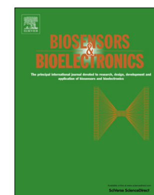
Biosensors and Bioelectronics 53 (2014) 82–89

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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



Photopatterning of self assembled monolayers on oxide surfaces for the selective attachment of biomolecules

Pompi Hazarika [a], Jonathan M. Behrendt [a], Linn Petersson [b], Christer Wingren [b], Michael L. Turner [a,*]

[a] School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK
[b] Department of Immunotechnology, CREATE Health, Lund University, BMC D13, Lund SE-221 84, Sweden

A R T I C L E I N F O

Article history:
Received 23 July 2013
Received in revised form
29 August 2013
Accepted 2 September 2013
Available online 20 September 2013

Keywords:
Self-assembled monolayer (SAM)
Silicon
Photo-pattern
Proteins
Biomarker detection

A B S T R A C T

The immobilization of functional biomolecules to surfaces is a critical process for the development of biosensors for disease diagnostics. In this work we report the patterned attachment of single chain fragment variable (scFv) antibodies to the surface of metal oxides by the photodeprotection of self-assembled monolayers, using near-UV light. The photodeprotection step alters the functionality at the surface; revealing amino groups that are utilized to bind biomolecules in the exposed regions of the substrate only. The patterned antibodies are used for the detection of specific disease biomarker proteins in buffer and in complex samples such as human serum.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In immunosensors, antibodies are used as the recognition element to bind a specific target antigen (Holford et al., 2012). Antibody based immunoassays are one of the most important tools used in medical diagnostics as arrays of antibodies enable parallel detection of multiple proteins in a single sample and these arrays can be used to identify panels of disease biomarkers (Kopf and Zharhary, 2007; Wingren and Borrebaeck, 2009). An essential step in the fabrication of these arrays is the immobilization of the antibody to a substrate (Acton et al., 2011). Self-assembled monolayers (SAMs) of organic compounds have been previously used to immobilize biomolecules on a surface (Baldrich et al., 2008; Hodneland et al., 2002; Reynolds et al., 2009; Sigal et al., 1996; Valsesia et al., 2006), as SAMs are an inexpensive and versatile method for introducing functional groups to surfaces (Flink et al., 2000; Gooding et al., 2003; Love et al., 2005; Ulman, 1996). They have been applied in many fields from the fabrication of electronic devices to the creation of biosensors (Aswal et al., 2006; Chaki and Vijayamohanan, 2002; Cloarec et al., 1999; Dahmen et al., 2003; Smits et al., 2008). Commonly used SAMs include thiols on metal surfaces (Lee et al., 2005; Love et al., 2005; Ulman, 1996) such as gold, silver, and copper; and organosilanes on hydroxylated surfaces like metal oxides (Onclin et al., 2005; Senaratne et al., 2005). An advantage of oxide substrates compared to gold (Dulkeith et al., 2002) is that they do not quench fluorescence thus making these surfaces suitable for study by fluorescence based techniques.

The covalent linkage of organosilane SAMs to oxide surfaces allows chemical modification of the surface functionality whilst maintaining the integrity of the SAM (Herzer et al., 2010). Biomolecules attached to silane surfaces can be used in label free sensitive detection by photonic based sensors (Nair and Vijaya, 2010). Surface immobilized biomolecules such as antibodies must retain their biological recognition properties after immobilization. Selective binding and the correct orientation of the immobilized antibodies are important, whilst at the same time non-specific adsorption of biomolecules to the surfaces should be avoided (Jung et al., 2008; Onclin et al., 2005).

In this paper we successfully address these issues and present an approach for immobilization and patterning of human recombinant single chain fragment variable (scFv) antibodies (Ahmad et al., 2012; Soderlind et al., 2000) on oxide surfaces whilst retaining their biological activity. We report a method for obtaining antibody arrays using photo-patterned SAM and detection of biomarker proteins using surface bound antibodies. A photo labile SAM is covalently attached to a silicon dioxide surface and used to pattern antibody scFv features by covalent or non-covalent linkages. The patterned antibody features are used to detect the disease biomarker complement protein C1q (Hong et al., 2009) in buffer (pure form) and in human serum (crude, complex sample). This approach leads to very little non-specific binding of the antibodies and antigens to the surfaces, and the

* Corresponding author. Tel.: +44 161 2754625; fax: +44 161 2754273.
E-mail address: michael.turner@manchester.ac.uk (M.L. Turner).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.001

surface bound biomolecules show exceptional stability. This is a simple method that can be used to reveal functionalities on oxide surfaces that are suitable for biomolecule immobilization. It has many potential applications in bioelectronics, particularly in the development of more robust and sensitive biosensors.

