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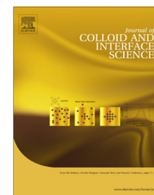

Protein immobilization on nanoporous silicon functionalized by RF activated plasma polymerization of Acrylic Acid

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ABSTRACT

Plasma Enhanced Chemical Vapor Deposition (PECVD) technique is used to polymerize Acrylic Acid for the surface functionalization of porous silicon samples with different pore dimensions. The polymer shows free —COOH groups also at the pores inner surface, suitable for the immobilization of fluorescent labeled Protein A. The stability of the polymer, its role in the protection from aging of the porous matrix and the efficiency of the functionalization for the binding of protein A have been characterized by ATR–FTIR, SEM, Optical Contact Angle and Fluorescence Microscopy. The polymerization process is well controllable and suitable for the functionalization of porous silicon leaving free carboxylic groups at the surface ready for the immobilization of biochemical species for sensing applications.

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1. Introduction

In the last decades, porous silicon (pSi) has attracted a wide attention for its applications in biological and chemical sensing [1–4]. Porous silicon is produced by electrochemical etching of crystalline silicon (c-Si) in a solution of hydrofluoric (HF) acid, and the pore diameter can be controllably varied from a few nanometers up to several hundred nanometers by adjusting the etching parameters [5]. A key feature of pSi is its very high surface area to volume ratio (up to 600 m^2/cm^3) [4], making it an ideal platform for sensing and especially biosensing due to its well-assessed biocompatibility [6]. The pores inner surface of freshly etched samples shows a considerable hydrophobicity [7,8] due to the native Si_ySiH_x termination that, by the way, are highly reactive and prone to spontaneous oxidation. These groups are easily attacked also by weak oxidizing agent when they diffuse inside the porous matrix, causing remarkable and irreversible aging of the structure. Notwithstanding pSi well known hydrophobicity, that may limit the diffusion of water based solutions, such as those used in biosensing experiment [9–11], some H_2O molecules diffuse into the porous system, causing oxidation, due to nucleophilic attacks of water molecules on the silicon hydrides groups [12]. For this issue, when pSi is used as platform for biochemical sensing devices, surface modification is used both to stabilize the pSi surface and to tailor the surface chemistry for specific applications.

For many years, thermal oxidation and annealing at high temperatures were the only useful stabilizing treatments for pSi. During the last years several other, and even better, stabilizing treatments have been introduced. Among them, the chemical modification of pSi surface can be widely exploited in many research fields for applications ranging from the surface passivation and stabilization to the development of new strategies for immobilization and detection of both chemical and biological species [1,13,14]. Most common methods are based on the reaction of hydride groups with suitable organic species [15], mostly deriving from well-known organosilanes chemistry performed on flat silicon-containing substrates for sensing [16,17]. These methods, such as hydrosilylation involving radical initiator, heat or light promoted hydrosilylation, Lewis acid mediated reactions, organolithium derivatization, cathodic/anodic electrografting and microwave-assisted chemical reaction [18–22], require more or less complex experimental procedures sometimes carrying, as a drawback, contamination and/or oxidation of the modified surface. More recently, a NH_3 plasma treatment has evidenced that a gas phase procedure can be used as simple and fast method for surface modification of pSi [23].

Plasma-Enhanced Chemical-Vapor Deposition (PECVD) technique, performed in vacuum conditions at RT, is a clean process allowing to the formation of highly cross-linked polymerized material by use of a monomer in the plasma state [24,25]. Plasma polymerization can be employed to modify surfaces with a thin functional polymeric layer by properly choosing the monomer containing the desired functional groups, and, furthermore, unchanging the bulk properties of the starting material. In previous works [26,27] and reference therein, we demonstrated that it is

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possible to modify the surface, for instance, of crystalline silicon (c-Si), corning glass, polyethylene (PE) and SiO_2 terminated 1-D Photonic Crystals by a Plasma Polymerized Acrylic Acid (PPAA) thin coating, that exposes a high density of functional carboxylic groups able to graft amino terminated ss-DNA and proteins (A/G). In our study, PPAA functionalization has been carried out on freshly prepared pSi layers. The plasma polymerization process is optimized by investigating the role of the PPAA deposition time with respect to the pSi pore size. Plasma modified pSi layers were characterized by water contact angle measurements (WCA), ATR–FTIR spectroscopy and FE-SEM analyses and the protein binding was determined by micro-fluorescence and ATR–FTIR characterization.

The experimental results, here reported and described, suggest that PECVD can be used to easily functionalize the pSi matrix in order to produce a novel hybrid organic–inorganic material for biosensing applications.

2. Experimental

2.1. Materials

Silicon wafers were purchased from Cemat Silicon SA. Hydrofluoric acid (HF, 50%) and absolute ethanol were supplied by Carlo Erba, Acrylic Acid (AA, 99%), Pentane (99%), Tween80®, Toluidine Blue O (TBO) – Technical Grade from Sigma Aldrich and sodium hydroxide (NaOH) were obtained from Fluka. The phosphate buffer saline (PBS) 1 × solution was from GIBCO®. The AlexaFluor546-conjugated Protein A (PtA-AF546) was purchased from Invitrogen.

