Biosensors and Bioelectronics 36 (2012) 222–229

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Quantification of surface etching by common buffers and implications on the accuracy of label-free biological assays

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ARTICLE INFO

Article history:
Received 13 March 2012
Accepted 11 April 2012
Available online 21 April 2012

Keywords:
DNA microarrays
Proteins microarrays
Label-free detection
Optical interferometry
Glass stability
Buffer effect

ABSTRACT

High throughput analyses in biochemical assays are gaining popularity in the post-genomic era. Multiple label-free detection methods are especially of interest, as they allow quantitative monitoring of biomolecular interactions. It is assumed that the sensor surface is stable to the surrounding medium while the biochemical processes are taking place. Using the Interferometric Reflectance Imaging Sensor (IRIS), we found that buffers commonly used in biochemical reactions can remove silicon dioxide, a material frequently used as the solid support in the microarray industry. Here, we report 53 pm to 731 pm etching of the surface silicon oxide over a 12-h period for several different buffers, including various concentrations of SSC, SSPE, PBS, TRIS, MES, sodium phosphate, and potassium phosphate buffers, and found that PBS and MES buffers are much more benign than the others. We observe a linear dependence of the etch depth over time, and we find the etch rate of silicon dioxide in different buffers that ranges from 2.73 ± 0.76 pm/h in 1 M NaCl to 43.54 ± 2.95 pm/h in $6 \times$ SSC. The protective effects by chemical modifications of the surface are explored. We demonstrate unaccounted glass etching leading to erroneous results with label-free detection of DNA microarrays, and offer remedies to increase the accuracy of quantitative analysis.

1. Introduction

Glass substrates often serve as the solid support in the microarray industry, as they are inexpensive, easy to handle, optically transparent, and expected to be chemically inert. Device miniaturization and immobilization of multiple capture agents on a solid substrate have enabled scientists to study DNA–DNA, DNA–protein, and antibody–antigen interactions in a high-throughput manner (Haab et al., 2001, MacBeath, 2002, Schena et al., 1995, Syvanen, 2001). Furthermore, investigations of various biochemical reactions on microarrays, such as DNA amplification, primer elongation, single base extension and reverse transcription, have emerged (Erdogan et al., 2001, Kinoshita et al., 2007, Mitterer et al., 2004). Whether a biochemical reaction takes places on a solid support or in standard laboratory glassware, it is inevitable that these surfaces will be subjected to the same reaction conditions as the biochemical species of interest. Thus, it is imperative that the surfaces be stable during the entirety of the reaction. For instance, it was shown that a variation of the surface roughness of the glass substrates can contribute to the poor immobilization efficiency of peptide arrays, resulting in a 80% decrease in signal from idealized conditions (North et al., 2010). In addition, unexpected surface inconsistencies can cause artifacts that are not observed in reactions traditionally carried out in solution, leading to an increased number of false positives or false negatives (Sauer et al., 2009).

Silicon dioxide, or more commonly referred as silica or glass, is a very popular choice of material, thanks to its well-known surface chemistry and ease of use in semiconductor micromachining technology. Thus, it is often the choice for sensor surfaces in label-free sensing technologies. While many sensors assume stable glass substrates in their platforms, silica dissolution in even relatively inert solutions with neutral pH has been reported (Icenhower and Dove, 2000, Kato, 1968). Dissolution rates for glass have been reported for time periods ranging from one to a few hundred days; however, there are no studies of silica dissolution during biochemical reactions lasting for several hours to a day. This lack of data for short times is likely due to the fact that quantifying surface etching over such short periods of time is very challenging with conventional methods, such as AFM, which require a vertical resolution better than the surface roughness. Using the Interferometric Reflection Imaging Sensor (IRIS) (Ozkumur et al., 2008), we found that SiO_2 can be etched when the surface is exposed to buffers commonly used in molecular biology protocols. This surface erosion may

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http://dx.doi.org/10.1016/j.bios.2012.04.020

compromise data acquired by label-free sensors without the investigator's knowledge, potentially leading to false or inaccurate findings. Because of the inability to measure and quantify small changes on the surface using conventional methods, the phenomenon we present here may have gone unnoticed.

We examined the silica dissolution in multiple buffers at concentrations relevant to biochemical assays. We report, for the first time, the etch rate of thermally grown silicon dioxide in buffers commonly used for microarray research. The etch depth of SiO_2 is measured for various etch times with IRIS, and the effect of the buffer concentration on the etch rate is investigated. The experiments were performed at room temperature, consistent with typical incubation protocols. Our results demonstrate that nearly all buffer solutions we examined etch the silicon oxide surface, in some cases more than 1 nm in 24 h. Because of the relevance to the biosensor field, we investigated whether chemically modified surfaces for biomolecule immobilization suffer from similar dissolution rates as bare silicon oxide surfaces. Furthermore, we explored several immobilization buffers on functionalized silica surfaces to determine their effects on DNA microarray printing. Finally, we offer solutions to the silica etching problem in buffers for quantitative analyses of DNA microarrays.

