Applied Surface Science 289 (2014) 571–580

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Spatially controlled immobilisation of biomolecules: A complete approach in green chemistry

Eva Grinenval, Guillaume Nonglaton*, Françoise Vinet

CEA-LETI, MINATEC Campus, DTBS/SBSC, 17 rue des Martyrs, F-38054 Grenoble Cedex 9, France

ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form 24 October 2013
Accepted 10 November 2013
Available online 20 November 2013

Keywords:
Green chemistry
Surface patterning
Surface chemistry
Molecular vapour deposition
Silane
Fluorescence

ABSTRACT

The development of "green" sensors is a challenging task in the field of biomolecule sensing, for example in the detection of cardiac troponin-I (cTnI). In the present work a complete approach in green chemistry was developed to create chemically active patterns for the immobilisation of biological probes. This key technology is discussed on the basis of the twelve green chemistry principles, and is a combination of surface patterning by spotting and surface chemistries modified by molecular vapour deposition. The (1*H*,1*H*,2*H*,2*H*)-perfluorodecyltrichlorosilane (FDTS) was used as a novel anti-adsorption layer while the 3,4-epoxybutyltrimethoxysilane (EBTMOS) was used to immobilise probes. Oligonucleotides and the anti-cTnI antibody were studied. The spatially controlled immobilisation of probes was characterised by fluorescence. The demonstrated surface modification has broad applications in areas such as diagnostics and bio-chemical sensing. Moreover, the environmental impacts of surface patterning and surface chemistry were discussed from a "greenness" point of view.

1. Introduction

Sensors constitute important technological advances in many application fields such as health [1–3], environment [4–6], and industry [7–9]. They are often developed without regard for their environmental impact, and there has been a growing interest in the development of sensors using "green" chemistry [10–12]. This concept was introduced in 1991 by the U.S. Environmental Protection Agency (EPA), and is defined as "the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances" [13]. In this definition, the word "hazardous" is taken in the broadest sense: The danger may be physical (flammable, explosive . . .), toxicological (carcinogenic, mutagenic . . .), or global (ozone depletion, climate change . . .). This definition was later expanded to twelve principles by Anastas and Warner [14], who helped to develop and popularise the concept. Principles are reminded in Table 1. In 2005, the acronym "PRODUCTIVELY" was used to capture the essence of these principles [15]. Green chemistry has been developed to provide a framework to ensure the prevention of pollution related to chemical activities.

The mechanism by which the sensor detects a target molecule is comprised of a probe linked to the substrate via a coupling agent, as shown in Fig. 1. The grafting of the coupling agent and the probe at the substrate surface involves surface chemistry. Moreover, in a sensor, probes are frequently spatially controlled, *i.e.* localised to a precise place. The spatially controlled immobilisation of probes is an important requirement in many applications, such as diagnostic systems [16], protein microarrays [17] and nanostructured biosensors [18]. A combination of surface chemistry and surface patterning is necessary to spatially control the immobilisation of probes. Two areas should be differentiated on the substrate in order to achieve two different surface chemistries: The first one should be suitable with the probe immobilisation and the second should provide an anti-adsorption layer. The anti-adsorption layer prevents undesired probe immobilisation and nonspecific adsorption of the target molecule.

Surface patterning using photolithography is already known in the literature for various applications [19–24]. After a surface-cleaning pre-treatment, photolithography consists of exploiting the properties of certain polymers to break (in the case of a positive photoresist) their molecular chains under UV light, so that the exposed material can be dissolved by an appropriate development solution. By letting UV light irradiate the photoresist through a mask with defined regions, the mask geometries are transferred to the light-sensitive layer. An alternative surface patterning method can be easily performed by spotting a thermal resist onto silicon substrates. Both approaches to surface patterning will be discussed from a "greenness" point of view.

Once the surface patterning is finished, the subsequent chemical modification of the surface is performed in order to immobilise antibodies or probes. There are several ways to modify the surface

* Corresponding author. Tel.: +33 438 78 91 29; fax: +33 438 78 51 64.
E-mail addresses: eva.grinenval@gmail.com (E. Grinenval), guillaume.nonglaton@cea.fr (G. Nonglaton), francoise.vinet@cea.fr (F. Vinet).

0169-4332/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsusc.2013.11.046

Table 1
The 12 principles of green chemistry.

