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Nanoporous silicon matrix used as biomaterial

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Abstract

In the last years due to porous silicon (PS) biocompatible properties, PS layers were investigated as support for cell adhesion, as matrix for drug delivery, or for bio (optical) sensors. In this paper we have investigated different types of surface modification of nanoporous silicon in order to be used as support for immobilization of DNA molecules. Good results were obtained by DNA immobilization on Au/PS layer.

Keywords: Nanostructured material; Thin films; Nanofabrications; X-ray diffraction; DNA immobilization

1. Introduction

Since room-temperature photoluminescence [1] and electroluminescence from porous silicon (PS) were reported, it has been intensively studied in order to develop nanophotonic devices. In the last years due to PS biocompatible properties, PS layers were investigated as support for cell adhesion [2], as matrix for drug delivery [3,4] or for bio (optical) sensors [5,6].

Two aspects of porous silicon are of particular relevance for in vivo implants applications: it can be used as a sensitive biosensor for proteins, antigens, and DNA and it can be modified with a wide range of biological or organic molecules. These two features should allow PS to serve as a versatile biomaterial. Although efforts in this area are still in early developmental stages, combining the biocompatibility of the material with its highly bio-sensitive capabilities leads to new applications in tissue-based bioassays, drug delivery, and health-monitoring applications.

In this paper, PS based diagnostic test for non-invasive DNA analyses was studied. There are known different chemical techniques to link the oligonucleotides to solid substrates, directly or by a linker aid. In this paper we have used a –SH group and cystein linker and we have demonstrated that $–NH_2$ modified oligonucleotides are covalently attached on the functionalized Au/PS by the $–NH_2$ end groups.

2. Experimental results

The experimental work was focused on, obtaining a nanoporous silicon surface functionalized for DNA attachment.

The micro-reservoirs used as support for biological samples were engraved on p+(1 0 0) silicon wafers by anisotropic etching in 20% KOH solution at 85 °C using SiO_2 layer as mask [7]. Nanoporous silicon (PS), 30% porosity, with pore size less than 20 nm was realised on silicon reservoir surface by partial electrochemical dissolution in a 25% hydrofluoric acid in alcohol ethylic solution at 20 mA/cm^2 current density for 5 min. PS has very complex, anisotropic crystalline architecture characterised by a high surface area.

The as-prepared PS surface is hydrophobic; in order to assure its stability and biocompatibility, the following procedure was applied. The wafers were washed in deionised water, ethanol and acetone for 10 min in each. After that, an annealing process in two steps was performed: 30 min at 300 °C and 45 min at 800 °C in dry oxygen atmosphere. The direct result of the surface passivation process is the replacement of Si–H groups with Si–O groups.

A gold (Au) layer of 10 nm thicknesses was deposited on PS reservoir surface by a vacuum evaporation method. The Au/PS systems was investigated by SEM (Fig. 1), SIMS (Fig. 2) and X-ray diffraction (Fig. 3a), in order to obtained information about the morphology, composition and structure.

It is known [8,9] that (1 1 1) orientation of the gold layer is necessary for a good attachment of DNA molecule; the Au texture on PS substrate was investigated at 500 °C, 700 °C and 900 °C in N_2/H_2 atmosphere. X-ray

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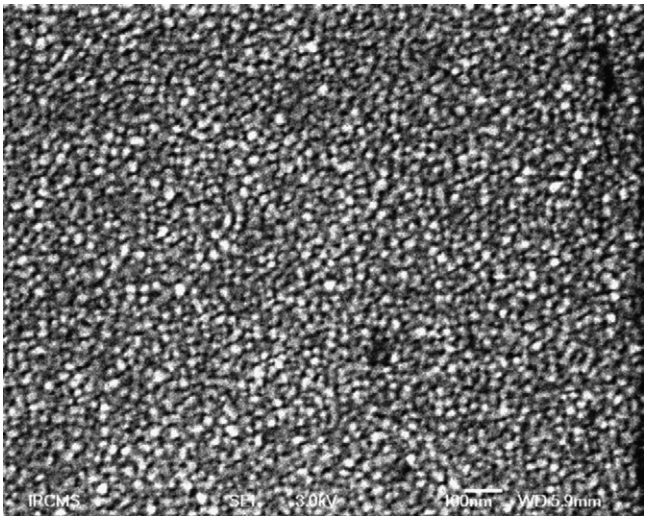

Fig. 1. SEM image of the Au/PS.

measurements were performed using a rotating crystal method, a Mo X-ray tube ($\lambda_{k\alpha l} = 0.709324$ Å) and a Si (4 0 0) perfect wafer as a monochromator. The operating parameters of the X-ray tube were $I = 22$ mA, $V = 40$ kV, integration time $t = 10$–40 s/step, 2θ step 0.01–0.1°.

After the annealing steps (500, 700, 900 °C) the Au film on PS substrate was found to be in a mixed amorphous-crystalline state with different textures: (1 1 1) and (2 2 0); at 900 °C the (1 1 1) Au texture became predominant. The Au layers are nanostructured with a medium nanocrystallite dimension $D_{\sqrt[3]{V}} = 17.46 \pm 0.096$ nm, $D_{hkl} = 20.36 \pm 0.13$ nm (Fig. 3b).

