Surface Functionalization of Nanostructured Porous Silicon by APTS: Toward the Fabrication of Electrical Biosensors of Bacterium *Escherichia coli*

G. Recio-Sánchez[1,2], G. Domínguez-Cañizares[1,3], M. Manso[1,2], I. Preda[1], V. Torres-Costa[1,2], A. Gutiérrez[1,3], L. Soriano[1,3] and R.J. Martín-Palma[1,2,3,]*

[1]Departamento de Física Aplicada, Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain, [2]CIBER-bbn, Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain, [3]Instituto de Ciencia de Materiales "Nicolas Cabrera", Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain

Abstract: Nanostructured porous silicon (nanoPS) basically consists in a network of silicon nanocrystals with high specific surface. Its intrinsic high surface reactivity makes nanoPS a very suitable material for the development of biosensors. In this work, the surface of nanoPS was functionalized by the use of (3-aminopropyl)triethoxysilane solutions in toluene. *Escherichia coli* (*E. coli*) antibodies were subsequently immobilized on the functionalized surfaces. Finally, fragments of this bacterium, which are specifically recognized by the antibodies, were immobilized. Moreover, devices with a metal/nanoPS/semiconductor/metal structure were fabricated aiming at the electrical biosensing of *E. Coli* bacterium. The experimental results showed a strong variation of the current as a function of the presence/absence of bacterium *E. Coli* and surface concentration.

Keywords: Biosensor, functionalization, nanostructured porous silicon, electrical detection.

1. INTRODUCTION

Nanostructured materials are receiving increasing attention as candidates for the fabrication of high-sensitivity biosensors, given the accurate control of their physico-chemical properties and overall behavior as a result of the precise control over their size, shape and composition [1-4]. However, the successful development of solid-state biosensors requires the fabrication of reliable interfaces between biomolecules and the surface of the transducer. This task is usually accomplished by the use of stable interlayers [5,6]. Among other properties, an effective interlayer should contain a large density of functional groups which would provide specific reactivity with the biomolecules to be detected. Some of the most widely used functional groups include thiols (-SH), amines ($-NH_2$), and carboxylic acids (-COOH). In most cases, the interaction with biomolecules occurs through covalent bonding.

The particular structure, surface morphology and overall properties of nanostructured porous silicon (nanoPS) [7-9] have stimulated much research on this material, resulting in many different actual and proposed applications (several examples can be found in [refs. 10-13]), including biosensing [14]. NanoPS can be loosely described as a network of silicon nanocrystals with quite large specific surface. The optical and electrical behavior of nanoPS are in general extremely sensitive to its surface properties and composition [15-17] and, in particular, are highly sensitive to the presence and concentration of biomolecules in contact with its surface [18-20]. Therefore, the integration of nanoPS in optical and electrical biosensors would result in increased sensitivity. In addition, the formation process of nanoPS is fully compatible with current semiconductor processing technologies. Furthermore, the functionality of nanoPS in biological media has been previously outlined. Its particular surface structure is highly attractive for the nucleation of bioceramic apatite in simulated body fluids [21] and acts as a catalytic environment for protein nucleation [22].

In this work, the surface of nanoPS was functionalized for its subsequent use in the field of electrical biosensing. In previous works, nanoPS thin films were functionalized by means of a thermally-activated chemical deposition system, using (3- aminopropyl) triethoxysilane (APTS) [6, 23]. Aiming at the development of a simplified functionalization process, funtionalization of nanoPS was performed in this work by using solutions of APTS in toluene. X-ray photoelectron spectroscopy (XPS) was used for the determination of the surface composition before and after the functionalization process. After antibody immobilization, fragments of *E. coli*, which are specifically recognized by the antibodies present on the surface of nanoPS, were immobilized. Finally, it is shown that the presence of *E. coli* fragments on the surface of nanoPS can be detected by changes in the electrical behavior of nanoPS-based devices with the structure metal/nanoPS/Si/metal.

