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Study of the Nanostructurated Silicon Chemical Functionalization

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Abstract. Chemical functionalization of semiconductor surfaces cover a broad range of potential applications ranging from surface passivation and stabilization to development of new strategies for chemical or biological species immobilization on the surface and detection / study of various interactions characterizing biological and chemical systems. Organic derivatization of semiconductor surfaces is an active field of research due to the importance of these materials in modern technologies. The microfabrication processes developed on silicon substrate are well-known and they allow obtaining structures suitable for integration into microelectronics platforms with chemical and biological functionality. In this paper, we report the functionalization with organic molecules of porous silicon internal surface which work as coupling agent (3- aminopropyl triethoxysilane- APTS) for biomedical applications. The test structures were characterized from morphological and compositional point of view using optical microscopy with fluorescence module and Fourier-transform infrared (FTIR) spectroscopy techniques. Moreover, porous silicon with fluorescent agent on its surface was analyzed using the UC4 Microarray Scanner from Genomic Solutions in the view of future using in DNA microarray technology.

1. Introduction

Silicon (Si) received a lot of attention due to its specific semiconductor properties and futhermore because it allows the development of a broad range of micropatterning processes in order to achieve functional features for future integration in complex systems. Porous Silicon (PS) is a nanostructured material based on silicon fabricated

by electrochemical etching of crystalline silicon which consists of assembly of holes (pores) and fibrils, rendering a large surface to volume ratio in order of 500 m^2/cm^3.

The quantum confinement phenomena lead to new properties, like photoluminescence or electroluminescence [1], important for future applications as optoelectronics. Also, the high specific surface area with huge chemical reactivity of PS makes this material a good candidate for the development of a wide variety of chemical or biological sensors. For the biomedical applications of porous silicon (PS), biomolecules have to be first immobilized on its surface through different linkers with functional groups [2]. The biomaterial [3] is that material used in a medical system that interacts with the biological systems. Some types of materials can be "bioinerts", "biocompatibles" or resorbables', regarding their *in vivo* response. In the case of the traditional biomaterials, one of the important problems which appears, is related to the difficulty to modify the biomaterial surface in order to take place the interaction between cells and its surface [4]. Chemical structure and physical morphology of the support surface are important factors for the molecule attachment.

Some of the mechanisms which have an influence over the biocompatibility are: proteins adsorption, cells growth, the stability of the structures during the hydrolysis process and the pH of the biological systems. Studies regarding the biomolecule immobilization on solid supports [5], including adsorption, covalent attachments or immobilization by using a ligand molecule, have been developed. It has been demonstrated that both the surface type and the immobilization method used have a huge influence over the biological activity of the attached proteins.

The DNA microarray-based analysis of single nucleotide polymorphisms (SNPs) is important for the correlation of genetic variations and individual phenotypes, and for locating disease-causing genes. To facilitate the development of surfaces suitable for immobilization of oligonucleotides, we report in this paper a novel method of silicon based structures preparation with surface suitable for immobilization of DNA. It is well established that the protocols employed for the immobilization of pre-fabricated nucleic acids largely affect the performance of the microarray. For instance, the binding capacity of the arrays surface can be increased significantly by the use of appropriate linker systems [6]. Most commonly, the automated deposition of nucleic acids on amino-terminated surfaces, such as 3-aminopropyltriethoxysilane (APTS) or poly-L-lysine (PLL)-coated slides, is applied to generate microarrays. A general problem associated with the preparation support structures for microarray technology is the limited stability of such arrays, leading to interference with stringent hybridization conditions and frequent regeneration steps. Furthermore, the spots of such surfaces often reveal inhomogeneous signal distributions [7]. Thus, robust and homogenously chemically activated surfaces are required which allow for the covalent immobilization of oligomer probes.

