Journal of Colloid and Interface Science 452 (2015) 180–189

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

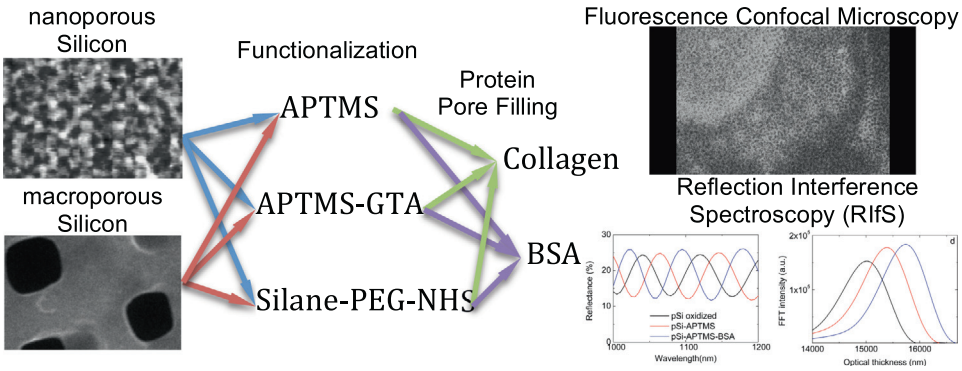
www.elsevier.com/locate/jcis

Protein attachment to silane-functionalized porous silicon: A comparison of electrostatic and covalent attachment

Malgorzata Baranowska, Agata J. Slota, Pinkie J. Eravuchira, Maria Alba, Pilar Formentin, Josep Pallarès, Josep Ferré-Borrull *, Lluís F. Marsal *

Departament d'Enginyeria Electrònica, Elèctrica i Automàtica, Universitat Rovira i Virgili, Avda. Països Catalans 26, Tarragona 43007, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 January 2015
Accepted 14 April 2015
Available online 23 April 2015

Keywords:
Porous silicon
APTMS
GTA
Silane–PEG–NHS
Collagen
Bovine serum albumin
Reflection interference spectroscopy (RIfS)

ABSTRACT

Porous silicon (pSi) is a prosperous biomaterial, biocompatible, and biodegradable. Obtaining regularly functionalized pSi surfaces is required in many biotechnology applications. Silane–PEG–NHS (triethoxysilane–polyethylene-glycol–N-hydroxysuccinimide) is useful for single-molecule studies due to its ability to attach to only one biomolecule. We investigate the functionalization of pSi with silane–PEG–NHS and compare it with two common grafting agents: APTMS (3-aminopropylotrimethoxysilane) as electrostatic linker, and APTMS modified with glutaraldehyde as covalent spacer. We show the arrangement of two proteins (collagen and bovine serum albumin) as a function of the functionalization and of the pore size. FTIR is used to demonstrate correct functionalization while fluorescence confocal microscopy reveals that silane–PEG–NHS results in a more uniform protein distribution. Reflection interference spectroscopy (RIfS) is used to estimate the attachment of linker and proteins. The results open a way to obtain homogenous chemical modified silicon supports with a great value in biosensing, drug delivery and cell biology.

1. Introduction

Porous Silicon (pSi) is a nanostructured material obtained by the electrochemical etching of crystalline silicon. In the process, a silicon wafer is used as the anode and a platinum wire as the cathode while the electrolyte is hydrofluoric acid-based with other different components [1–7]. Depending of the type silicon and of the anodization conditions it is possible to tune the size and depth of the pores. Porous silicon has a broad range of applications in the biomedical field such as biosensing, drug delivery and tissue engineering [8–12], due to its unique physical and chemical properties such as high surface area, tunable pore size, biodegradability and

* Corresponding authors. Fax: +34 977 559 605.
E-mail addresses: josep.ferre@urv.cat (J. Ferré-Borrull), lluis.marsal@urv.cat (L.F. Marsal).

http://dx.doi.org/10.1016/j.jcis.2015.04.022
0021-9797/

biocompatibility, and photonic effects [13–15]. Furthermore, the pSi surface can be easily functionalized with many different chemicals to suit any application [1,16].

Porous Si has since long attracted the attention of researchers in biosensing [17–20]. Porous Si was used for detection of many biomedical substances such as glucose, DNA, protein, viruses and bacteria [21–25]. Another property that makes pSi especially adequate for biomedical applications is that it completely dissociates in aqueous solutions with pH above 7 to silicic acid, what raises great interest in drug delivery research [26]. The porous matrix enables many different possibilities of functionalization, drugs adsorption, and loading of different molecules that make it attractive for the delivery of chemotherapeutics [27]. The delivery is impacted by the geometry, the surface modification path and the degradation [28]. Drug delivery using pSi particles has been explored by studying the release kinetics of various molecules including cis-platin [29] dexamethasone [30], ibuprofen [31] and doxo-rubicin [32].

Bioactive properties of pSi were first shown when hydroxypeptide crystals were successfully grown on its surface [33]. Factors such as the substrate topography or the surface treatment can have effects on cell functions such as adhesion, proliferation, migration and differentiation [34]. It has been proved that surface treatments in pSi can improve cell attachment in comparison with only oxidized pSi [35]. In this sense, silanization can change surface properties and improve cell attachment. The effect of this kind of functionalization of the pSi on cell attachment was investigated by Low et al. [15], who revealed that aminosilane and further collagen coating improve cell adhesion.