2. EXPERIMENTAL

Nanostructued porous silicon layers were fabricated by electrochemically etching boron-doped (*p*-type) silicon wafers (orientation: <100>, resistivity: 0.1–0.5 Ωcm). Low resistivity ohmic contacts were formed by thermally evaporating Al on the backside of the Si wafers and subsequently annealing at 400 °C for 5 min. The wafers were cut into 1×1 cm^2 pieces which were mounted into a sample holder with an exposed area to the electrochemical etching solution of 0.64 cm^2. The composition of the solution was 1:2 HF (48 wt %):ethanol (98 wt %). The wafers were galvanostatically etched under illumination from a 100 W halogen lamp. The etching current density was typically 80 mA/cm^2 and the etching time 25 seconds, leading to the formation of 2 micron-thick nanoPS layers with pore sizes in the range on a few tens of nanometers [7].

Before functionalization, the nanoPS layers were left in contact with the atmosphere for 24 hours in order to promote silicon oxidation. Surface funtionalization was performed by the immersion of the nanoPS layers in (3-aminopropyl)triethoxysilane (APTS):toluene (2:1000) solutions for 15 min. APTS has been previously demonstrated to allow the effective surface functionalization of nanoPS [6,23]. In this process, SiO_2 present 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.

X-ray photoelectron spectroscopy (XPS) characterization was performed in a conventional surface analysis chamber equipped with a Mg-anode X-ray source, and a VG-CLAM4 hemispherical electron analyzer. The base pressure in the analysis chamber was in the 10^{-10} mbar range.

Address correspondence to this author at the Departamento de Física Aplicada, Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain; Tel: +34 914974028; Fax: +34 914973969;
E-mail: rauljose.martin@uam.es

1573-4137/11 $58.00+.00

Escherichia coli antibodies (Abcam) were immobilized on the functionalized surface by the immersion of the nanoPS-based devices in antibody: phosphate buffered saline (PBS) 1x in a range of concentrations from 0 to 100 µg/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 µg/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.

A Hitachi S3000-N scanning electron microscope (SEM) was used for morphological characterization. This characterization system is equipped with an ISIS 300 microanalysis system, thus allowing energy-dispersive x-ray (EDX) analysis. Electrical characterization (*I*-*V* curves) was carried out in the dark by using a Hewlett Packard pA meter/dc voltage source, Model 4140B.

3. RESULTS

NanoPS thin-films were fabricated by the electrochemical etch of Si wafers for subsequent surface functionalization and *E. coli* antibody immobilization. The chemical composition of the nanostructured surfaces was studied by X-ray photoelectron spectroscopy (XPS) at three different stages (Fig. **1**): immediately after the fabrication of nanoPS (fresh nanoPS), after being in contact with the atmosphere for 48 hours (oxidized nanoPS), and after the functionalization process (functionalized nanoPS). In this regard, it is worth noting that nanoPS is left intentionally in contact with the atmosphere an extended period of time in order to promote Si nanocrystal oxidation, aiming at boosting amine group interaction with SiO_2.

Fig. (**1**) shows the C 1s, Si 2p, O 1s, and N1s XPS spectra acquired from fresh nanoPS, oxidized nanoPS and functionalized nanoPS layers. The experimental results show in all cases carbon, silicon and oxygen peaks (Figs. **1a**, **1b**, and **1c**). The C1s/Si2p and O1s/Si2p ratios determined from XPS measurements are presented in Table **1**. As it can be seen in Fig. (**1b**), exposure of fresh nanoPS

