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Atomic Force Microscopic Studies of Site-Directed Immobilization of Antibodies Using Their Carbohydrate Residues

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Introduction

Control over the deposition of proteins and peptides to solid-phase materials is important in many areas of technology.[1,2] Whitesides and co-workers introduced an experimental system based on self-assembled monolayers with well-defined structures and chemical composition as a model to study the nonspecific adsorption of several representative proteins.[3,4] However, although there are extensive studies on protein adsorption at different surfaces, controlled methods for site-directed attachment have been poorly developed.[5,6] The present paper develops sequentially a "model" system for the site-directed immobilization of antibodies. The oxidation of the antibody carbohydrate residues by periodate is a versatile approach introduced by O'shannessy for the modification of antibodies, which are able to be attached to drugs and labels or chromatography supports.[7,8] Surface derivation of silicon wafers with (3-aminopropyl)triethoxysilane (APTES) in dry toluene resulted in the covalently bonded siloxane film with surface coverage that was relatively controllable by regulating the reaction conditions.[9,10] A simple, convenient, and efficient method for site-directed incorporation of aldehydes generated on the carbohydrate side chains at the C terminal of IgG is explored as a means of ensuring site-directed immobilization of IgG on the APTES treated silicon wafer surface. We used atomic force microscopy (AFM) as an analytical tool combined with enzyme immunoassay (EIA) and X-ray photoelectric spectroscopy (XPS) to investigate the presence, spatial distribution, and antigen binding capacity (AgBC) of antibodies site-directly immobilized onto the APTES-treated silicon wafer surface.

Experimental Section

The silicon wafers (n-type, 4 mm × 4 mm × 1 mm) were cleaned with the standard procedure.[6] Amine group derivatization of silicon wafers was described elsewhere.[9,10] The antibody to hepatitis B surface antigen (anti-HBs) was prepared in our laboratory and used as a model system in the studies, and the oxidation of antibodies was described in another paper.[8] Site-directed immobilization of oxidized

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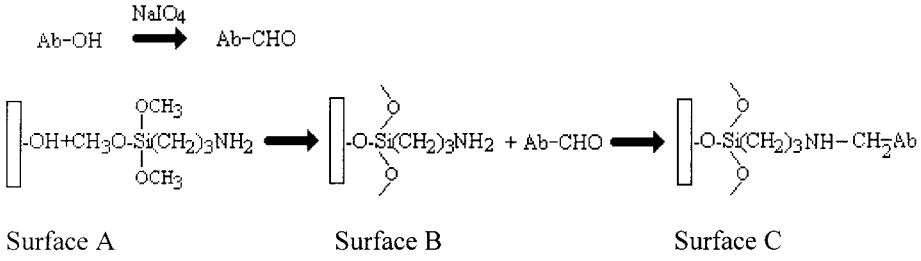
Scheme 1. Steps of the Oxidation of Antibody Carbohydrate Residues by Periodate, the Derivatization of a Silicon Substrate by APTES, and the Subsequent Site-Directed Immobilization of Antibodies