2. Experimental methods

The nanostructured silicon was realized using an electrochemical etching process in ethanoic hydrofluoric acid solution [8]. The structure and morphology of PS layers,

which depends on porosity, is strongly influenced on both, substrate characteristics – resistivity and crystalline orientation – and process parameters – HF concentration and anodic current density. It was used p-type silicon substrate boron – doped 0.005 Ωcm and oriented according to the crystalline direction (100). Before electrochemical anodization of silicon, the samples were firstly cleaned in a Piranha solution (a 3:1 mixture of sulfuric acid and 30% hydrogen peroxide),and then rinsed abudently with deionised water (18 MΩ). The porosity close to 50% (mesoporous silicon) was achieved using equal parts by volume of HF and ethanol (1 HF: 1 C_2H_5OH) and a constant anodic etching current density of 3 mA/cm^2. The anodization process was carried out in an teflon – HF corosion resistent material - electrochemical cell.

Often, freshly etched porous silicon may be unstable due to the rate of its oxidation by the atmosphere unsuitable for cell attachment purposes. Therefore, surface has to be modified to improve stability and to offer the functional group for different biomolecule attachment. This work is focused on PS surface functionalization using an organic molecule - alkylsilane – which contains amine groups, 3-aminopropyltriethoxysilane (APTS) [9]:

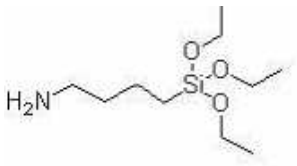


Fig. 1. Schematic presentation of APTS molecular structure.

In order to improve the APTS functionalization process, post-anodization processes were performed to achieve a chemical stability of internal surface groups, and furthermore to obtain the silanols (≡Si-OH) groups which are easier replaced with amino functional groups (-NH_2) on the chemical active surface of mesoporous silicon [10]. Three types of structures have been subjected to APTS treatment and the modifications in terms of their functionality have been compared: fresh PS – A1, A2 samples– partial oxidized PS (p) OPS – B1, B2 samples– and oxidized PS – C1, C2 samples. For partial oxidation of PS – (p) OPS– the PS samples were storage for two weeks and respectively for complete oxidation an additional few minute's boiling of PS in H_2O_2 was realized. Silanization process with APTS (purchased from Sigma- Aldrich) was performed by immersing the sample in a 1M solution of APTS in methanol. Modifications of internal surface chemical map during these processes have been studied by FTIR. In order to study the improving of fluorescence recorded signal by nanostructuration of Si surface, a simple deposition of fluorescein was performed. Due to specifically reaction of fluorescein with amino groups from APTS, the microarray technology allows study of proposed structures functionalities in terms of envised biomedical applications. The intensity of the fluorescent signal of the spots before and after washing was analyzed using Gene TAC Analyzer (Genomic Solutions). Microarrays were scanned at 635 nm (CY5 channel) and 532 nm (Cy3 channel) (UC4

Microarray Scanner) and the fluorescent signal was recorded using a scanning laser confocal fluorescence microscope from Genomic Solutions.

The technological processes developed for this study are presented in the Table 1.

Table 1

Sample no.	**Structure**	**Treatments**
A1	PS / Si	– Fluorescein deposition on PS
A2	PS / Si	– Fluorescein deposition on PS – DI water cleaning
B1	(p)OPS / Si	– APTS functionalization – Fluorescein deposition
B2	(p)OPS / Si	– APTS functionalization – Thermal treatment at 100°C, for 20 minutes – Fluorescein deposition on surface
C1	OPS / Si	– APTS functionalization – Thermal treatment at 100°C for 20 minutes – Fluorescein deposition
C2	OPS / Si	– APTS functionalization – Thermal treatment at 100°C for 20 minutes – Fluorescein deposition – DI water cleaning

3. Results and discussions

3.1. Characterization of the fresh PS surface (A Sample)

The starting material in our study is porous silicon and it is characterized by a trenched structure with tens of nanometers fibrils and pores. The morphology of PS layer was characterized using scanning electron microscopy (SEM) and the image of PS surface is presented in Fig. 2:

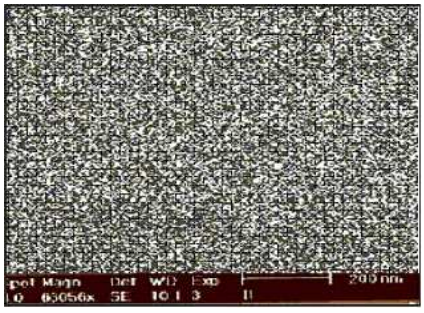


Fig. 2. SEM image of mesoPS surface.

