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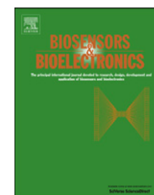

A new controlled concept of immune-sensing platform for specific detection of Alzheimer's biomarkers

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ABSTRACT

We propose a concept of very specific immune-sensing platform dedicated to the quantification of biomarkers of Alzheimer disease (AD) in biological fluids. High sensitivity is required for the earliness of AD diagnostic, mainly based on clinical evaluation at present time. Accordingly, a controlled and adaptative surface functionalization of a silicon wafer with carboxylated alkyltrichlorosilane has been developed. The surface has extensively been characterized by AFM and X-ray Photoemissive Spectroscopy. The surface modification has been chemically assessed by XPS at each functionalization step. The survey spectra of silicon surface, after, 1, 3, 6 and 24 h of silanisation, highlight a significant enhancement of the functionalization efficiency upon time. The oxidation reaction has also been investigated by XPS and showed components related to the carboxylic group. AFM measurements pointed out a morphological modification consistent with a homogenous development of the carboxylic group and an almost protein monolayer on the surface. Moreover, we evaluated the biological activity of the grafted antibodies involved in (AD) biomarker detection onto this silanized surface by fluorescent microscopy. A sandwich immunoassay dedicated to the sensitive detection of one biomarker of Alzheimer disease (AD), the amyloid peptide 1–42 (Aβ 1–42), was carried out. The results demonstrated that the controlled silanized surface provides a novel and viable way to detect biomarkers with high specificity and open the route to an original development of immune-sensing applications on such surfaces.

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1. Introduction

Self-assembled monolayers of organosilanes have focused much attention in the fields of immune-sensor technology. Organosilanes have been reported in the literature to be used for protein immobilization (Kanan et al., 2002; Gabriel et al., 2007; Rusmini et al., 2007; Hervas et al., 2012). They generally offer favorable properties such as biocompatibility of the surface and protein protection from denaturation during the immobilization process (Rusmini et al., 2007; Buchapudi et al., 2011). On the other hand, the main difficulty for protein immobilization on a solid support, particularly for antibodies, is to maintain their three dimensional conformation and orientation. Indeed, antibody orientation is crucial for an immunoassay in which the antibody is linked to a solid surface. A proper orientation allows the exposition of the binding sites to the sample solution and ensures a specific recognition. Most of the immobilization strategies, lead to random immobilization of the antibodies that can limit the antigen binding capacity (Caruso et al., 1996; Pei et al., 2010; Seitz et al., 2011). Among these, one of the most popular strategies employed consists of an amine coupling on carboxylated or active ester substrates via their amino groups (Rusmini et al., 2007). This method has been widely used on flat carboxylated surfaces (Pei et al., 2010; Samanta and Sarkar, 2011). However, the grafting density, the orientation and the biological function of the grafted antibodies, following the immobilization strategy described previously, on silane modified silicon surfaces have to be investigated. In this way, a fine understanding and control of the chemical surface preparation are needed (Faucheux et al., 2004; Popat et al., 2002).

In this paper, we present an extensive characterization of chemically silanized silicon surface by contact angle, XPS and AFM. All these techniques constitute essential tools to control the chemical surface modification in view of a successful bio-functionalization (Boussert et al., 2009; Inoue and Ishihara, 2010). We also aimed at clarifying the immobilization mechanism relying on self-assembled silanes for antibodies and silicon surface. In this perspective, the grafting density and orientation of antibodies on the prepared

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immuno-sensing platform have been studied, by an immuno-enzymatic assay. Further, the ability of immobilized antibodies to establish specific interaction has been evaluated by fluorescence microscopy. This chemical platform was finally applied to the sensitive detection of one AD biomarker, the amyloid peptide 1–42 (Aβ 1-–42), by fluorescence based sandwich immunoassay. Limit of detection (LOD) and specific binding density have also been evaluated. Indeed, Amyloid-β 1–42 (Aβ1–42) peptide is a major component of senile plaques deposited in the brain of individuals with Alzheimer's disease (AD) (Hardy and Selkoe, 2002; Israel et al., 2012), Motter et al. (Motter et al., 1995. Annal Neurol. 38, 643–648) first reported that Aβ1–42 concentration are decreased in CSF of AD patients.

2. Materials

Octane (99.9%), potassium permanganate (99%), potassium carbonate (99%), and sodium periodate (99%) were purchased from VWR and were used without further purification. The 7-octenyltrichlorosilane (90%) was provided by Flurochem Ltd. and stored at temperature smaller than 6 °C. Hydrogen chloride and ethanol (99.9%) were HPLC grade and purchased from Fisher Scientific.

Polyclonal, Fab2-specific rabbit anti-mouse immunoglobulin G (IgG) was obtained from Invitrogen, Saint-Aubin, France. Mouse monoclonal antibodies directed against amyloid peptides were provided by tebu-bio, Le Perray-en-Yvelines, France. Model immunoglobulin G (anti-mouse IgG), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (sulfo-NHS) were obtained from Sigma Aldrich. All the chemicals employed were of analytical reagent grade and were used as received.