The Au/PS structures were silanized with 1 mM octadecyl-trichloro-silane in freshly distilled toluene overnight at room-temperature. The structures were then washed twice with dry toluene and once with ethanol, followed by excessive washing with D.I. water. Cysteamine (2-aminoethanethiol,) was dissolved at a concentration of 100 mM in pure ethanol.

The DNA immobilization capacity of Au/PS layer on silicon wafers was tested; a fluorescent oligonucleotide 5′-TET-TGCAACCCACCTGAATGA-3′ (10 pmol/μl in 10% DMSO) was linked to the surface by baking at 80°C, for 2 h. After several washing steps in D.I. water, each cavity was analyzed by a fluorescent microscope (Nikon).

Subsequently, an oligonucleotide 5′-TACGTGAAGTGCCAGCACA-3′ was linked on Au/PS reservoirs and a fluorescent amplimer was hybridized with the probe. The DNA fragment was obtained by PCR amplification of human D1S-2644 microsatellite with the oligonucleotides pairs above functioning as primers. The fluorescence after hybridizations was revealed by fluorescence microscopy.

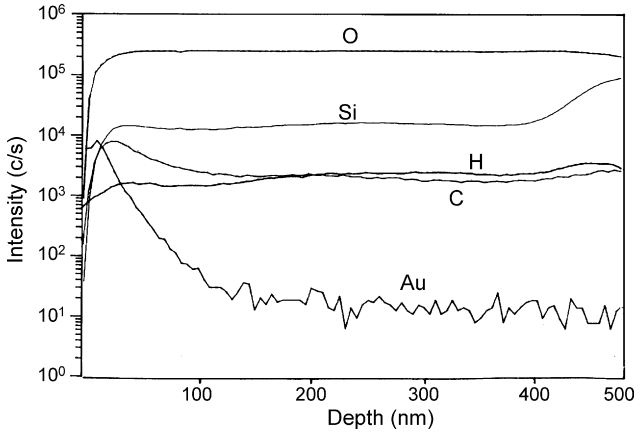

Fig. 2. SIMS depth profile of the Au/PS composite layer.

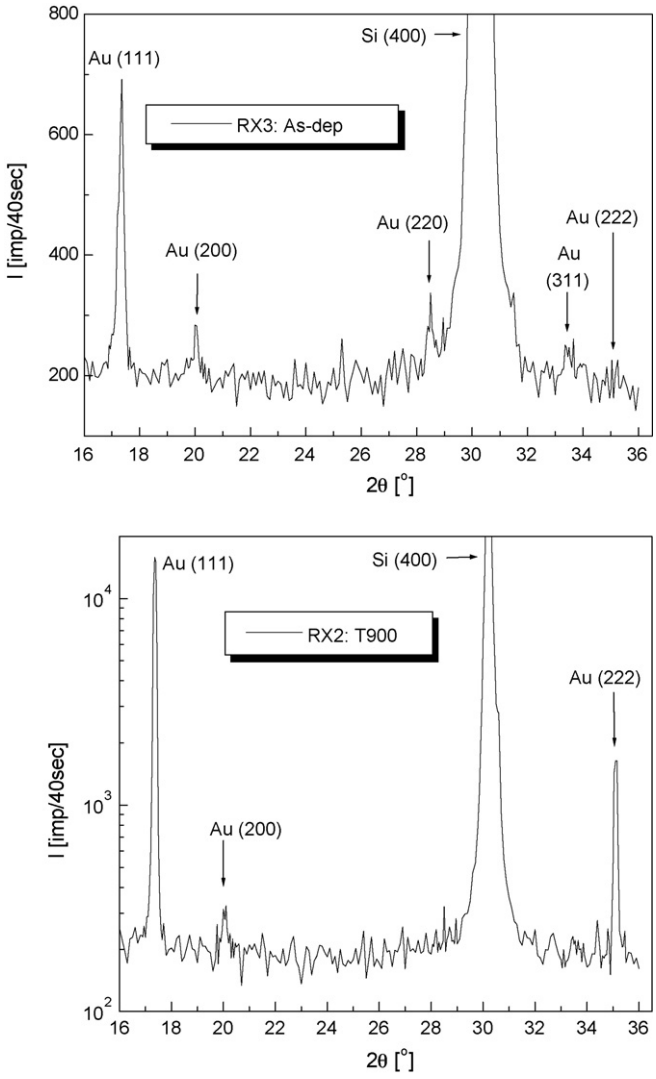

Fig. 3. X-ray diffraction pattern of Au/PS: (a) before annealing and (b) after annealing at 900 °C.

3. Conclusions

The reservoirs of DNA amplification test structures have the surface porosified and covered with an Au layer in order to immobilize the DNA fragments amplified by PCR reaction. PS is a very suitable material for composite layer preparation because it offers a surface topography controllable with nm resolution in three dimensions and allows chemical surface modifications.

XRD analysis on thermally treated Au/PS layers has evidenced the Au textured surface. The orientation factor is increased on the probes after the annealing treatment at 500 °C – (1 1 1) texture. Au/PS structures were functionalized by the thiol group of the cystein linker.

We have demonstrated that $-NH_2$ modified oligonucleotides are covalently attached on the functionalized Au/PS by the $-NH_2$ end groups.

DNA probes immobilization on Au/Si surface reactor is very uniform both before and after hybridization compared to chemically functionalized probes with polylisine or polypyrrol.

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