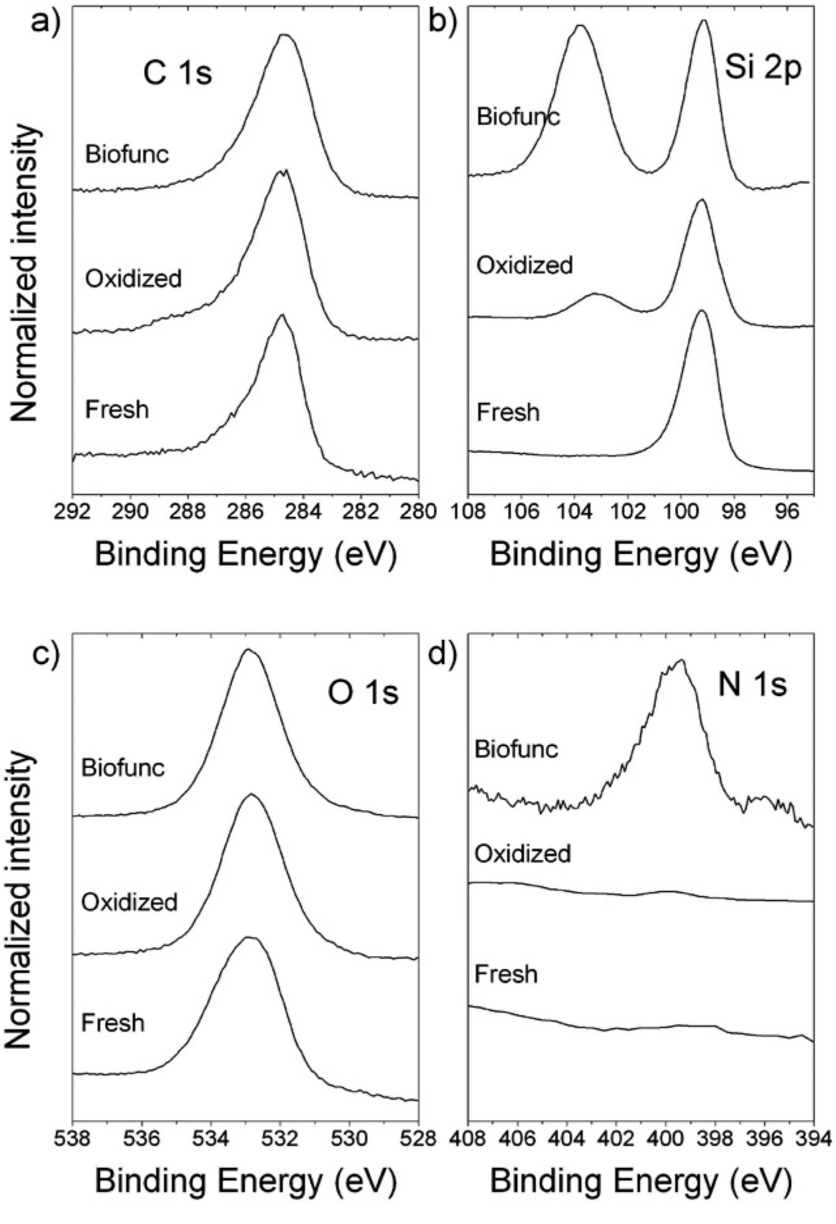


Fig. (1). XPS spectra showing the main elements present on the surface of fresh, oxidized and functionalized nanoPS layers: (**a**) C 1s, (**b**) Si 2p, (**c**) O 1s and (**d**) N 1s.

layers to the atmosphere produces slight oxidation of the Si atoms, resulting in a new peak located at about 103.2 eV, whereas the functionalization process produces strong oxidation of the Si atoms in the nanoPS layers. Accordingly, a peak located at around 103.8 eV is now found indicating that Si suboxides close to SiO_2 were formed. This tendency is also reflected in Table **1** where the C1s/Si2p and O1s/Si2p ratios determined from XPS quantitative analysis are presented. As such, the initial O1s/Si2p ratio corresponding to fresh nanoPS increases upon exposure to the atmosphere from 0.162 to 0.684. The subsequent functionaliztion process further oxidizes the initial nanoPS layer (the O1s/Si2p ratio equals 1.283 in this case).

Table 1. C1s/Si2p and O1s/Si2p Ratios as Determined from XPS Measurements

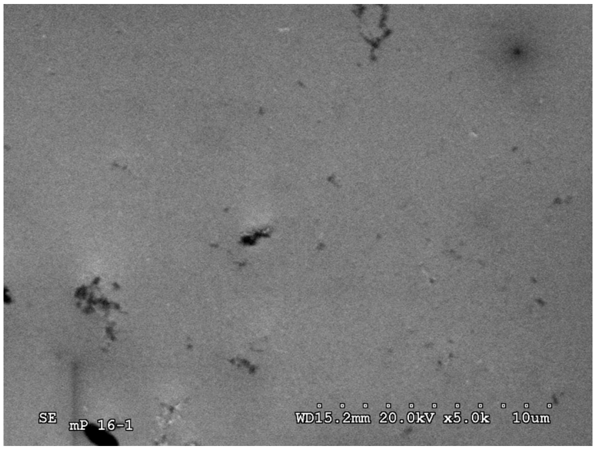
	C1s/Si2p Ratio	O1s/Si2p Ratio
Fresh nanoPS	0.098	0.162
Oxidized nanoPS	0.595	0.684
Functionalized nanoPS	0.949	1.283