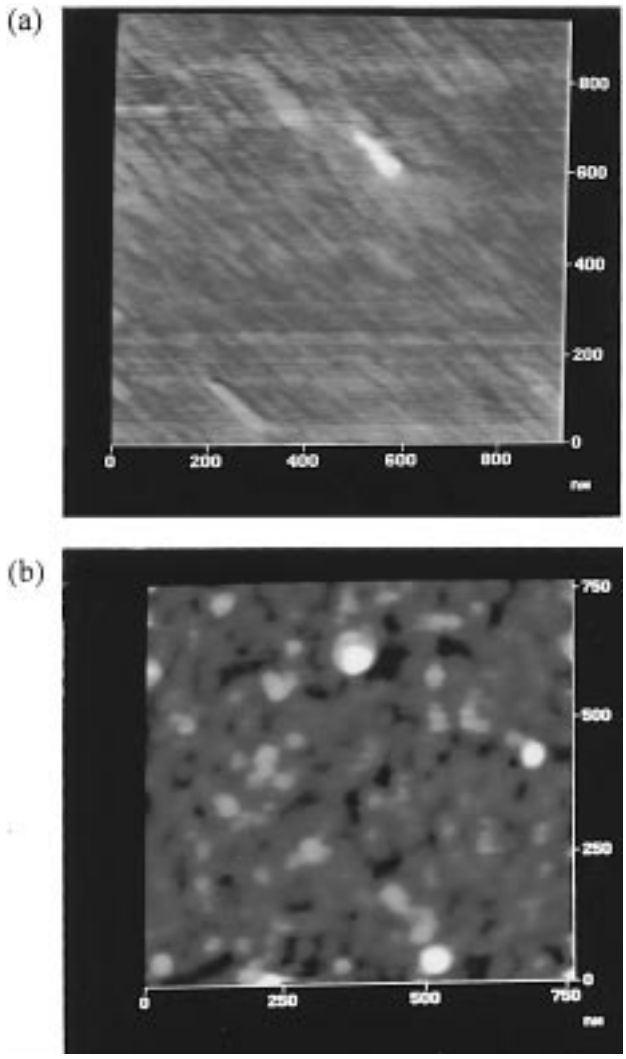


Figure 1. AFM images of (a) a cleaned silicon wafer surface at 800 nm × 800 nm. (b) APTES film on a silicon wafer surface at 750 nm × 750 nm.

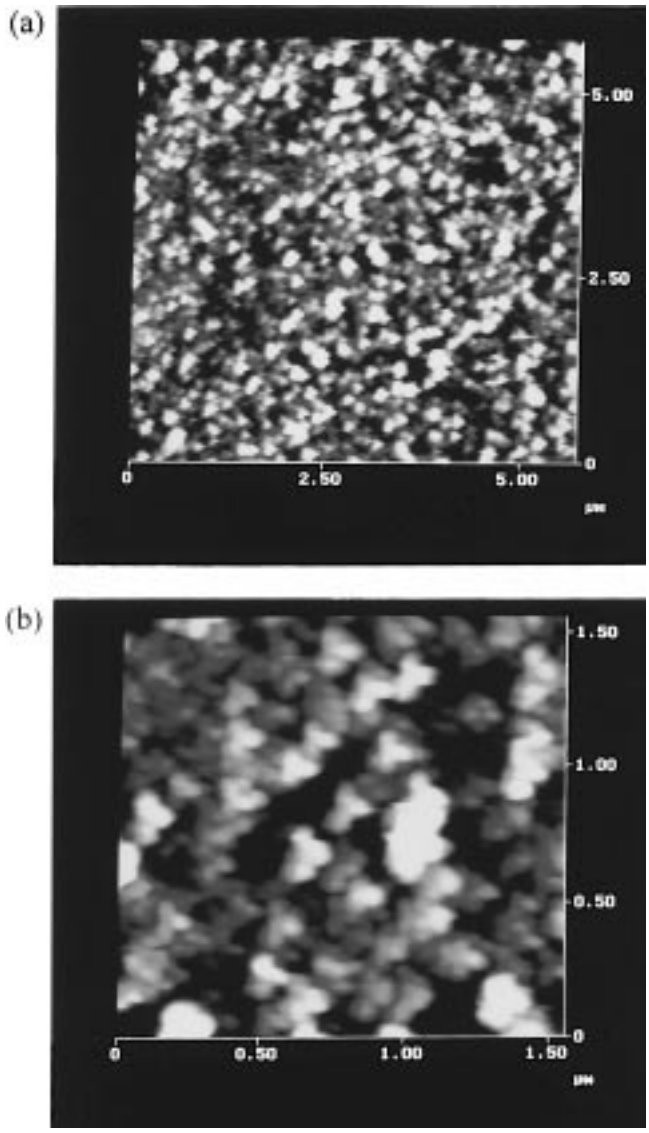


Figure 2. AFM images of IgG antibodies site-directly attached on the APTES film on the silicon wafer surface at (a) 5.0 μm × 5.0 μm and (b) 1.50 μm × 1.5 μm.

