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Towards the Development of Electrical Biosensors Based on Nanostructured Porous Silicon

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Abstract: The typical large specific surface area and high reactivity of nanostructured porous silicon (nanoPS) make this material very suitable for the development of sensors. Moreover, its biocompatibility and biodegradability opens the way to the development of biosensors. As such, in this work the use of nanoPS in the field of electrical biosensing is explored. More specifically, nanoPS-based devices with Al/nanoPS/Al and Au-NiCr/nanoPS/Au-NiCr structures were fabricated for the electrical detection of glucose and *Escherichia Coli* bacteria at different concentrations. The experimental results show that the current-voltage characteristics of these symmetric metal/nanoPS/metal structures strongly depend on the presence/absence and concentration of species immobilized on the surface.

Keywords: porous silicon; glucose; *Escherichia coli*; biosensing

1. Introduction

The particular structure and surface morphology of nanostructured porous silicon (nanoPS), as well as the properties of the nanoPS/Si interface, together with the relatively good control over its physicochemical behavior provided by electrochemical processes, has stimulated much research

leading to many different applications in a number of fields ranging from microelectronics to biomedical applications. A number of examples can be found in references [1–3]. It is worth noting that the nanoPS formation process is fully compatible with current semiconductor processing technologies.

NanoPS can be regarded as a complex network of silicon nanocrystals embedded into a porous matrix [4–6]. The large specific surface area of nanoPS together with its large surface reactivity allows the fabrication, among other devices, of chemical sensors and biosensors. In particular, the electrical behavior of nanoPS is in general extremely sensitive to its surface properties and composition [7–9], and is highly sensitive to the presence or absence of biomolecules on its surface [10]. Therefore, the integration of nanoPS in electrical biosensors would result in increased sensitivity.

Moreover, its particular surface structure and large specific surface is highly attractive for the nucleation of bioceramic apatite in simulated body fluids [11] and can be used as a catalytic environment for protein nucleation [12]. An additional advantage is given by the fact that for some specific applications no surface functionalization is required for the subsequent covalent linkage of biomolecules.

In this work, nanoPS-based devices with a metal/nanoPS/metal structure were fabricated for the electrical detection of glucose and *Escherichia coli* (*E. coli*). Immobilization of glucose on nanoPS was successfully performed with no previous surface functionalization. However, biofunctionalization was required for the immobilization of *E. coli* fragments for subsequent detection. Based on our previous experience [13–15], biofunctionalization of nanoPS was performed by the use of solutions of aminopropyltriethoxysilane (APTS) in toluene. These solutions were found to be highly adapted for the subsequent immobilization of *E. coli* antibodies through the high density of amino groups induced on the surface of nanoPS. After antibody immobilization, fragments of *E. coli*, which are specifically recognized by the antibodies present on the surface of nanoPS, were immobilized. The nanoPS-based devices were found to show a strong dependence of their electrical behavior on the presence and concentration of glucose and *E. coli* fragments.

2. Experimental

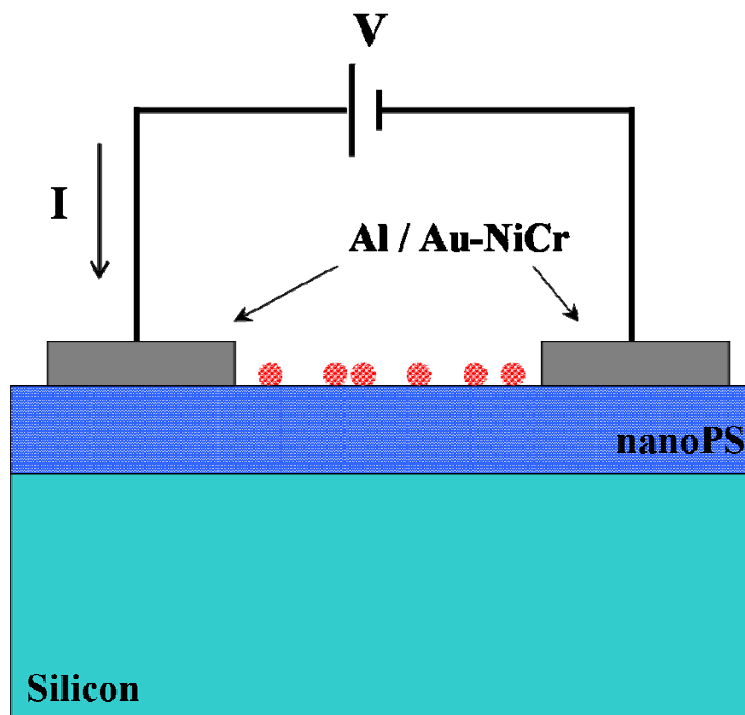
2.1. Fabrication of Electrical Biosensors Based on Nanostructured Porous Silicon