The functionalization process results also in increased C1s/Si2p ratio, from 0.098 (fresh nanoPS) to 0.595 (oxidized) to 0.949 (functionalized). In the first case, carbon most likely comes from contamination, while the increment observed in the later case might be associated to CH_2 and CH_3 groups coming from the APTS molecule. With regard to the oxidation process, the Si 2p core level spectra perfectly illustrate this process. Two very well defined components can be observed in samples exposed to the atmosphere and to the functionalization process. The first contribution, in common with the fresh nanoPS sample, is located at binding energy (BE) of 99.1 eV and arises from underlying Si-Si. After exposure to the atmosphere a contribution at BE of 103.2 eV can be attributed to a thin Si-OH saturation layer. After the functionalization, the adsorption of the APTS molecules introduces new SiO_x contributions at slightly higher BE of 103.8 eV.

However, the most significant experimental result which would have a key effect on the effective immobilization of *E. coli* antibodies arises from the presence of nitrogen after the functionalization process. The peak observed in Fig. (**1d**) univocally indicates the presence of amino groups linked to the nanoPS layer and shows the effectiveness of the functionalization process. The functionalization implies the presence of silicon- aminopropyl bonds (Si- CH_2-CH_2-CH_2-NH_2). This new molecular structure on the surface introduces a C-N contribution to the C 1s core level spectrum of functionalized samples, which are reflected by an increase in the full width at half maximum (FWHM). Such an increase in the FWHM is specially observed in the high BE contribution of Si 2p peak and further justifies its attribution to an undetermined silicon sub-oxide SiO_x.

After having successfully functionalized the surface of nanoPS, *E. coli* antibodies were immobilized on the nanostructured surface and afterwards fragments of bacterium *E. coli* were immobilized. In this work the surface of nanoPS was analyzed by SEM after the functionalization process and after the immobilization of fragments of bacterium *E. coli*. Fig. (**2**) shows the surface morphology in both cases, from which the presence of fragments of bacterium *E. coli*. on the surface of nanoPS is observed (Fig. (**2**), bottom).

Previous works have studied the adsorption process of bovine serum albumin (BSA) on porous silicon layers [24], as well as protein adsorption on functionalized porous silicon surfaces [25], by a combination of FTIR spectroscopy, SEM, ellipsometry and photoluminescence. However, in this work electrical measurements are used to track changes after functionalization and *E. coli* immobilization. As such, to assess the possible use of nanoPS for the electrical detection of bacterium *E. coli*, metallic contacts were deposited on the surface of nanoPS resulting in devices with the structure Au-NiCr/nanoPS/Si/Al. Current-voltage measurements (Fig. **3**) demonstrate that these devices show rectifying electrical behavior, as previously observed in similar Al/nanoPS/Si/Al structures [26,27]. Fig. (**3**) also shows a typical *I-V* curve of the Au-NiCr/nanoPS/Si/Al devices after functionalization, from which a rectifying behavior is again observed. However, current values for a given voltage are notably lower than in the previous case. Accordingly, the functionalization process carried out in APTS:toluene solutions, which is needed to provide a soft chemical binding layer for the *E. coli* antibodies, results in reduced conductivity. As such, reduced conductivity is associated to the presence of a large surface density of amino groups on the surface and near-surface of nanoPS.



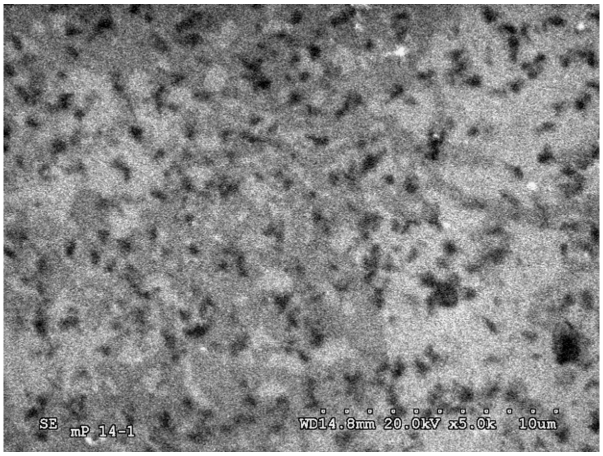


Fig. (2). Scanning electron microscopy (SEM) images of the surface of nanoPS after the funtionalization process (top), and after immobilization of antibodies (100 μg/ml) plus fragments of bacterium *E. coli* (bottom).