2. Material and methods

2.1. Synthesis

2-(2-Nitrophenyl)propyl(3-(triethoxysilyl)propyl) carbamate was synthesized using a published method (Ahmad et al., 2009). All chemicals were purchased from Sigma Aldrich and used as received with no further purification. Briefly, 2-(2-nitrophenyl)propan-1-ol was first synthesized by mixing benzyltrimethylammonium hydroxide (Triton B) solution in methanol (40 wt%, 37.0 mL, 82.1 mmol) with 2-ethylnitrobenzene (10.8 mL, 80.1 mmol) and paraformaldehyde (2.54 g, 84.6 mmol). The reaction mixture was heated at reflux at 80 °C and stirred for 20 h. Upon cooling to room temperature, the pH of the solution was adjusted to 7 through addition of aqueous hydrochloric acid (1 M), followed by extraction with ethyl acetate (3 × 100 mL). The combined organics were washed with water (300 mL) and brine (300 mL) and dried over magnesium sulfate and the solvent was removed by evaporation under reduced pressure. The crude product was separated by flash silica column chromatography (light petroleum ether/ethyl acetate, 4:1 then 2:1) to yield the compound as orange oil (4.38 g, 24.2 mmol, 30%). δH (400 MHz; $CDCl_3$), 1.34 (3H, d, $J=4$ Hz), 1.71 (1H, s(br)) 3.53 (1H, m), 3.80 (2H, m), 7.37 (1H, m), 7.50 (1H, m), 7.58 (1H, m), 7.76 (1H, m); δC (75 MHz; $CDCl_3$) 17.6, 36.4, 67.9, 124.1, 127.2, 128.2, 132.6, 138.1, 150.8; IR(ATR): $\nu=3378$ (br), 3072 (w), 2972 (m), 2935 (m), 2876 (m), 1608 (w), 1577 (w), 1519 (s), 1483 (w), 1351 (s); m/z (ES+) 202 ($[M+Na]^+$, $C_9H_{11}NO_3$, 100%).

The 2-(2-nitrophenyl)propan-1-ol (1.60 g, 8.88 mmol) was dissolved in dichloromethane (15 mL), and 3-isocyanatopropyltriethoxysilane (2.42 mL, 9.77 mmol) was added followed by triethylamine (124 µL, 0.88 mmol). The reaction mixture was heated at reflux at 60 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with a further 20 mL of dichloromethane and was washed with aqueous phosphate buffer (pH 7, 3 × 50 mL) and brine (50 mL). The organic phase was dried over magnesium sulfate and the solvent was removed by evaporation under reduced pressure. The crude product was separated by flash silica column chromatography (hexane/ethyl acetate, 3:1) to yield the desired final product as yellow oil (2.39 g, 5.58 mmol, 63%). δH (400 MHz; $CDCl_3$), 1.88 (2H, t, $J=8$ Hz), 1.22 (9H, t, $J=8$ Hz), 1.34 (3H, d, $J=8$ Hz), 1.58 (2H, m), 3.11 (2H, q, $J=8$ Hz), 3.70 (1H, m), 3.81 (6H, q, $J=8$ Hz), 4.10 (1H, m), 4.24 (1H, m), 4.84 (1H, s(br)), 7.36 (1H, m), 7.48 (1H, m), 7.57 (1H, m), 7.73 (1H, m) δC (75 MHz; $CDCl_3$) 7.6, 17.5, 18.3, 18.4, 23.2, 33.3, 43.4, 58.5, 124.0, 127.3, 128.0, 132.6, 137.5, 153.7, 156.1; IR(ATR): $\nu=3342$ (m), 3074 (w), 2974 (m), 2972 (m), 2885 (m), 1708 (s), 1608 (w), 1577 (w), 1525 (s), 1483 (w), 1355 (m), 1239 (m), 1073 (s); m/z (ES+) 452 ($[M+Na]^+$, $C_{19}H_{32}N_2O_7Si$, 100%).

