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Biofunctional porous silicon micropatterns engineered through visible light activated epoxy capping and selective plasma etching

C. Rodriguez [a], O. Ahumada [c], V. Cebrián [c], V. Torres Costa [a, b], M. Manso Silván [a, *]

[a] Departamento de Física Aplicada and Instituto de Ciencia de Materiales Nicolás Cabrera, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[b] Centro de Microanálisis de Materiales, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[c] Mecwins, Tres Cantos, 28760 Madrid, Spain

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ABSTRACT

Porous silicon (PSi) is a versatile matrix with tailorable surface reactivity, which allows processing a diversity of biofunctional structures. An assembly process has been activated by catalyzing PSi oxidation with visible light, enabling subsequent binding with (3-glycidyloxypropyl)-trimethoxy-silane (GPTMS) in the submonolayer regime. A multispectroscopic approach has been followed to fully characterize the surface capped PSi. XPS has been used to trace the process of light induced oxidation and GPTMS assembly on supported PSi. Field emission SEM confirms that the surface topography is not modified by the activated assembly. To complement the chemical analysis of the bound GPTMS, FTIR and solid state NMR were used on functionalized PSi particles. Finally, the surfaces of GPTMS capped PSi have been successfully micropatterned by a masked Ar plasma etching process. The process gives rise to surface hydrophilic/hydrophobic contrasts, which are efficient in the selective binding of activated gold nanoparticles. The contrasts were applied to the local recognition of mouse serum proteins adsorbed on GPTMS functionalized PSi through an immunofluorescence assay. The results confirm the effectiveness of GPTMS capped PSi as adsorptive layer for immunosensing.

1. Introduction

Functionalization of semiconductor nanostructures with organic monolayers is an attractive process that can serve to make the properties of the original semiconductor compatible with a tailored surface chemistry [1,2]. Nanostructured porous silicon (PSi) can be described as a matrix of silicon quantum dots immersed in an amorphous Si/silica network. PSi is an extremely versatile material which allows control over its pore structure leading to high specific surface areas (hundreds of m^2/g) [3]. PSi is generally bioengineered on a supported Si wafer [4], but has been described also in the form of particles in a wide range of applications [5]. The pore structure and porosity of PSi can be adapted depending on the specific envisaged application. Although both micro- and mesopores can be used to achieve high porosity, mesoporous structures are generally preferred because they exhibit better mechanical stability in comparison to the microporous ones [6]. Furthermore, the pores present an open structure and are accessible to biomolecules such as enzymes, which enables its biocatalytic functionalization [7].

Besides, the controlled surface reactivity of PSi opens new opportunities to tune the material for specific biomedical applications [8]. Assembly has been previously proposed by visible light activated hydrosilylation (reaction between hydrogen-terminated silicon and molecules containing double bonds) [9,10] but surface modification with organosilane assemblies allows diversifying the chemical functionalities and provides biomolecular selectivity [11]. Relevantly, the assembly of organosilanes on PSi surfaces requires a previous oxidation that has been traditionally promoted by thermal process or peroxidation [12]. Relevantly, recent reports have demonstrated that oxidation of PSi can be catalyzed upon exposure to visible light, leading to an increase of free electrons at the PSi interface, which can efficiently reduce metals [13]. Here we propose that the electron density induced upon light activation can catalyze the heterogeneous condensation reactions of (3-glycidyloxy-propyl)-trimethoxy-silane (GPTMS) with the surface of the oxidized PSi, avoiding previous thermal or chemical oxidation [12]. Epoxy groups in GPTMS are able to react with different nucleophilic groups providing strong linkages not drastically altering protein

* Corresponding author.
E-mail address: miguel.manso@uam.es (M. Manso Silván).

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function [14], which is exploited to develop protocols for enzyme immobilization.

Plasma processes have been extensively used for biofunctionalization purposes in a diversity of configurations. The most extended mode, plasma polymerization (an alternative name for plasma enhanced chemical deposition of strictly organic monomers) has been used for deposition of amines [15,16], carboxylic acid [17,18] and other chemical functionalities. However, this process is not compatible with the epoxy group, very unstable face to ionic, electronic and radiative activation in the plasma. The alternative to this process has been a previous plasma activation of the target substrate and an ulterior reaction with a glycidyl monomer (plasma grafting process) [19]. In spite of the demonstrated functionality in adhesion applications, the grafting of these epoxy functionalities is more often performed by radiation induced (UV) activation, allowing a selective patterning of the surface [20]. This process is however not straightforward with the silane chemistry, in view of the low partial pressure of the epoxy silane precursors at typical thermodynamic conditions. Selective plasma etching, a process carried out under partial masking of the surface, appears as the most attractive way to micropattern organosilane functionalized surfaces for traditional microelectronic [21] or biomedical applications [22].

In this work, we aim at studying the visible light activated surface functionalization of PSi with one of the most common epoxy silanes (GPTMS). A significant effort is devoted to the characterization of the changes taking place at the PSi surface during functionalization. Moreover, we point at the biofunctionality of the GPTMS capping process by performing a selective plasma etching step, followed by the application of an immune assay.