Current-voltage measurements were taken after probing *E. coli* fragments on the Au-NiCr/nanoPS/Si/Al devices from PBS solutions of increasing concentration of *E. coli* fragments, ranging from 10 μg/ml to 100 μg/ml. Fig. (**4**) shows the experimental results. In this case, increased *E. coli* surface concentration results in increased conductivity, suggesting that the higher the concentration, the larger the surface concentration of *E. coli* fragments present on the surface of nanoPS. The increase of conductivity might have its origin in the intrinsic conductivity of bacterium *E. coli* [28].

Summarizing, the experimental results show that the electrical behavior of devices with the structure Au-NiCr/nanoPS/Si/Al is greatly affected by the funtionalization process and by probing with

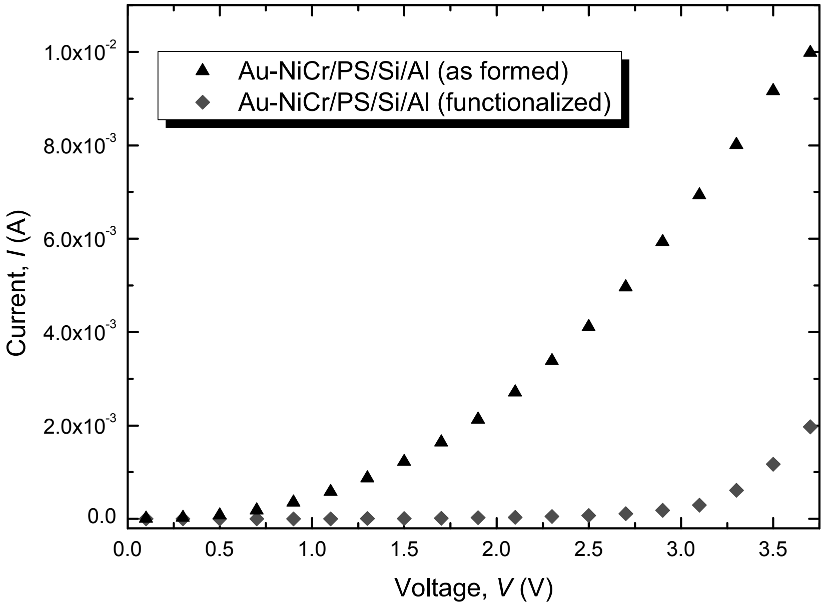


Fig. (3). Typical current-voltage (I-V) curves through the Au-NiCr/nanoPS/Si/Al structures before and after functionalization in APTS:toluene solutions. A rectifying behavior is observed in both cases.

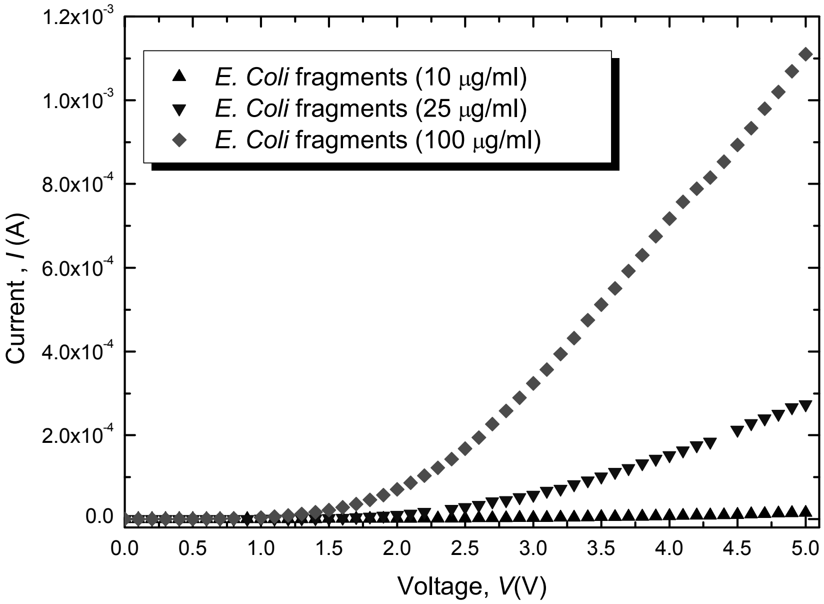


Fig. (4). Current (I) as function of applied voltage (V) through the Au-NiCr/PS/Si/Al devices after probing with of *E. coli* fragments at different concentrations on devices on which *E. coli* antibodies were previously immobilized in solutions at 100 μg/ml.

fragments of bacterium *E. coli*. It must finally be mentioned that close devices with the structure Al/nanoPS/Si/Al have previously shown their effectiveness in the electrical detection of glucose, given their strong dependence on the presence and concentration of glucose [20].