Layers of nanoPS were grown by electrochemical etching of boron-doped (*p*-type) silicon wafers (orientation: $\langle 100 \rangle$, resistivity: 0.1–0.5 Ωcm). Low resistivity ohmic contacts were formed by coating the backside of the Si wafers with Al and subsequently annealing at 400 °C for 5 min. The wafers were cut into 1×1 cm² pieces which were mounted into a sample holder with an exposed area to the electrochemical etching solution of 0.64 cm². The electrolyte consisted of HF (48 wt %):ethanol (98 wt %) solutions with different HF to ethanol ratios. The wafers were galvanostatically etched using etching current densities ranging from 10 mA/cm² to 100 mA/cm², and etching times from 20 to 180 seconds. Illumination from a 100 W halogen lamp was used to promote the generation of electron-hole pairs, this contributing to reduce the typical crystallite size.

Metal (Al or Au-NiCr) top contacts were used as 1-mm-diameter diode pads for the fabrication of the electrical biosensors. Since Au often does not adhere well to the surface of nanoPS, extremely thin NiCr layers (~10 Å) were previously sputter-deposited onto the nanoPS layers. The overall process

leads to two different biosensing structures: Al/nanoPS/Al and Au-NiCr/nanoPS/Au-NiCr. Figure 1 shows the structure of the nanoPS-based devices used for the electrical biosensing of glucose and *E. Coli* fragments.

Figure 1. Cross-sectional view of a typical metal/nanoPS/metal biosensing device and configuration used for the electrical characterization.



2.2. Immobilization of Glucose

NanoPS-based devices with the structure Al/nanoPS/Al were incubated with glucose (D-(+)-glucose, Sigma-Aldrich). As such, glucose was diluted in distilled water at different concentrations ranging from 1 $\mu\text{g/L}$ to 1000 $\mu\text{g/L}$. The nanoPS-based devices were incubated in 2 mL glucose solutions for 20 minutes. Removal of non-adsorbed glucose molecules was accomplished by the immersion of the samples in 2 mL of water for five minutes. This process was repeated twice to ensure effective removal of non-adsorbed glucose molecules.

2.3. Biofunctionalization and Immobilization of *E. Coli*

Aiming at the subsequent immobilization of *E. coli* antibodies, surface biofunctionalization was performed by the immersion of the nanoPS-based devices in aminopropyltriethoxysilane (APTS)-toluene (2:1,000) solutions for 15 min. Amine groups were chosen since they are present in proteins, and their chemical interaction with other functional groups is relatively well understood. Furthermore, APTS has been previously demonstrated to allow the effective surface functionalization of nanoPS [13,15]. In this process, the presence of SiO_2 on the surface of nanoPS (formed by the oxidation as a result of exposure to ambient air), reacts with the APTS-based solution leading to a large surface density of amino groups.

E. coli antibodies (Abcam) were immobilized on the functionalized surface by the immersion of nanoPS-based devices with the structure Au-NiCr/nanoPS/Au-NiCr in antibody: phosphate buffered saline (PBS) 1x in a range of concentrations from 0 to 100 $\mu\text{g/mL}$. After antibody immobilization, *E. coli* fragments (KPL) were immobilized following the same procedure, *i.e.*, by the immersion of the nanoPS-based devices in *E. coli* fragments: PBS solutions of different concentration (ranging from 2.5 to 100 $\mu\text{g/mL}$) for 30 minutes. Before and after each incubation step, the nanoPS-devices were rinsed in distilled water to eliminate any non surface linked antibodies or fragments and/or remaining PBS crystals.

