Single-Step DNA Immobilization on Antifouling Self-Assembled Monolayers Covalently Bound to Silicon (111)

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Hydrosilylation of alkenes with epoxide-terminated tri(ethylene oxide) moieties on Si−H surfaces yields homogeneous monolayers for the efficient coupling of biomolecules. The wetting properties of the epoxide-functionalized surface allow for the spotting of solutions of biomolecules, making the surface amenable to microarraying. Immobilization of thiolated DNA was achieved in a single step to fabricate biorecognition interfaces showing the hybridization of complementary DNA at low concentrations and negligible binding of noncomplementary DNA.

Introduction

Silicon, as a material that is highly compatible with microfabrication and the mass production of integrated devices, is ideal for the fabrication of optical[1,2] and field effect transistor-based[3,4] biosensors. The modification of silicon to form the biorecognition interface is typically via silane chemistry on the oxidized silicon surface. However, silane chemistry is prone to multilayer formation and has limited stability in aqueous media. Si−C linked monolayers formed by reaction of alkenes with Si−H terminated silicon surfaces[5,6] (that is, without an intervening oxide layer) provide an alternative self-assembly system that is extremely robust, highly controllable, and well suited to the modification of silicon micro- and nanostructures.[7]

For the development of reliable biosensors, the design of the biorecognition interface is crucial. The ideal monolayer system should allow simple and efficient immobilization of the biomolecule, preferably with a single reactive group compatible with a range of nucleophiles, and provide an environment optimal for the binding of the analyte while resisting the nonspecific adsorption of other species as reported for Si−C linked monolayers terminated with oligo(ethylene oxides) (OEOs).[8−13]

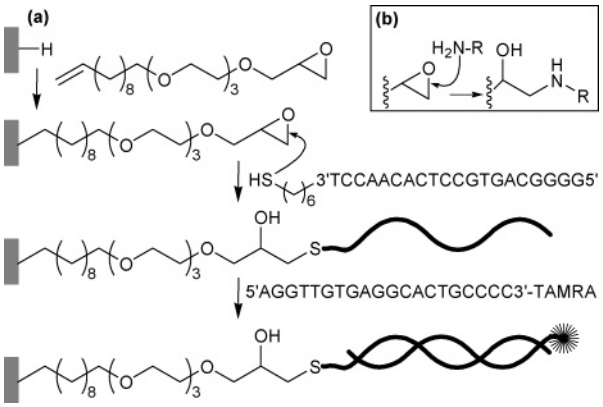

Figure 1. (a) Scheme of DNA immobilization via thiolysis followed by hybridization. (b) Alternative coupling scheme via aminolysis.

In addition, for Si−C monolayers the molecular species should ideally be stable under both the photochemical and thermal conditions that can be used for monolayer formation. Here we report on the modification of Si−C surfaces terminated with tri(ethylene oxide) glycidyl ether moieties on Si (111). Hydride-terminated silicon substrates were immersed in solutions of 2-((2-(2-(2-(undec-10-enyloxy)ethoxy)ethoxy)ethoxy)methyl)-oxirane (epoxyalkene) in 1,3,5-triethylbenzene at 200 °C.[14] Subsequently covalent attachment of biomolecules containing free amines or thiol groups was achieved simply by exposing the biomolecule to the monolayer without any activation step (Figure 1). Not only does the alkene possess an antifouling[15,16] moiety but the epoxide is stable to both high temperature and long storage time. Furthermore, the epoxide-terminated surface is reasonably hydrophobic, which, when coupled with the one-step biomolecule attachment strategy, is ideal for microarray preparation by spotting solutions of biomolecules onto the surface.