The SEM image reveals that the pores have a uniform distribution in PS layer and their diameter is around 20 nm, corresponding to a mesoporous structure. The

internal surface chemical composition of as prepared PS was characterized using FTIR technique (Fig. 3):

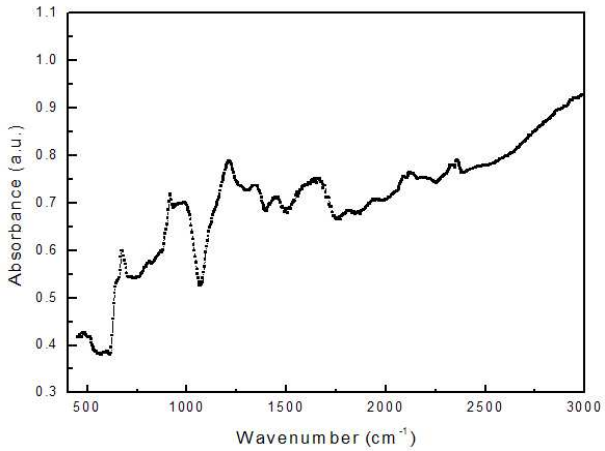
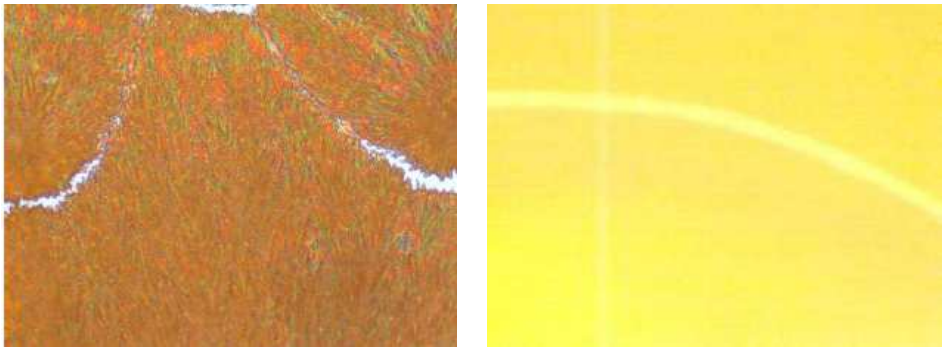


Fig. 3. IR spectrum of fresh porous silicon.

The FTIR absorbance spectrum shows the absorbance characteristics of the hydride-terminated surface, which consists of: ν(Si-H_x) stretching modes at 2088 cm^{-1} for ν(Si-H), 2116 $cm^{-1}$for ν(Si-H_2), and 2137 cm^{-1} for ν(Si-H_3) and a δ(Si-H_2) scissor mode at 910 cm^{-1}[11, 12]. Modes at 626 and 665 cm^{-1} are assigned to Si-H_x vibrations [11, 13]. A small signal at 1108 cm^{-1} is assigned to the Si-O-Si stretch mode [14].

The presence of fluorescent agent on PS surface was analyzed by optical microscopy coupled with fluorescence module (Fig. 4 – A2 sample):



(a) (b)

Fig. 4. Optical image of fluorescein deposited on mesoPS surface before (a) and after (b) washing in DI water.

The images reveals the poor adhesion of fluorescein on fresh PS surface due to the presence of hydride bonds and it was improved by oxidation, because the modification to hydroxyl facilitate noncovalent bonding with this protein.

The UC4 Microarray Scanner spots are presented in Fig. 5:

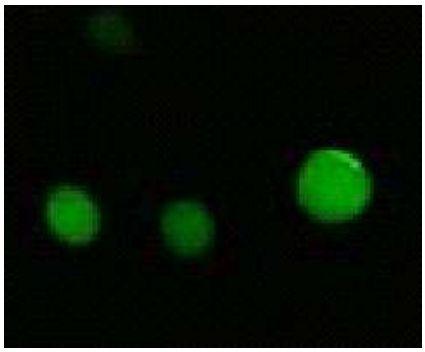


Fig. 5. The Microarray Scaner image of spots on PS surface with fluorescein.

The non-functionalized PS sample presents a lightening signal, but after washing the sample in water, the fluorescent signal was not observed anymore.