2. Experimental

2.1. Preparation of PSi

The back side of p-type boron-doped (resistivity 0.05–0.1 Ω cm) (100) oriented Si wafers were first coated with an aluminum layer to provide low resistance ohmic electrical contacts. They were subsequently cut into 15 mm × 15 mm pieces and mounted into a teflon cell. PSi was then formed by electrochemical etch of the silicon wafers in an aqueous electrolyte composed of a mixture of hydrofluoric acid (HF) (40%) and absolute ethanol (98%) (volume ratio 1:1). The current density was fixed at 80 mA/cm^2, and the anodization time at 20s, leading to a 1 μm thick film. PSi particles were also formed by applying fixed current density of 78 mA/cm^2 and periodically (every 50s) pulsed for 1s to a higher value (104 mA/cm^2). The waveform was repeated for 30 cycles, producing highly porous and mechanically fragile layers spaced at predetermined points in the porous film [23]. The thickness induced instability of the PSi/Si interface causes a fragmentation of the layer, which can be easily scrapped from the surface. The introduced artificial cleavage planes allowed extracting PSi particles used for FTIR and solid state NMR (SS-NMR) characterization.

2.2. Functionalization of the surface

Epoxy capped PSi structures were synthesized using GPTMS (Sigma Aldrich). The organosilane-based solution was prepared by diluting GPTMS in MeOH (0,2% v/v) and the PSi films and particles were incubated in this solution for 30min, 1 h, 2 h and 3 h, under visible light (150 W halogen lamp with maximum of 10% power emission at 700 nm and power emission below 2% for radiation under 360 nm). The silane concentration used was selected to remain in the submonolayer regime and minimize homogeneous silanization reactions with solvent moisture. The samples were then cleaned by rinsing in methanol and dried under N_2 atmosphere. The whole process was carried out in a glove box.

2.3. Plasma etching

The selective plasma etching of GPTMS capped PSi was carried out in a capacitively coupled plasma using a laser machined 200 μm thick Si mask for patterning. The samples were loaded in a vacuum chamber equipped with a RF plasma system (R301, 13,56 MHz, 300 W maximal power) and a roots pump reaching 6.5×10^{-2} mbar background pressure. A 5 min treatment at 50 W power with 30 sccm Ar (6.5×10^{-1} mbar) plasma was then performed. After etching, the Ar flow was cut, the background pressure reached and the chamber vented under N_2. The Si mask was removed and the patterned GPTMS capped PSi used without further treatment.

2.4. Selective adhesion assays

We performed two different assays in order to demonstrate the adhesion selectivity of the plasma processed contrasts: a) using activated colloidal Au nanoparticles (GNPs) and b) through an immune assay. The first one consisted in incubating the GPTMS micropatterned PSi in a −COOH terminated GNPs solution (5 μg/mL in PBS) for 1 h at 37 °C, and the further optoplasmonic detection of the GNPs bound to the surface by using dark field optical microscopy (Nikon Eclipse Ni). The second experiment involves non-specific binding of proteins from mouse serum with the GPTMS groups and their detection with fluorescently labelled goat-antimouse antibody without previous blocking. We diluted the mouse serum in PBS (1/100) and we deposited the samples upside down on droplets of 50 μL for 1 h. After rinsing the sample with PBS for three times we incubated the fluorescently labelled antibody diluted in PBS in the dark. The samples were then cleaned with PBS, water and EtOH to eliminate any un-specifically adsorbed antibody. The surfaces were observed in an inverted Fluorescence microscope (Olympus) equipped with a CCD camera.

2.5. Characterization

Field emission SEM images from PSi surfaces were obtained in a Philips XL30S microscope operated at 10 keV. Water contact angles (WCAs) were measured in the sessile drop configuration using a KSV 100 goniometer, droplets of 3 μl and 5 different measurements on independent areas for statistical purposes. XPS measurements were carried out with a SPECS GmbH photoelectron spectrometer with energy analyzer PHOIBOS 150 9MCD. XPS spectra were recorded using non-monochromatic Mg K_α excitation at pass energies of 75 eV for survey and 25 eV for high-resolution core-level spectra. The electron emission angle was 0° and the source-to-analyzer angle was 90°. The data were processed using CasaXPS v16R1 (Casa Software, UK).

The vibrational spectroscopy characterization of the functionalized PSi particles was performed by FTIR using a Brucker Vector 22 (resolution 8 cm^{-1}, 4000-400 cm^{-1}, 32 scans at 10 kHz) in transmission configuration after preparation of KBr disks. SS-NMR spectra were recorded with a Bruker AV-400-WB equipped with a 4 mm triple probe channel using ZrO rotors (10 kHz). A cross-polarization (CP-MAS) pulse sequence was used between ^{1}H and ^{13}C with dipolar decoupling of ^{1}H at 80 kHz. The work frequencies were 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C. The spectral width was 35 KHz, the ^{1}H excitation pulse 2.75 μs, the contact time 3 ms, the relaxation time 4s and 1 k scans were accumulated. Adamantano (CH_2 29,5 ppm) was used as secondary reference relative to TMS as primary reference. For the ^{29}Si CP/MAS experiment, a ^{1}H excitation pulse of 3 μs was used (π/2). The spectral width was