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(15) The term "antifouling" describes the ability of surfaces to resist the nonspecific adsorption of biomolecules. In this study, antifouling properties are discussed with respect to fluorescent labeled oligonucleotides. The importance of reducing nonspecific adsorption of DNA to microarray surfaces was recently demonstrated using poly(ethylene glycol)-modified surfaces that exhibited 13 times lower background fluorescence than surfaces without poly(ethylene glycol).[16] Here we observed negligible background fluorescence without the need to use stringent conditions or detergents in the washing step after the hybridization reaction.
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We demonstrate the utility of this surface chemistry via the attachment of both amino acids and oligonucleotides. DNA was chosen because the modification of Si−C linked monolayers with DNA has been studied extensively, providing a useful comparison to our surface chemistry. The current, mainly multistep, strategies include the introduction of maleimide groups followed by the coupling of thiolated oligonucleotides,[7,17−20] the reaction of aminated oligonucleotides with terminal NHS esters,[21−23] and automated solid-phase DNA synthesis on hydroxyl-terminated monolayers.[24−27] The novelty of the epoxyalkene-derived monolayer is that it combines antifouling moieties with one-step DNA immobilization. Importantly, the coupling point for the oligonucleotide is located at the *top* (distal end) of the OEOs; consequently, the thermodynamics and kinetics of hybridization are not hindered[28] as would be expected for coupling points buried inside the monolayer.[8]

Experimental Section

Materials. 1,3,5-Triethylbenzene (97%) was purchased from Fluka, redistilled from sodium under vacuum, and stored over molecular sieves under an argon atmosphere. Thiolated spacer modified oligonucleotide 5′GGGGCAGTGCCTCACAACCT-$(CH_2)_6$-SH 3′, tetramethyl rhodamine (TAMRA)-labeled complementary DNA1 5′AGGTTGTGAGGCACTGCCCC-TAMRA3′, and noncomplementary DNA2 5′GGATGGACGAAGCGCTCAGG-TAMRA3′ were purchased from GeneWorks Pty Ltd, SA, Australia.

Preparation of Si−C Linked Monolayers. Si (111) wafers (n- or p-type, 1−10 Ω cm) were cleaved into pieces and cleaned in concentrated H_2SO_4/30% H_2O_2 (semiconductor grade, 3:1, v/v) at 90 °C for 20−30 min followed by copious rinsing with Milli-Q water. Hydrogen-terminated Si (111) surfaces were prepared by etching in a deoxygenated 40% solution of NH_4F for 15−20 min.[29,30] The formation of monolayers was achieved via thermal reaction in a deoxygenated solution of the alkene in 1,3,5-triethylbenzene under an inert atmosphere at 200 °C for 2−6 h. After the reaction, the sample was rinsed several times with dichloromethane and ethyl acetate and blown dry under a stream of argon.

Derivatization of Epoxy-Terminated Surfaces. *1. Aminolysis of the Epoxide.* The samples were immersed in 50 mM ammonium acetate buffer at pH 8.0 containing 20 mM glycine for 1 h. The samples were then rinsed with Milli-Q water and ethanol and blown dry under argon.

2. Thiolysis of the Epoxide. The samples were immersed in 50 mM phosphate buffer at pH 8.0 containing 20 mM *N*-acetylcysteine overnight. The samples were then rinsed with Milli-Q water and ethanol and blown dry under argon.

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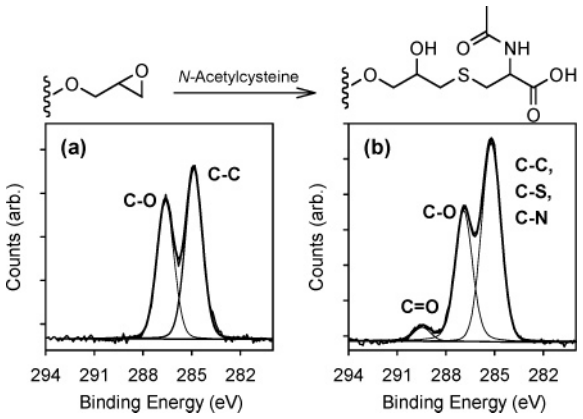

Figure 2. XP spectrum of the C 1s region of an epoxide-terminated monolayer (a) before and (b) after coupling of *N*-acetylcysteine.

DNA Immobilization and Hybridization. Epoxide-derivatized Si (111) surfaces were spotted with 10 μM DNA in 0.1 M $NaHCO_3$ buffer at pH 8.3 containing 0.3 M NaCl using a micropipettor and were incubated for 12 h at 40 °C in a humid chamber. The samples were rinsed with Milli-Q water and ethanol, dried, and incubated for an additional hour at 60 °C in 1% 2-mercaptoethanol (v/v) in 0.1 M $NaHCO_3$ buffer at pH 8.3 containing 0.3 M NaCl to react any remaining epoxides on the surface. The samples were rinsed, dried, and hybridized under coverslips with 0.1−10 μM complimentary DNA1 or noncomplementary DNA2 in 10 mM Trisma buffer at pH 7.0 containing 10 mM $MgCl_2$ and 1 M NaCl at room temperature for 4 h in the dark, followed by rinsing (twice) with 50 mM Na_2HPO_4 buffer at pH 7.0 containing 0.1 M NaCl.