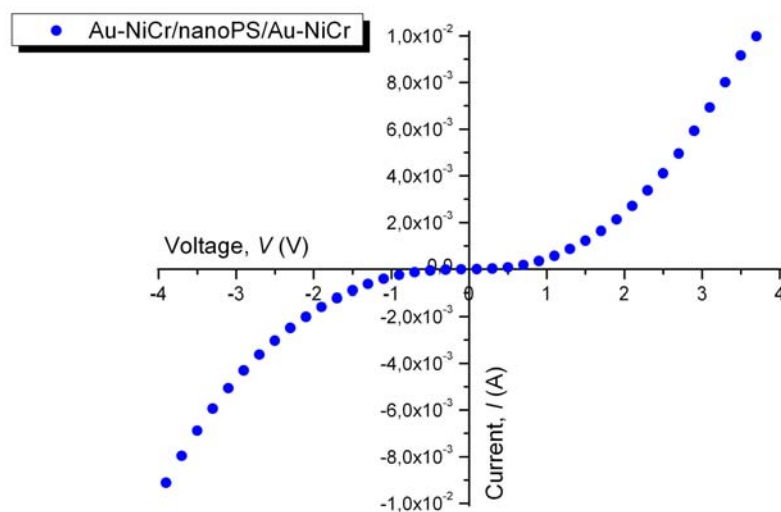
2.4. Electrical Characterization

Electrical characterization (I-V curves) was carried out in the dark by using a Hewlett Packard pA meter/dc voltage source, Model 4140B. Figure 1 shows the configuration used for the determination of the electrical behavior (I-V curves) of the nanoPS-based devices.

3. Results

Current-voltage measurements (*I-V* curves) were taken between two consecutive contacts deposited on top of the surface of nanoPS (Figure 1) immediately after the fabrication of the metal/nanoPS/metal devices. Figure 2 shows a typical *I-V* curve for the Au-NiCr/nanoPS/Au-NiCr devices, from which it is observed that these structures show a double rectifying behavior. A similar behavior has been previously observed in symmetrical nanoPS-based devices with the structure metal/nanoPS/Si/nanoPS/metal using different metals (Au/Cr, W, Ta, WTi, Cr and Ti), finding that the current through the PS-based structures greatly depends on the metallic contact and that Au contacts with an extremely thin Cr underlayer provide the highest values of current [16].

Figure 2. Typical *I-V* curve for the Au-NiCr/nanoPS/Au-NiCr symmetrical structures.

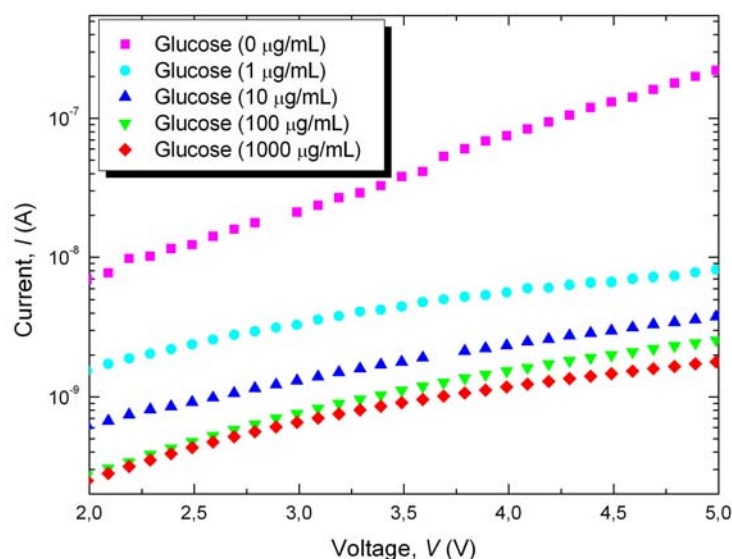


3.1. Electrical Biosensing of Glucose

The singular structure and composition of the surface of nanoPS has been demonstrated to favor immobilization of glucose [10]. Accordingly, for this particular application no previous surface functionalization is required. The large surface activity of nanoPS observed in this and previous works [15] can be partially attributed to its high specific surface which provides numerous available bonding sites, as a result of the electrochemical formation process in HF-based solutions and subsequent oxidation processes.

Typical current-voltage (I - V) characteristics acquired immediately after the immobilization of glucose on the surface of nanoPS are shown in Figure 3. It is notable that resistivity has noticeably increased, most likely due to surface oxidation of the porous structure. The different curves were recorded for several concentrations of glucose in distilled water, ranging from 0 $\mu\text{g/mL}$ to 1,000 $\mu\text{g/mL}$. From Figure 3 it is observed that increasing glucose concentration results in lower current for a given voltage. Additionally, the horizontal distance between the curves is smaller for increasing glucose concentration, indicating that the devices are approaching the saturation limit. In particular, the curves corresponding to 100 $\mu\text{g/mL}$ and 1,000 $\mu\text{g/mL}$ practically overlap for low voltages.