3.2. Characterization of the partially oxidized PS (B Sample)

Storage of PS sample in atmosphere, even for a short period of time, leads to a partial oxidation due to the oxygen presence and the hydride-terminated surface is replaced principally with oxides groups, like O-Si-H and O_3-Si-H, as is presented in Fig. 6 FTIR spectrums:

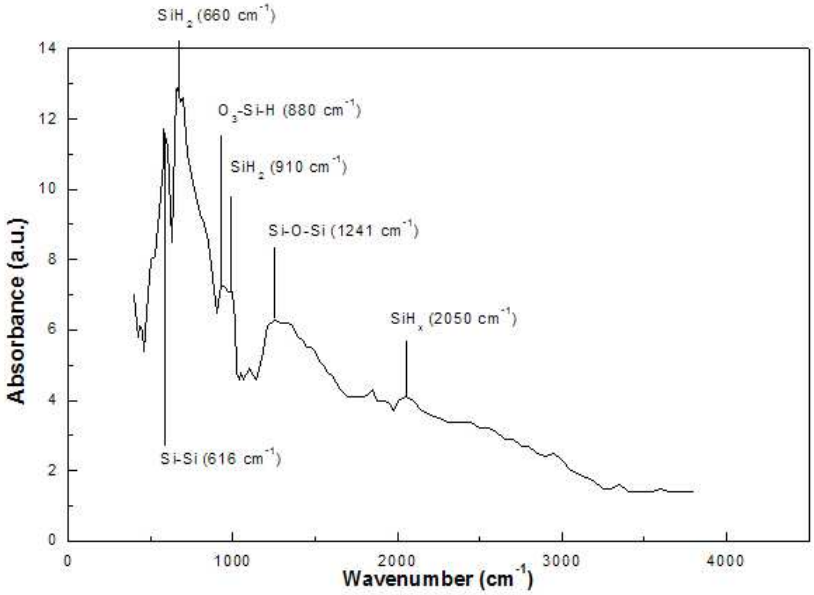


Fig. 6. FTIR spectrum of (p) OPS.

These bonds are instable and supplementary processes were required to promote the surface stability of PS. The surface functionalization was performed by 3-aminopropyltriethoxysilane (APTS) deposition immersing the samples in a 1M solution of APTS and methanol for 30 minutes. An additional thermal treatment was performed at 100°C for 20 minutes, leading to surface covered with amine groups.

In Fig. 7 the absorbance IR spectra corresponding to APTS deposition onto porous silicon surface is presented:

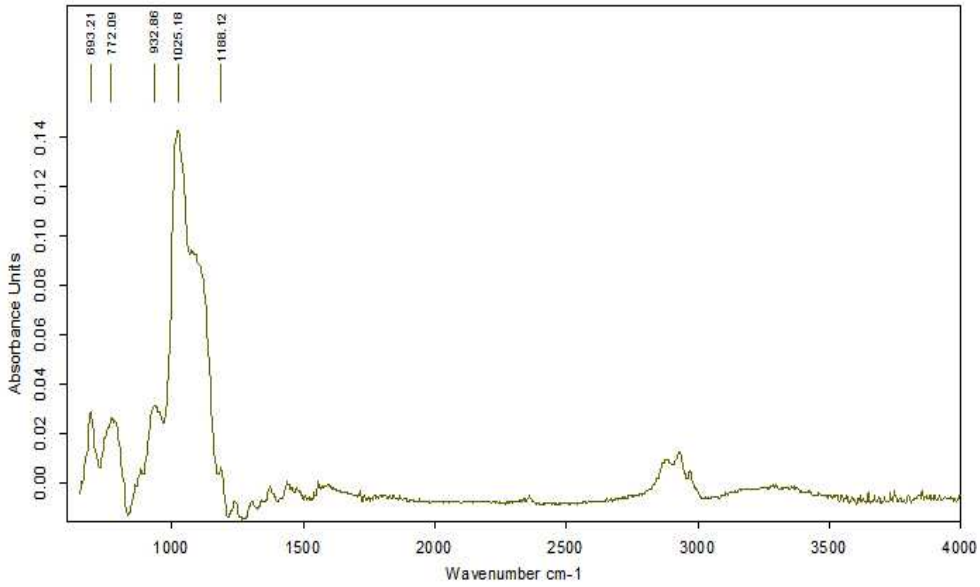
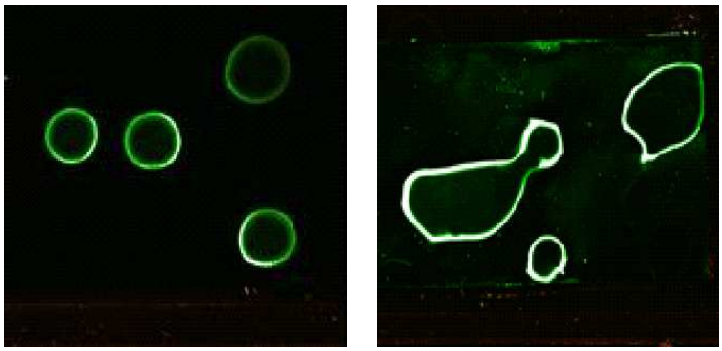


