowed us to know the biosensor behavior and also the contribution of biological processes in the transfer characteristics (Figure 3).

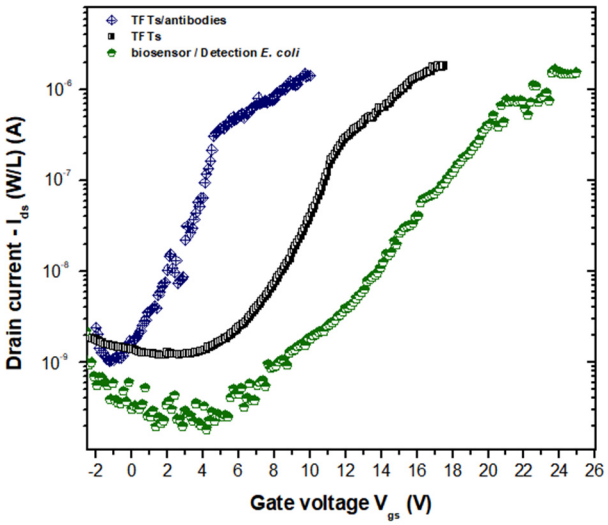

Figure 3. Transfer characteristics of biosensor before and after of bacterial detection.

When antibodies are immobilized an important change in the transfer characteristics is observed and it is attributed directly to the presence of antibodies in the biosensor. It is suggested that when antibodies are on the active layer, they are able to change the behavior in the conduction channel resulting in that observed displacement. Since the antibodies have an intrinsic charge derived from their aminoacid residues, this has an influence on the needed voltage to reach the on–off regions [10]. Afterwards, when bacterial detection takes place, the displacement towards positive voltages affects the previous contribution shown by the antibodies, which is mainly related to the bacterial membrane adhered in the antibody variable region. It is suggested lipopolysaccharides and other components of the bacterial membrane exert a resistive effect on the conduction channel. Based on these observations, the perceived changes on the transfer characteristics of detection are

directly relate d to the used bacterial concentration (1×10^8 CFU/mL). Therefore, it is suggested that lower bacterial concentrations will allow to observe different displacements between both curves (Figure 3 blue and green).

These results show clear signals of the bacterial detection from the biosensor. One of the most important points of our work is the perspective of the FET devices, whose electrical behavior should not be altered in function of the biological components [10], that is just accomplished in this research. Although a random type of antibody immobilization was performed, in previous reports [8], it was demonstrated the functionality of antibodies by carrying out a molecular validation of detection for that, it is suggested that the antibodies have the ability to detect *E. coli* because their active sites are available for the antibody-antigen recognition.

4. Conclusions

The methodological process for immobilization of antibodies has been found to be reproducible. The process of chemical modification of the transistor active layer does not affect the electrical behavior or any of the components, which allows a transfer curve to be obtained and the process conditions are reproducible. It is worth mentioning that the use of plastic substrates and processing at low temperature enables the development of immunosensor devices with an important projection for low cost and sustainable technologies.

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

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