Figure 3. Current (I) as a function of applied voltage (V) for Al/nanoPS/Al structures after glucose immobilization from solutions at different concentrations.



The experimental results indicate that the immobilization of glucose on the surface of nanoPS leads to changes in its surface charge distribution, resulting in lower conductivity through the Al/nanoPS/Al devices. These results agree with previously reported measurements of electrical conductivity of glucose in water [17]. Moreover, the reduction in conductance is not attributed to water used for the dissolution of glucose, since water has been previously demonstrated to increase the conductivity of nanoPS [18,19]. Accordingly, this effect has been attributed to changes in the dielectric constant, dipole moment and possible chemisorption or physisorption on the surface of nanostructured porous silicon.

3.2. Electrical Biosensing of *E. coli*

As opposed to the case of glucose immobilization, biofunctionalization is required to provide a soft chemical binding layer for the subsequent immobilization of *E. coli* antibodies. The presence of reactive amine functional groups on the surface of functionalized nanoPS has been previously confirmed by X-ray photoelectron spectroscopy (XPS) characterization, from which it was determined that the main elements present in the surface of nanoPS are nitrogen, oxygen, silicon and carbon [13].

Electrical characterization was performed after surface biofunctionalization in APTS-toluene solutions, from which a double-rectifying behavior was again observed, as in the case of as-formed nanoPS-based devices. However, surface biofunctionalization results in decreased conductivity for a given voltage for both forward and reverse polarization. *E. coli* antibody immobilization was performed by incubation in *E. coli* antibody:PBS solutions at 25 µg/mL leading to increased conductivity. It was also determined that higher antibody concentration results in larger current for a given voltage.

The surface of nanoPS was analyzed by scanning electron microscopy (SEM) immediately after the biofunctionalization process and after the immobilization of fragments of bacterium *E. coli* (Figure 4). Surface analysis shows the successful immobilization of fragments of bacterium *E. coli* on the surface of nanoPS (Figure 4, bottom). As such, it was verified that the antibodies and fragments of bacterium have been linked to the nanostructured surface.

Figure 4. Scanning electron microscopy (SEM) images of the surface of nanoPS immediately after the biofunctionalization process (top), and after immobilization of antibodies (100 µg/mL) plus fragments of *E. coli* bacterium (bottom).

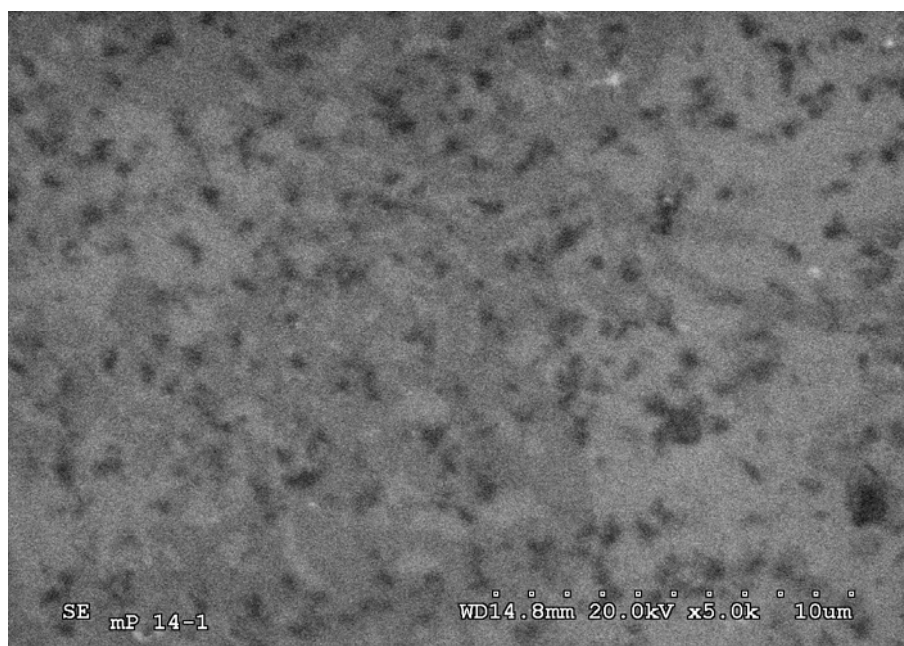
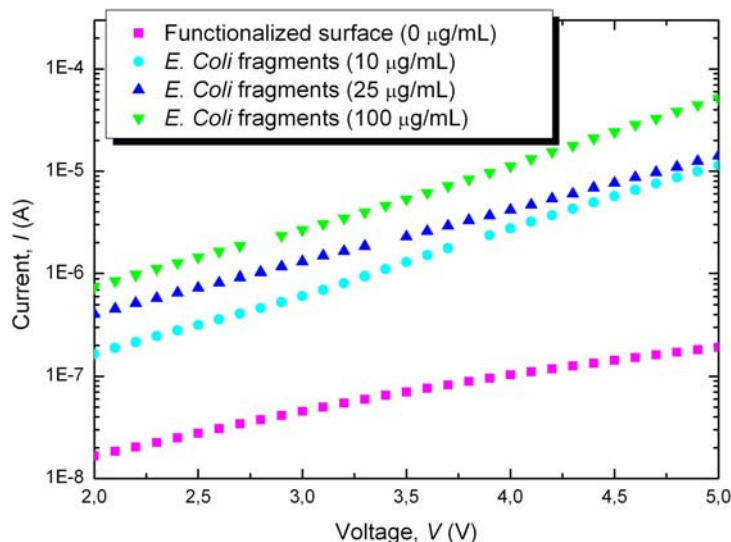