Sample Characterization. XP spectra were obtained using an EscaLab 220-IXL spectrometer with a monochromated Al Kα source (1486.6 eV), hemispherical analyzer, and multichannel detector. The spectra were accumulated at a takeoff angle of 90° with a 0.79 mm^2 spot size at a pressure of less than 10^{-8} mbar. Fluorescence images were acquired using a Fujifilm FLA-5000 scanner with an LPG filter and SHG laser excitation of 532 nm for TAMRA emission of 555 nm. Contact angle measurements were determined using a Rame-Hart contact angle goniometer. The experimental uncertainty in the contact angle was estimated to be ±4°.

Results and Discussion

After modification of the Si−H surface with the epoxyalkene, XPS was used to verify the composition of the monolayer and assess the quality of the sample. The XP survey spectrum (Figure S2a, Supporting Information) showed the presence of carbon and oxygen as expected for the grafted monolayer in addition to the silicon peaks from the underlying substrate. The silicon 2p narrow scan (Figure S2b, Supporting Information) did not show a significant peak between 102 and 105 eV characteristic of oxidized silicon, confirming the high quality of the sample. The carbon 1s envelope (Figure 2a) was fitted with two peaks at 285.0 and 287.0 eV corresponding to the C−C and C−O linked carbons. The oxygen 1s narrow scan (Figure S2d, Supporting Information) revealed the presence of at least two different oxygen species attributed to the ether and epoxy oxygens from the monolayer and oxygen associated with trace levels of oxidized silicon. X-ray reflectivity measurements of silicon functionalized with the epoxyalkene in mixed monolayers confirmed that smooth organic films of monolayer thickness (∼16 Å) with a low interfacial roughness (∼2 Å) at the silicon−monolayer and monolayer−air interfaces were formed. ^{1}H NMR spectra of the reaction mixture after the hydrosilylation reaction at 200 °C showed no evidence of the opening of the epoxide ring under these conditions. This is significant because other activated groups commonly used for coupling of biomolecules are unstable

Table 1. Static Water Contact Angles (θ) for the Epoxy-Terminated Surface before and after Reaction with Thiols

surface modification	θ/deg
as prepared	74
2-mercaptoethanol	58
thiolated DNA, then 2-mercaptoethanol	<10

under high temperatures or other harsh conditions and therefore require multistep chemistries.

To investigate the reactivity of the surface-bound epoxy groups and establish conditions for DNA immobilization, reaction with amino acids via sulfhydryl- or amino groups was investigated followed by XPS of the modified surface. The *N*-acetyl derivative of cysteine was chosen as a model for the thiolysis reaction because reaction could occur only via the sulfhydryl group but not via the (protected) amino group. Nitrogen was detected in the XP survey spectrum after reaction of the epoxy-functionalized surface with *N*-acetylcysteine at pH 8.0. The nitrogen 1s narrow scan (Figure S3, Supporting Information) contained a peak at 400.5 eV assigned to the binding energy of the acetamide group. There were two changes in the carbon 1s narrow scan (Figure 2b). First, the increase and slight broadening of the peak at ~285.0 eV compared to that observed prior to the coupling reaction was attributed to the contributions from the C−C, C−S, and C−N bonded carbons of *N*-acetylcysteine. Second, the spectrum showed a new peak at 289.3 eV assigned to the carbonyl carbons of the acetamide and carboxylic acid moieties of *N*-acetylcysteine. The peak area ratio of the carbonyl peak in the carbon 1s scan to the nitrogen 1s peak was 2:1 as expected for *N*-acetylcysteine. On the basis of elemental composition, it was estimated that approximately 50% of the terminal epoxy groups had reacted with the thiol. This yield is comparable to that achieved for the immobilization of thiols to a Si−C linked monolayer modified with maleimide groups in a multistep process.[31] Thiolysis was further investigated using 2-mercaptoethanol with a view to using this reaction to remove unreacted epoxides remaining on the surface after coupling of the biomolecule (see below). The static water contact angle of the epoxide surface (74°) was decreased after reaction with 2-mercaptoethanol (58°) (Table 1) at elevated temperature and pH, suggesting the appearance of hydroxyl groups from the thiolysis (and possibly competing hydrolysis under basic conditions).