1	Prevention	It is better to prevent waste than to treat or clean up waste after it has been created
2	Atom economy	Synthetic methods should be designed to maximise the incorporation of all materials used in the process into the final product
3	Less hazardous chemical syntheses	Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment
4	Designing safer chemicals	Chemical products should be designed to affect their desired function while minimising their toxicity
5	Safer solvents and auxiliaries	The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used
6	Design for energy efficiency	Energy requirements of chemical processes should be recognised for their environmental and economic impacts and should be minimised. If possible, synthetic methods should be conducted at ambient temperature and pressure
7	Use of renewable feedstocks	A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable
8	Reduce derivatives	Unnecessary derivatisation (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimised or avoided if possible, because such steps require additional reagents and can generate waste
9	Catalysis	Catalytic reagents (as selective as possible) are superior to stoichiometric reagents
10	Design for degradation	Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment
11	Real-time analysis for pollution prevention	Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances
12	Inherently safer chemistry for accident prevention	Substances and the form of a substance used in a chemical process should be chosen to minimise the potential for chemical accidents, including releases, explosions, and fires

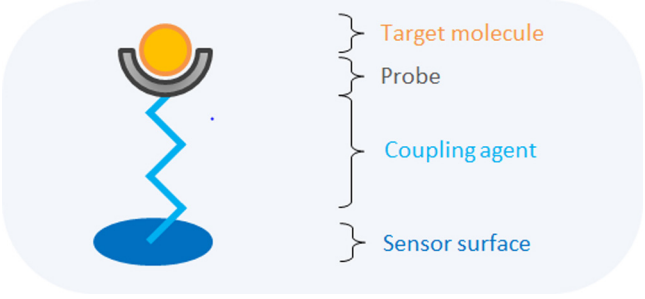

Fig. 1. Elements constituting the detection mechanism of a sensor.

chemistry of a substrate, including dip coating [25], spin coating [26], spray coating [27] or electrochemical deposition [28]. Although widely used, these techniques are usually not consistent with green chemistry principles by producing excessive waste and utilizing hazardous chemical syntheses. Alternative techniques such as chemical and physical vapour deposition [13] are preferred for surface modification. In this article, our attention is thus focused on the chemical modification of silicon oxide (SiO_2) based on green chemistry concepts for the immobilisation of probes. The proof of concept was validated with routine sequences of oligonucleotides and then utilized for the detection of the anti-troponin-I antibody. Troponin-I (cTnI) is an interesting cardiac biomarker released to the blood stream when a heart muscle injury occurs. The principles of Anastas and Warner incorporated during the implementation of our chemical process of surface modification will be detailed gradually through the different steps.

2. Material and methods

2.1. Surface patterning using photolithography or spotting

Standard protocols were used to create patterns onto 760 μm thickness silicon substrates of $25 \times 25\,mm^2$ area with a thermal silicon oxide surface layer. As briefly explained in the introduction, standard UV photolithography was adopted. After a surface-cleaning pre-treatment using remote RF oxygen plasma (500 sccm O_2 flow, 250 W, 300 s) a commercial positive thin photoresists (AZ1512HS from AZ) was spun onto the substrate, and was then baked for 90 s at 100 °C and subjected to UV light exposure. The substrate was then developed in AZ developer (1:1 developer:H_2O). The substrate was finally rinsed in DI water (resistivity of 18.2 MΩ cm) and dried under an air stream. Alternatively, the spotting technique consists of the use of a commercial thick positive resist (JSR-335 from JSR Corporation). After a similar surface-cleaning pre-treatment with O_2 plasma, the resist was spotted on a silicon substrate using a commercial ultra-low volume dispensing system (SciFlexarrayer S3 from Scienion AG, Berlin, Germany). Dispense capillary PDC2030-S6050 from Scienion was used and the substrate was then baked for 30 s at 110 °C to cure the resist.

2.2. Anti-adsorption chemistry: Surface chemistry used as an anti-adsorption layer

The anti-adsorption chemistry is based on (1*H*,1*H*,2*H*,2*H*)-perfluorodecyltrichlorosilane FDTS (ABCR, 97%). The silanisation was performed with commercial molecular vapour deposition equipment (MVD100 from Applied MST, San José, US). The deposition conditions of FDTS were the following: In the first step, one cycle of tetrachlorosilane $SiCl_4$ (Sigma Aldrich, 99,998% Semiconductor grade) at 18 Torr was injected, followed by four cycles of H_2O at 18 Torr. This step took place for a duration of 600 s at 35 °C. In a second step, two cycles of FDTS at 0.5 torr were injected, followed by one cycle of H_2O at 18 Torr. This step took place for a duration of 900 s at 35 °C and aimed at grafting FDTS to the surface by a silanisation reaction.

2.3. Immobilisation chemistry: Surface chemistry used for the probe immobilisation

An epoxide function was grafted onto the support by silanisation with the commercial epoxysilane: 3,4-epoxybutyltrimethoxysilane EBTMOS (Sikémia, ≥95%). Similarly to the anti-adsorption chemistry, the silanisation was performed with the same molecular vapour deposition equipment (*vide supra*). The deposition conditions of EBTMOS on a full silicon slide were as follows: In the first step, one cycle of $SiCl_4$ at 18 Torr was injected, followed by four cycles of H_2O at 18 Torr. This step took place for a duration of 600 s at 35 °C. In a second step, four cycles of EBTMOS at 0.5 torr were injected, followed by two cycles of