Fig. 7. Spectrum of APTS deposition on PS.

The presence of two bands at 1025 and 1188 cm^{-1} assigned to the symmetric stretch mode of (Si–O) demonstrates formation of the siloxane bands on PS internal surface. These bands indicate that the siloxane bond formation has occurred between Si-H bonds and porous silicon surface. Also, bands from 772 and 693 cm^{-1} correspond to the N-H bond deformation out of plane mode and symmetric stretch of Si-C, respectively. Finally, the absorbance bands observed at high frequency region are assigned to the symmetric and antisymmetric vibration mode of CH_2, of the alkyl silane chains (2972 and 2930 cm^{-1}). Analyzing the spectra of APTS deposition onto PS surface, we can tell that APTS can be used to functionalize the porous silicon surface in order to attach biomolecules.

Furthermore, the presence of amino groups was verified by scanning laser confocal fluorescence equipment. The samples were analyzed before and after DI water washing in order to see if the APTS attachment on PS sample is stable. The results are presented in Figs. 8 (a) and (b).

From these images it can be observed that the PS lightening signal of remains also after washing the sample in water. So, it can be said that the amino groups are stable attached to the silicon surface.



(a) (b)

Fig. 8. Microarray Scaner images of APTS on deoxidized PS, (a) Before washing in water and (b), after washing.

3.3. Characterization of the oxidized porous silicon (C Sample)

Complete oxidation of PS was realized using an additional few minutes boiling in H_2O_2. In Fig. 9 is presented the spectrum of oxidized PS, from which can be seen the presence of Si-O-Si (843 cm^{-1} and 1053.5 cm^{-1}), O_3Si-H (893 cm^{-1}), respectively Si-O-H (2150 cm^{-1} and 3500 cm^{-1}).

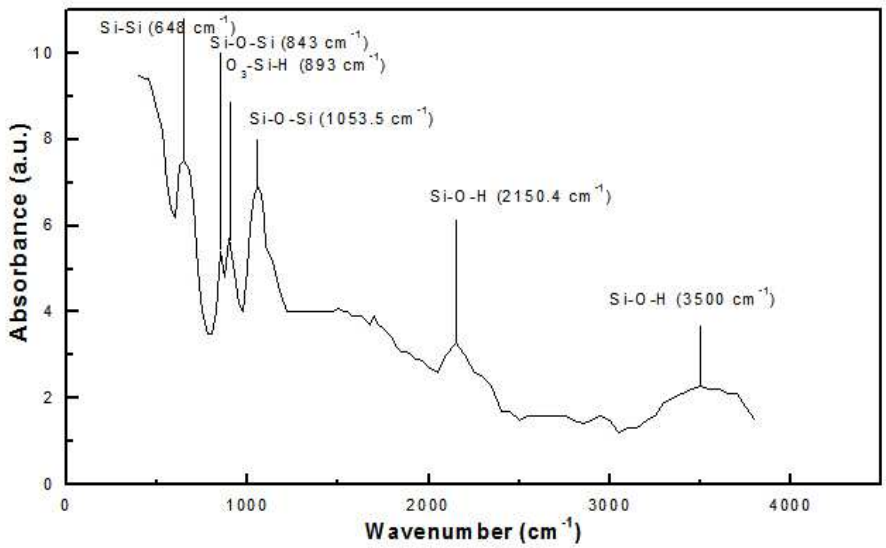


Fig. 9. IR spectra for oxidized PS (OPS) sample.