2.2. SAM fabrication

Oxidized silicon wafers (thickness 525 µm; resistivity 0.001–0.005 Ω cm) with thermally grown oxide layer (thickness 300 nm) were purchased from IDB Technologies Ltd. The wafers were cut into 1 cm × 1 cm size pieces for surface modification. The substrates were rinsed with ethanol, iso-propanol and acetone and dried with a stream of N_2. The substrates were treated with O_2-plasma (Diener Electronic GmbH.) for 2 min (60 W) at 25 °C. The cleaned substrates were transferred to a N_2 glovebox for deposition of SAM. Each substrate was submerged in a solution of the organosilane in anhydrous toluene (2 ml, 1 mM) for 48 h. After the incubation period, the samples were rinsed with toluene and ethanol, dried with N_2 and annealed on a hot plate at 120 °C for 45 min. Substrates before and after SAM deposition were characterized by water contact angle measurement (DSA 100, Kruss), true non-contact mode atomic force microscopy (AFM; XE-100, Park Systems) and X-ray photoelectron spectroscopy (XPS; Kratos).

2.3. Photo-patterning and conversion of amino to carboxyl group

The SAM deposited substrates were exposed to UV-light at 365 nm (Lumen Dynamics LX-400 UV Spot Curing System, Jenton International UK) through a mask with transparent features (squares) of various sizes; 10 µm × 10 µm, 30 µm × 30 µm, 50 µm × 50 µm, 70 µm × 70 µm and 100 µm × 100 µm (features printed on glass using a chrome coating) to remove the photo-labile protecting groups. Removal of the protecting group reveals amino groups on the substrate surface in the exposed regions for binding of the biomolecule. By revealing the surface functionality only in the exposed region it is possible to reduce non-specific binding of proteins on the rest of the substrate. Substrates were photo-exposed without mask when used for contact angle measurements and XPS. All substrates were photo-irradiated for 1 min in air to generate the amino functionality on the surface. After photo-irradiation, the substrates were rinsed with ethanol and dried with N_2. In order to bind proteins to the surface, the amino groups were converted into carboxyl groups by treatment with succinic anhydride. Each photo-patterned substrate was submerged in a solution (2 ml) of succinic anhydride (0.2 M, Aldrich) in a mixture of anhydrous acetonitrile and triethylamine (0.2 M, Fluka). The samples were incubated overnight at room temperature and then rinsed thoroughly with ethanol and dried with N_2. The photo-patterned substrates were characterized by contact angle (water) and XPS.

2.4. Binding of streptavidin (STV) to SAM modified substrate

The carboxyl groups on the substrate surface can be activated using a mixture of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (0.4 M, Aldrich) and *N*-hydroxysulfosuccinimide (sulfo-NHS) (0.1 M, Aldrich) in deionized water. The EDC/NHS solution (60 µl) was incubated on each substrate for 30 min at room temperature, followed by rinsing with PBS (68 mM Na_2HPO_4, 32 mM NaH_2PO_4, 150 mM NaCl, pH 7.4). Alexa 546 labeled-STV (5 µg/ml, Invitrogen) in PBS–Tween (68 mM Na_2HPO_4, 32 mM NaH_2PO_4, 150 mM NaCl, 0.05% tween-20, pH 7.4; 60 µl) was then applied on to the substrates and incubated for 2 h at room temperature in the dark. After the incubation period, the substrates were rinsed with PBS–Tween (pH 7.4) and deionized water and dried with N_2. The substrates were characterized using a fluorescence microscope (Olympus) and non-contact mode AFM.

2.5. Production and purification of scFv antibodies

Two human recombinant scFv antibody fragments, directed against C1q and C3 were selected from the n-CoDeR-library (Soderlind et al., 2000). The scFvs were produced in 100 ml of *E. coli* cultures and purified from expression supernatants using affinity chromatography on Ni^{2+}-NTA agarose (Qiagen). Bound molecules were eluted with 250 mM imidazole (pH 8), extensively dialyzed against PBS (pH 7.4), and stored at 4 °C until further use. The degree of purity and integrity of the scFv antibodies were evaluated using 10% SDS-PAGE (Invitrogen). The protein concentrations were determined by measuring the absorbance at 280 nm.

Download English Version:

https://daneshyari.com/en/article/866676

Download Persian Version:

https://daneshyari.com/article/866676

Daneshyari.com