3. Experimental methods

3.1. Chemical surface preparation

The surface functionalization consists in grafting the surface of a silicon wafer and creates alkylsilane with carboxylic groups at the end of carbon chains (Fig. 1). The reaction occurs in liquid phase using an alkyltrichlorosilane (7-octenyltrichlorosilane) diluted in organic solvent as reported by the work of Sagiv (1980). The proportion of silane in the solvent is 1:600 in volume and the system is maintained under moisture-controlled atmosphere to prevent the polymerization of silane (Ulman, 1996). In the other hand, the organosilane presents a carbon double-bond chain end; an oxidation reaction of terminal vinyl group can be achieved by a mixture of oxides in solution: potassium permanganate and sodium metaperiodate (Müller and Chetham, 2007).

The silicone samples were sliced from B-doped Si (100) wafer, with a resistivity in the range 1–50 cm, in 1 cm^2: they present a natural layer of native SiO_2 with few dispersed silanol groups (Si–OH). The silanization solution is based on a mixture of 7-octenyltrichlorosilane and octane solvent freshly prepared with a respectively ratio of 1/600 (v/v). The expected monolayers were formed at ambient temperature (~19 °C) in a glovebox with a N_2 permanent flow (humidity never exceeded 5%): samples were emerged in the prepared silanization solution enclosed in a lapping beaker into stirring plateau during 24 h. Finally, the samples were rinsed using the same solvent. For the oxidation step, the oxidant mixture is prepared with $KMnO_4$ (0.5 mmol), K_2CO_3 (1.8 mmol), $NaIO_4$ (19.5 mmol) in ultra-pure water. After 12 h of reaction time, the samples are washed in solution of $NaHCO_3$ (0.3 mol L^{-1}), DI water, HCl (0.1 mol L^{-1}) water, and ethanol.

3.2. Antibody immobilization and immunoassay procedure

All experiments have been carried out on the 1 cm^2 surfaces previously silanized and oxidized. Firstly, samples were converted to activate ester by reacting with 5 mg/ml EDC and 5 mg/ml S–NH–S 20 µl (1 µg/µL). Diluted anti-mouse IgG antibody was added to the solution with 200 µl of EDC and 200 µl of S–NH–S and the covalent attachment was performed overnight at room temperature. The surfaces were rinsed twice with PBS containing Tween 20 (0.01%). Silanized surfaces were then blocked with 2% BSA solution for 1 h. Immuno-reaction between immobilized IgG and anti-mouse IgG tagged with FITC or mouse IgG directed against Fab fragment hrp conjugate were performed. After 2 h incubation, the samples were washed with PBS containing 0.02% Tween 20 three times. For the immune enzymatic assay, the procedure followed consists in: after washing for 4–5 times, each well was added with 100 µL anti-mouse IgG (secondary antibody) HRP conjugate and incubated. Then, each well was incubated with 100 µL 3,3′,5,5′-tetramethylbenzidine (TMB) solution for 15 min, avoid of light after washing for 4–5 times. The absorbance at 450 nm was measured after blocking with a stop solution. All these experiments were compared with a silanized surface without grafted antibodies. The sandwich assay was performed by adding 20 µl (1 µg/µL) of antigen sample completed by 180 µl of PBS for 2 h at room temperature. Then a washing was performed with PBS, and 0.2% Tween 20 during 10 min three times. The sandwich immunoassay followed the procedure depicted in Fig. 2(C). After the covalent capture of the antibody, different concentrations of amyloid peptide were added on the surfaces. The plate was washed as described above after incubation for 2 h.

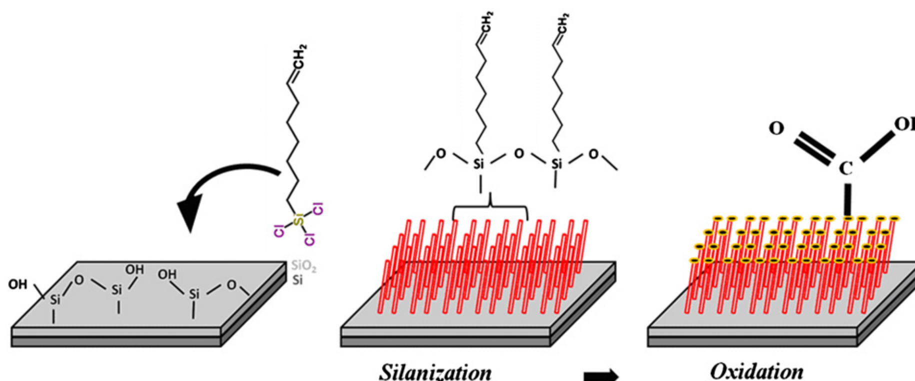

Fig. 1. Schematic drawing of the 7-octenyltrichlorosilane grafting reaction on silicon wafer. All reactions were performed in the glovebox set-up where hydrometric conditions are controlled by a permanent N_2 flux.

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