4. CONCLUSIONS

NanoPS layers were fabricated by the electrochemical etch of Si wafers. These nanostructured layers were subsequently oxidized, and functionalized by immersion in APTS/toluene solutions. The presence of amine groups on the surface of nanoPS was demonstrated. Successful immobilization of *E. coli* antibodies and fragments of bacterium *E. coli* on the surface of the previously functionalized nanoPS layers was demonstrated. As such, APTS/toluene solutions were found to be highly effective for the immobilization of *E. coli* antibodies given the large density of amine groups induced on the surface of nanoPS.

Additionally, electrical biosensing devices with the structure Au/Ni-Cr/nanoPS/Si/Al were fabricated. These devices showed their effectiveness in the electrical detection of changes on the surface of nanoPS, including the presence of *E. coli* antibodies. Accordingly, the potential of nanoPS-based devices in the field of biosensing has been demonstrated.

Moreover, the successful functionalization of nanostructured silicon surfaces is expected to have a significant impact, not only on the development of high-sensitivity biosensors, but also in such fields as bio-catalysis, tissue engineering, and the like.

ACKNOWLEDGEMENTS

The authors gratefully thank L. García-Pelayo for his invaluable technical support in the realization of the present work. Partial funding has been provided by the Comunidad de Madrid (Spain) under project Microseres and by the Ministerio de Ciencia e Innovación (MICINN) through contract FUNCOAT-CSD2008-00023.

REFERENCES

[1] Alivisatos, P. The use of nanocristals in biological detection. *Nat. Biotechnol.,* **2004**, *22*(1), 47-52.

[2] Martin-Palma, R.J.; Manso, M.; Torres-Costa, V. Optical biosensors based on semiconductor nanostructures. *Sensors,* **2009**, *9*(7), 5149-5172.

[3] Li, G. "Protein-based biosensors using nanomaterials"; In: Kumar, C., Ed. "Nanotechnologies for Life Sciences", Wiley-VCH, "Nanomaterials for Biosensors", **2007**, vol. *8*, pp. 278-310.

[4] Zhang, D.; Zhao, J.; Li, G. Protein-based voltammetric biosensors fabricated with nanomaterials. *Protein Pept. Lett.*, **2008**, *15*, 764-771.

[5] Williams, R.A.; Blanch, H.W. Covalent immobilization of protein monolayers for biosensor applications. *Biosens. Bioelectron.,* **1994**, *9*, 159-167.

[6] Martin-Palma, R.J.; Manso, M.; Perez Rigueiro, J.; Garcia Ruiz, J.P.; Martinez Duart, J.M. Surface biofunctionalization of materials by amine groups. *J. Mater. Res.*, **2004**, *19*, 2415-2420.

[7] Martín-Palma, R.J.; Pascual, L.; Herrero, P.; Martínez-Duart, J.M. Direct determination of grain sizes, lattice parameters, and mismatch of porous silicon. *Appl. Phys. Lett.* **2002**, *81*, 25-28.

[8] Martín-Palma, R.J.; Pascual, L.; Herrero, P.; Martínez-Duart, J.M. Monte Carlo determination of crystallite size of porous silicon from X-ray line broadening. *Appl. Phys. Lett.,* **2005**, *87*(21), 211906-1-3.

[9] Pascual, L.; Martín-Palma, R.J.; Landa-Cánovas, A.R.; Herrero, P.; Martínez-Duart, J.M. Lattice distorsion in nanostructured porous silicon. *Appl. Phys. Lett*., **2005**, *87*, 251921-1-3.

[10] Bisi, O.; Ossicini, S.; Pavesi, L. Porous silicon: a quantum sponge structure for silicon based optoelectronics. *Surf. Sci. Rep.,* **2000**, *38*, 1-126.

[11] Lehmann, V. *Electrochemistry of Silicon: Instrumentation, Science, Materials and Applications;* Wiley-VCH: Weinheim, **2002**.