The feasibility of coupling via aminolysis was confirmed by reaction of the epoxy-terminated monolayer with glycine in an aqueous buffer (pH 8.0). XPS showed the presence of a N 1s peak at 400.7 eV (Figure S4, Supporting Information) assigned to the secondary amine formed by the reaction of glycine with the epoxide.

Having established the applicability of this interface for the coupling of biomolecules through both thiolysis and aminolysis, we verified the ability to detect biological targets selectively by thiolated oligonucleotide immobilization and subsequent hybridization as a model system. Epoxyalkene-derivatized surfaces were spotted with a solution of thiolated DNA and incubated overnight in a humid chamber. The relatively hydrophobic nature of the epoxide-terminated surface allows spotting without spreading of the solution. Any epoxides remaining on the surface were removed by thiolysis using 2-mercaptoethanol yielding hydroxyl groups. Terminal hydroxyl functionality promotes the projection of the DNA into the solution for the formation of the

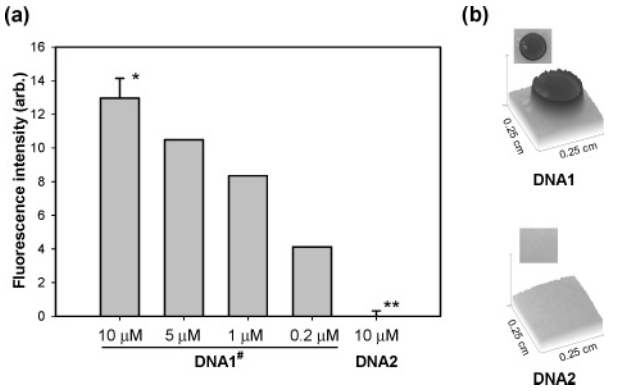

Figure 3. (a) Fluorescence intensity as a function of the target concentration of DNA1 for (*) $n = 4$ and (**) $n = 3$ independently prepared samples. (#) The total DNA concentration remained constant at 10 μM by adding varying amounts of noncomplementary DNA2. (b) Fluorescence images of DNA1 and DNA2 hybridization to oligonucleotides on Si (111).

DNA duplex[28,32] and is an important factor in reducing biofouling.[33] The DNA-modified surface was highly hydrophilic with a contact angle of less than 10°.

Hybridization of the DNA-modified surface with complementary (DNA1) and noncomplementary (DNA2) TAMRA labeled oligonucleotides resulted in elevated fluorescence for the complementary species with essentially no increase in the noncomplementary species (Figure 3). To demonstrate the specificity of the surface for the complementary target in solution, the concentration of complementary DNA1 was varied from 0.2 to 10 μM while adjusting the total DNA concentration to 10 μM with noncomplementary DNA. In these experiments, the lowest target concentration was easily detectable and is lower than those reported for DNA tethered to other Si−C monolayers.[7,17−27] We find that our surfaces provide high sensitivity to complementary species in solution with minimal background and without the use of detergents that could potentially compromise duplex stability.

Conclusions

Our results show that monolayers incorporating a tri(ethylene oxide) moiety and a terminal epoxy function could be biofunctionalized with ease in a single step by spotting biomolecule solutions onto the surface. Thiolated oligonucleotides immobilized in this fashion show specific hybridization of the target at submicromolar concentrations with insignificant binding of DNA to passivated regions on the surface. These properties are useful for the development of silicon-based microarrays and biosensors. The fact that this is achieved using Si−C linked monolayers, without an intervening oxide layer, is of particular interest for field effect devices because the biorecognition event occurs in closer proximity to the semiconductor surface than when an oxide player is present.

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Supporting Information Available: Synthetic methods for preparation of the functionalized alkenes, additional XPS data, and X-ray reflectivity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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