2.2. Samples preparation

The pSi single layers were prepared by two-step electrochemical etching process from single-side polished (100)-oriented p^+-type silicon wafers (B-doped, resistivity 1–4 mΩ cm). The first anodization was carried out at a constant current density of 63 mA cm^{-2} for 1 min in ethanolic HF solution ($HF:H_2O:C_2H_5OH$ = 1:1:3). The pSi layer was subsequently removed in NaOH 1 M. The wafers were etched again under the following process conditions:

1. High porosity (75%) layer with mean pores diameter about 30 nm (HpL), 2 μm thick: anodization in ethanolic HF solution ($HF:H_2O:C_2H_5OH$ = 1:1:3) at a constant current density of 125 mA cm^{-2} for 35 s.
2. High porosity (78%) layer with mean pores diameter about 70 nm (HpXL), 2 μm thick: anodization in ethanolic HF solution ($HF:H_2O:C_2H_5OH$ = 1:1:8) at a constant current density of 6 mA cm^{-2} for 10 min.

The pSi samples were then rinsed several times with pure ethanol and pentane in order to avoid cracking of the high porous structures.

2.3. Samples functionalization and binding capability investigation

Plasma Polymerized Acrylic Acid (PPAA) thin films have been prepared in a Plasma Enhanced CVD reactor (Chamber Base Pressure = 28 mTorr; RF = 13.56 MHz) equipped with a delivery frame suitable to inject vapors coming from liquid reactants (monomeric precursors). Acrylic Acid vapors (flow = 3 sccm) were diluted in Argon (flow = 20 sccm). Polymerization was performed by a pulsed plasma discharge [28] applying a discharge RF power of 200 W, a duty cycle of 10% (on time = 10 ms, off time = 90 ms) and testing three different deposition time (t_{dep} = 2.5, 5 and 10 min). Each PPAA-pSi samples have been soaked (30 min) in deionized water, Milli-Q grade (dH_2O) to remove unstable surface oligomers formed at the end of the plasma process [26].

PtA-AF546 were deposited on samples after plasma process using a solution of the protein with an incubation time of 1.5 h (0.2 mg/ml – drop volume = 5 μl). The samples were washed in 2 mL of Tween80® (0.05%) and then in 2 mL of dH_2O, on a rocker shaker, (10 min each rinsing) in order to remove weakly bound protein from the surface.

The binding capability of —COOH functionalities of PPAA-pSi samples have been tested with TBO. This dye is normally used to evaluate the surface density of carboxylic groups on a surface by means of colorimetric titration of the recovered solution. The amino group contained in TBO molecule reacts with a surface carboxylic group according to a 1:1 ratio [29].

PPAA-pSi samples were contacted with 2 ml of 0.5 mM TBO aqueous solution (pH 10) at 30° C for 5 h. To remove unreacted dye, substrates were rinsed with copious amount of 0.1 mM NaOH solution. Finally they are dried gently under N_2 flux.

2.4. Samples characterization

Freshly prepared pSi samples have been characterized in reflectance mode by means of UV–Vis–NIR Varian Cary-500 spectrophotometer (equipped with a specular reflectance unit with a 12° fixed angle of incidence). After anodization, samples porosity and thickness were evaluated performing a best-fit calculation of the reflectance spectrum by means of the Scout code (M.T Theiss Hard- and Software, Dr. -Bernhard -Klein-Str. 110, D-52078 Aachen Germany, Copyright Wolfgang Theiss, www.wtheiss.com), based on the transfer matrix method [30].

Samples morphology (pores size/amount and channels cross-section), before and after PPAA deposition, was observed by means of Field Emission Scanning Electron Microscope (FE-SEM SUPRA 25 ZEISS) using an accelerating voltage of 5 kV.

Surface chemical modifications were monitored, after anodization and following steps, by Fourier Transform InfraRed spectroscopy (FTIR). Spectra were collected on a Bruker Tensor 27 spectrometer in Attenuated Total Reflection (ATR) mode with 64 repetition for each spectrum with a resolution scan of 2 cm^{-1}.

Static (sessile drop) water contact angles (WCA) measurements have been performed by using OCAH200 (Dataphysics, Instruments GmbH). At room temperature, 1.5 μl of dH_2O was spotted onto the surfaces, images of the droplets were captured and SCA20 software was used to fit drop profiles through the Young–Laplace method and, indeed, to calculate contact angles between fitted function and base line. At least three drops were dispensed for each sample.

Wide-field Fluorescence Microscopy was performed in presence of the protein by a LEICA DM-LM microscope (objective 20×) equipped with a Hg vapor arc lamp (50 W) and L5 type combination filters suitable for Fluoresceine-like fluorophores. Image analysis was performed by ImageJ public domain software, available through the National Institutes of Health (Bethesda, MD, USA; available at http://rsb.info.nih.gov/ij/).

3. Results and discussion

3.1. PPAA deposition

In a previous work [26], conditions for optimized PPAA deposition were determined to form a stable and reproducible film onto various flat substrates such as c-Si, Polyethylene and corning glass. Plasma polymerization depends upon the power of the glow discharge, monomer pressure in the reactor chamber and deposition time.

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