2. Materials and methods

2.1. Chemicals and buffer preparation

All chemicals were purchased from Sigma-Aldrich unless stated otherwise. Multiple concentrations of sodium phosphate buffer (NaPB) were prepared with monobasic and dibasic sodium phosphate, and the pH was adjusted to 8.5. Potassium phosphate buffer (KPB) was prepared in a similar fashion. Phosphate buffer saline (PBS, Fisher Science) at 10 × was diluted, and saline-sodium citrate (SSC, pH 7.0) and saline-sodium phosphate-EDTA (SSPE, pH 7.4), both 20 × solutions, were diluted to appropriate concentrations. A stock solution of 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, containing 100 mM MES, 1 M [Na+], and 20 mM EDTA, was diluted to multiple concentrations. Tris(hydroxymethyl)aminomethane (Tris, pH 8.0) buffer were prepared at multiple concentrations, each with 150 mM sodium chloride (NaCl), and different concentrations of NaCl solutions were prepared.

2.2. Substrate preparation and etching experiment

Silicon wafers with 17 μm of thermally grown oxide (Silicon Valley Microelectronics) were protected with photoresist except for repetitive 100 μm circular voids that exposed the silica surface. The samples were immersed in ample amounts of buffer solutions (~15 mL) to avoid any depletion of etching agents for the duration of the experiments: 2, 6, 12, 18, and 24 h. All the experiments took place on a rotating shaker, and the buffer was replaced with a fresh solution after 12 h. Deionized water was used as the negative control. After the etch period, the samples were washed three times in deionized water on the rotator for 10 min, followed by another 5 min wash. The final stage of washing was done by a thorough rinse under a stream of deionized water, after which the samples were dried with ultra pure argon gas. The remaining photoresist was removed by sonicating the samples three times in acetone for 5 min, followed by a acetone, methanol, and deionized water rinse. The samples were dried with nitrogen gas and ashed with O_2 plasma for 10 min (500 W) to ensure the complete removal of any photoresist on the SiO_2 surface. Please refer to the supplementary material for the detail fabrication procedure and the schematic representation of the etching experiment (Fig. S1).

2.3. Interferometric Reflectance Imaging Sensor (IRIS) measurement

The working principle of IRIS is explained in detail elsewhere (Ozkumur et al., 2008). Briefly, a tunable laser is used to illuminate the substrate at 1 nm increments, from 766 nm to 784 nm. The reflected light from the SiO_2 surface and the Si–SiO_2 interface creates an interference signature, and the intensity images at all wavelengths are recorded onto a CCD camera. The data from all of the pixels of the camera are processed to find the optical thickness between the two reflecting surfaces, then translated into a height image of the entire field of view. A simple schematic of the IRIS setup is depicted in the supplementary material (Fig. S2). Using custom software, the relative optical thickness of the etched features was compared to the surrounding background.

2.4. Surface modifications

Silanization The samples were sonicated in acetone for 5 min, rinsed in methanol and deionized water, and dried with argon gas for cleaning. They were then submerged in 10% (w/v) NaOH for 10 min, rinsed with deionized water, and placed in deionized water for 2.5 min on the shaker. After drying with argon, the samples were placed in 3% (v/v) (3-glycidyloxypropyl) trimethoxy silane (epoxy silane, Sigma) in toluene for 3 min followed by a 5 min wash in fresh toluene. Samples were dried with argon gas, baked for 10 min at 100 °C, and stored in a dessicator prior to the experiments.

Polymer coating N,N-dimethylacrylamide (DMA)–acryloyloxy-succinimide (NAS)–3(trimethoxysilyl)-propyl methacrylate (MAPS) (which will be referred as copoly (DMA–NAS–MAPS)) was synthesized as described elsewhere (Cretich et al., 2004) and stored in a dessicator prior to the experiments. Following the same cleaning procedures described in the silanization method, the samples were cleaned with oxygen plasma for 2 min. The samples were then immersed in a 1:1 mixture of 1% (w/v) copoly (DMA–NAS–MAPS) in water and ammonium sulfate at 40% saturation concentration for 30 min, washed thoroughly with deionized water, dried with argon, and baked for 15 min at 80 °C.

2.5. DNA immobilization, hybridization, and denaturation

All of the oligonucleotides were purchased from Integrated DNA Technologies. The detailed sequence information is listed in the supplementary material (Table S1). All oligonucleotides were prepared to 20 μM in various concentrations of NaPB, KPB, and deionized water, and they were spotted with a bench-top robotic spotter (BioOdyssey Calligrapher Miniarrayer, Bio-Rad). For immobilization of duplex DNA, the oligonucleotides were hybridized in 150 mM NaPB prior to spotting. After the spotting was completed, the DNA microarrays were placed in a humidity controlled chamber (65% humidity) overnight to complete the biomolecule immobilization process. The DNA microarrays were washed the following day with the wash protocol that consisted of four times in 2 × SSC for 10 min, twice in 0.2 × SSC for 1 min, and two rinses in 0.1 × SSC prior to imaging.

The DNA microarrays were hybridized with the target oligonucleotide at 1 μM in 3 × SSC buffer in room temperature under constant agitation. Following hybridization, the DNA microarrays were washed with the same wash protocol described above. It was shown that washing in deionized water can denature the duplex DNA on the surface (Daaboul et al., 2011, Ozkumur et al., 2010). For complete denaturation of the duplex DNA, the DNA microarrays were washed three times in deionized water for 10 min each, followed by a thorough rinse in deionized water.