Figure 4. Cont.



Finally, current-voltage measurements were performed after probing *E. coli* fragments on the Au-NiCr/nanoPS/Au-NiCr devices from PBS solutions of increasing concentration of *E. coli* fragments, ranging from 10 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$. Figure 5 shows the experimental results, from which increased conductivity is observed for increased *E. coli* fragment concentration. This suggests that the higher the concentration, the larger the surface concentration of *E. coli* fragments on the surface of nanoPS and hence the device is more conductive. In the range of concentrations used in this work no saturation effects were found.

Figure 5. Current (I) as function of the applied voltage (V) through the Au-NiCr/nanoPS/Au-NiCr structure after probing with *E. coli* fragments at different concentrations on devices with *E. coli* antibodies previously immobilized in 25 $\mu\text{g/mL}$ solutions.



From the experimental results it can be inferred that the electrical behavior of devices with the Au-NiCr/nanoPS/Au-NiCr structure is greatly affected by the following three processes: functionalization in APTS-toluene solution, immobilization of *E. coli* antibodies and probing with fragments of *E. coli*.

In particular, the functionalization process results in decreased conductivity, which most likely has its origin in the presence of a large surface density of amino groups on the surface and near-surface of nanoPS. However, the immobilization of antibodies and fragments of *E. coli* leads to increased conductivity, suggesting that antibodies possess higher conductivity than nanoPS, and that they have successfully been linked to the biofunctionalized surface. In this respect, previous studies have determined the conductivity of colloidal *E. coli* particles of 1 micron in diameter to be 0.03 mho/m [20]. Additionally, increased conductivity suggests that the biomolecules might be not only present on the surface of nanoPS, but slight diffusion into the structure of nanoPS might have occurred. The variations on the conductivity are attributed, as in the case of glucose, to the effects caused by the immobilization of biomolecules in the dielectric constant and dipolar moments on the surface of nanoPS.

4. Conclusions

Electrical devices based on nanostructured porous silicon with the Al/nanoPS/Al and Au-NiCr/nanoPS/Au-NiCr structure were fabricated and characterized, showing a double rectifying behavior.

Glucose biomolecules were immobilized on the surface of nanoPS from solutions of glucose in distilled water at different concentrations. Electrical measurements (current-voltage characteristics) were performed through the nanoPS-based structures, from which a reduction of current for increasing glucose concentration (constant voltage) is observed.

Biofunctionalization was required for the subsequent immobilization of *E. coli* antibodies on the surface of nanoPS. However, the conductivity of the Au-NiCr/nanoPS/Au-NiCr devices is somewhat lowered by the biofunctionalization process. Finally, immobilization of antibodies and fragments of *E. coli* resulted in increased conductivity and showed a dependence on *E. coli* surface concentration. Accordingly, the Au-NiCr/nanoPS/Au-NiCr devices have been demonstrated as possible candidates for the subsequent development of biosensors for *E. coli* bacteria.

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