The specific chemical reaction which occurs at PS internal surface during oxidation is schematic presented in Fig. 10:

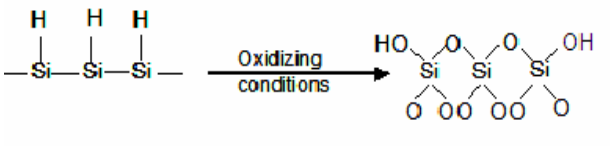


Fig. 10. Fresh porous silicon oxidation.

The oxidized porous silicon was functionalized with APTS.

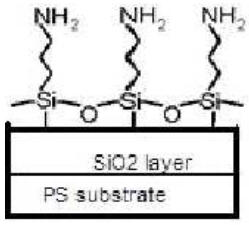


Fig. 11. The reaction between APTS and oxidized PS surface.

It was observed that the amino groups attached on oxidized PS surface are higher than in the case of porous silicon partially oxidized.

In Fig. 12 is presented the reaction between the fluorescein and the amine functionalized porous silicon.

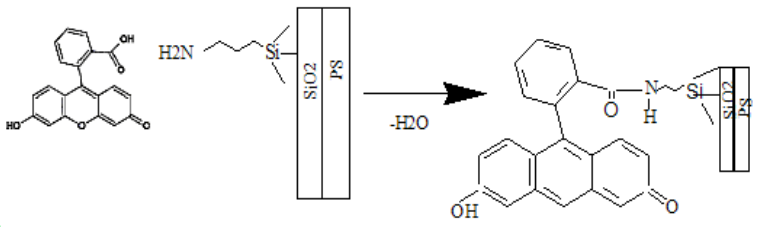
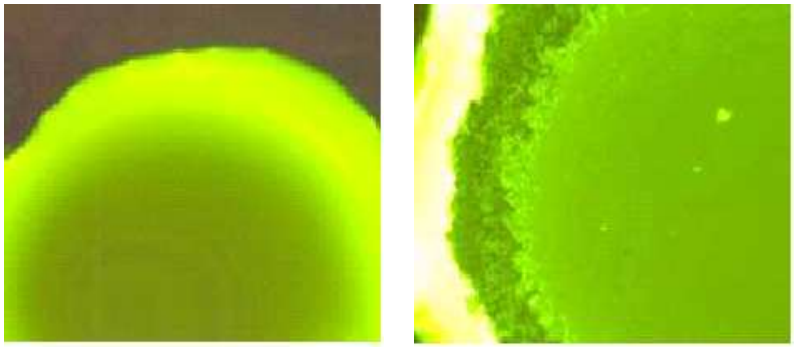


Fig. 12. The reaction between fluorescein and functionalized porous silicon.

In order to study the stability of the oxidized sample, there were analyzed by optical microscopy before and after DI water washing and the results are presented in Fig. 13 (a) and (b).



(a) (b)

Fig. 13. Optical microscopy images of APTS on oxidized PS, (a) Before washing in water and (b), after washing.

The presence of the fluorescence after washing is a clear indication of the stable covalent APTS bonding on PS oxidized surface, which is due to substitution of surface silanols (≡Si-OH) with amino functional groups (-NH_2) on the chemical active surface of mesoporous silicon.

4. Conclusions

In this paper, we report the functionalization with organic molecules of porous silicon internal surface which work as coupling agent (3- aminopropyl triethoxysilane-APTS) for biomedical applications. The test structures were characterized from morphological and compositional point of view using optical microscopy with fluorescence module and Fourier-transform infrared (FTIR) spectroscopy techniques. Moreover, porous silicon with fluorescent agent on its surface was analyzed using the UC4 Microarray Scanner from Genomic Solutions in the view of future using in DNA microarray technology. Chemical functionalization of Si nanostructured surface allows us to realize both passivation and stabilization of instable structures and to develop new systems for biochemical applications.

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