[12] Föll, H.; Christophersen, M.; Carstensen, J.; Hasse, G. Formation and application of porous silicon. *Mater. Sci. Eng. R Reports,* **2002**, *39*(4), 93-141.

[13] Kochergina, V.; Foell, H. Novel optical elements made from porous Si. *Mater. Sci. Eng. R Reports,* **2006**, *52*(4-6), 93-140.

[14] Martín-Palma, R.J.; Torres-Costa, V.; Manso, M.; Martínez-Duart, J.M. Finite-thickness photonic crystals based on nanostructured porous silicon for optical sensing. *J. Nanophotonics,* **2009**, *3*, 031504.

[15] Cadet, C.; Deresmes, D.; Villaume, D.; Stievenard, D. Influence of surface defects on the electrical behavior of aluminum-porous silicon junctions. *Appl. Phys. Lett.* **1994**, *64*, 2827.

[16] Lehmann, V.; Hofmann, F.; Moeller, F.; Gruning, U. Resistivity of porous silicon: a surface effect. *Thin Solid Films,* **1995**, *255*, 20-22.

[17] Balagurov, L.A.; Yarkin, D.G.; Petrova, E.A. Electronic transport I porous silicon of low porosity made on a p+ substrate. *Mater. Sci. Eng. B* **2000**, *69-70*, 127-131.

[18] Martín-Palma, R.J.; Torres-Costa, V.; Arroyo-Hernández, M.; Manso, M.; Pérez-Rigueiro, J.; Martínez-Duart, J.M. Porous silicon multilayer stacks for optical biosensing applications. *Microelectronics J.,* **2004**, *35*, 45-48.

[19] Arroyo-Hernández, M.; Martín-Palma, R.J.; Torres-Costa, V.; Martínez Duart, J.M. Porous silicon optical filters for biosensing applications. *J. Non-Crystal. Solids,* **2006**, *352*, 2457-2460.

[20] López-García, J.; Martín-Palma, R.J.; Manso, M.; Martínez-Duart, J.M. Porous silicon based structures for the electrical biosensing of glucose. *Sens. Actuators B,* **2007**, *126*(1), 82-85.

[21] Chayen, N. E.; Saridakis, E.; El-Bahar, R.; Nemirovsky, Y. Porous silicon: an effective nucleation-inducing material for protein crystallization. *J. Mol. Biol.,* **2001**, *312*(4), 591-595.

[22] Canham, L.T. Bioactive silicon structure fabrication through nanoetching techniques. *Adv. Mater.* **1995**, *7*(12), 1033-1037.

[23] Arroyo-Hernández, M.; Martín-Palma, R.J.; Pérez-Rigueiro, J.; García-Ruiz, P.; García-Fierro, J.L.; Martínez-Duart, J.M. Biofunctionalization of surfaces of nanostructured porous silicon. *Mater. Sci. Eng. C,* **2003**, *23*, 697-701.

[24] Tay, L.; Rowell, N. L.; Poitras, D.; Fraser, J. W.; Lockwood, D. J.; Boukherroub, R. Bovine serum albumin adsorption on passivated porous silicon layers. *Can. J. Chem.*, **2004**, *82*(10), 1545-1553.

[25] Tay, L.; Rowell, N. L.; Lockwood, D. J.; Boukherroub, R. In situ monitoring of protein adsorption on functionalized porous Si surfaces. *J. Vac. Sci. Technol. A,* **2006**, *24*, 747 (5 pages).

[26] Martín-Palma, R.J.; Pérez-Rigueiro, J.; Martínez-Duart, J.M. Study of carrier transport in metal/porous silicon/Si structures *J. Appl. Phys.,* **1999**, *86*(12), 6911-6915.

[27] Martín-Palma, R.J.; Pérez-Rigueiro, J.; Guerrero-Lemus, R.; Moreno, J.D.; Martínez-Duart, J.M. Ageing of alumiun electrical contacts to porous silicon. *J. Appl. Phys.,* **1999**, *85*(1), 583-586.

[28] Carstensen, E.L.; Cox, Jr., H.A.; Mercer, W.B.; Natale, L.A. Passive electrical properties of microorganisms. *Biophys. J.,* **1965**, *5*(3), 289-300.

Received: April 20, 2010 Revised: July 21, 2010 Accepted: December 6, 2010