Silanization involves the attachment of aloxysilane to the hydroxylated pSi surface [36]. Aminosilanes such as APTMS (3-aminopropylotrimethoxysilane) improve only adsorption of biomolecules to pSi, instead, if covalent attachment is desired, it can be obtained with a further reaction with a bifunctional reagent such as glutaraldehyde (GTA) [37]. GTA produces imine (—C=N—) bonding between the biomolecules and the grafting agent [38]. This molecule is a common covalent linker for proteins but it has the disadvantage of non-specific bonding of the proteinaceous reagents due to free aldehyde groups introduced by glutaraldehyde fixation [39]. Two Schiff bases ($R^1R^2C{=}NR^3$) present in this covalent linkage make it unstable and susceptible to hydrolysis [40].

An alternative linker that avoids the attachment of many biomolecules to each site is the silane–PEG–NHS, which has the ability to attach only one protein molecule. This property is important in single molecule studies as well as to obtain a homogeneous protein layer. N-hydroxysuccinimide (NHS) is reactive group which reacts with primary amine groups to form stable amide bonds (—CONH—) with the proteins. Polyethylene glycol (PEG) is a water soluble, nontoxic polymer which has the unique ability of reducing non-specific protein adsorption and improves surface biocompatibility [41]. Silane–PEG–NHS provides high stability and reactivity in aqueous solutions. This linker has been applied to glass and alumina surfaces, although up to our knowledge no works report applications to porous silicon [42,43].

Bovine Serum Albumin (BSA) and collagen are two common proteins used as models for the study of the organization of biomolecules on functionalized surfaces. BSA is a small size protein (few nanometers in length) which has applications in immunohistochemistry, cell culture and assays [44–46]. Even though this is the most common protein used in research, it is difficult to handle as is known to easily form aggregates [47]. In contrast, collagen is one of the long, fibrous structural proteins (around 300 nm long), commonly used to coat surfaces to stimulate cell adhesion [48]. This protein is biocompatible and hydrophilic, stable and has a low cost what makes it a suitable material for modification [49]. Collagen has been extensively used in medicine and dentistry as a wound healing tissue [50,51]. The researchers have shown that cells grow better on surfaces coated with collagen [52].

In this work we study the organization of two model proteins (BSA and collagen) onto porous silicon surfaces and its dependence on different factors such as the pore size, the protein size or the surface functionalization paths. We consider two kinds of pSi with very different pore sizes: nanoporous and macroporous silicon, and three kinds of surface functionalization: APTMS, APTMS–GTA and as far as our knowledge for the first time in pSi, silane–PEG–NHS. Characterization of the samples is carried out by confocal microscopy, scanning electron microscopy (SEM), reflection interference spectrophotometry (RIfS), and Attenuated Total Reflection (ATR)-FTIR. Confocal microscopy provides information about the protein organization on the samples surface while RIfS and SEM permit to examine the pore filling for the nanoporous or macroporous silicon, respectively.

These results are of relevance in various fields: knowledge of how two different proteins are located in pores and how the refractive index is changing depending on functionalization can be of value for biosensing and drug delivery. Besides, obtaining uniformly covered surfaces is important for cell adhesion and growth.

2. Materials and methods

2.1. Materials

Silicon wafers were obtained from Si-Mat Silicon Materials (Kaufering, Germany). Glutaraldehyde (GTA) was purchased from Electron Microscopy Sciences (Hatfield, PA, USA). Silane–PEG–NHS was obtained from Nanocs (New York, NY, USA). Hydrofluoric acid (HF), dimethyloformamide (DMF), potassium hydroxide, nitric acid, 3-aminopropylotrimethoxysilane (APTMS), collagen from bovine achilles tendon, bovine serum albumin (BSA), rhodamine B isothiocyanate, phosphate buffer, toluene, dimethyl sulfoxide (DMSO) and ethanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Mili-Q-water (18 MΩ cm) was used for rinsing and preparation of solutions.

2.2. Methods

2.2.1. Porous silicon production

A custom made Teflon cell with etching area 1.54 cm^2 was used for the fabrication of nanoporous and macroporous silicon. Prior to etching, the Si wafers were washed with 5% fluoric acid (HF), rinsed with distilled water and dried. For macroporous silicon, wafers with resistivity 10–20 Ω cm were used. The etching was carried out at constant current of 5 mA for 10 min in a solution of DMF: HF (10:1 v/v). For nanoporous silicon, wafers with very low resistivity (<0.001 Ω cm) were used. The etching was carried out at constant current of 100 mA for 2 min in a solution of 3:1 (v/v) 48% aqueous HF/ethanol. After etching the samples were washed with ethanol, rinsed with water and dried. The nanoporous silicon had 50–60 nm diameter pores while macroporous silicon showed pores with a diameter around 1–2 μm (see Supplementary information, Fig. S1).

2.2.2. APTMS-functionalization

Before functionalization all the samples were oxidized in a furnace by the following procedure: a first step consisting of a 1 h period at 300 °C in nitrogen, followed by three steps in synthetic air: a second step at 400 °C during 1 h, a third step at 500 °C for a further 1 h and finally a period of 15 min at 600 °C. After this, the samples were allowed to cool down. Next, the samples were immersed in 0.1 M KOH (potassium hydroxide) for 3 min and then in 0.1 M HNO_3 (nitric acid) for 10 min. This treatment created hydroxyl