antibodies (1 mg/mL) onto the APTES-treated silicon wafer surface was performed in acetate buffer at pH 5.2 containing 5 mM $NaBH_4$ for 12 h at 4 °C. AFM measurements were performed using a commercial system (Nanoscope IIIa, Digital Instruments, Santa Barbara, CA) in the contact mode, and typical forces for all measurements were on the order of approximately 1 nN or less. The presence and AgBC of antibodies site-directly immobilized on the APTES film were also assessed using EIA (CliniBIO 128, Austria) and XPS (Perkin-Elmer PHI 5300 ESCA spectrometer). All experiments were carried out in triplicate.

Results and Discussion

Scheme 1 shows the site-directed immobilization steps in this work: first, silanization of a cleaned silicon wafer (surface A) with APTES in toluene resulting in surface B with terminal reactive amino groups; second, reaction of surface B with oxidized antibodies resulting in surface C.

Figure 1a,b shows the representative images of the cleaned silicon wafer (control) and the covalently bonded siloxane film observed routinely using AFM. The thickness of the organic siloxane layer and the number of reactive NH_2 groups of the above APTES-treated surface had been quantified with XPS, ellipsometry, and [^{14}C]-formaldehyde radiology measurements by Xiao et

Table 1. Assays for the Presence and Specific AgBC of Anti-HBs Antibodies Site-Directly Immobilized

treatment of the surfaces	EIA[a]
surface C	0.32 ± 0.02
surface B (control)	0.01

[a] Results in absorbance units, the result of enzymatic activity of the immunocomplex on surfaces B and C.

al.[9,10] The silanization in dry toluene led to multilayer formation with a thickness of 5.2 nm and a surface coverage of 1.54 nmol of NH_2 groups/cm^2, and the siloxane film had the capacity of site-directly coupling antibodies, with 75% of the amino groups oriented outward from the surface and 25% oriented toward the surface.[9−11]

Figure 2a,b show the typical AFM images of IgG molecules site-directly immobilized onto the APTES-treated surfaces at size 5 μm × 5 μm and 1.5 μm × 1.5 μm, respectively, and both show that some distinct Y-like structure features are observed to form surface patterns, which are proposed to result from the IgG antibodies. Visual inspection of the data suggests that the size of one of the three leaflets is in the range of around 30 nm. From crystallographic data it is known that an IgG antibody (MW 146 000) is arranged in three discrete domains, specifically two Fab fragments and one Fc.[12−14] It is also known that the hinge region between the two Fab domains is extremely flexible, and as a consequence it is usually difficult to predict the exact conformation and hence the size of an IgG antibody when adsorbed to a surface. An estimate of the dimensions as would be observed by AFM can be made from X-ray crystallographic work on isolated Fab fragments[15] and transmission electron microscopy images (TEM),[16,17] giving an expected upper limit to the molecular dimension on the surface of approximately 19 nm. Although those values from AFM are somewhat larger than would be expected, this is not an unusual observation in AFM data, which is proposed to principally result from the effects of a finite probe size and not from deformation of the molecular species due to the imaging forces employed.[18]

EIA results of various surfaces are shown in Table 1. The presence and the specific AgBC of anti-HBs antibodies on surface C were demonstrated. A comparison of the detailed XPS spectra of C 1s, O 1s, N 1s, and S 2p between surfaces B and C will be described in another paper.[11] The results from XPS also proved convincingly that the antibodies were site-directly immobilized onto the APTES-treated silicon wafer surface.

Conclusion

This paper demonstrates that the APTES-treated silicon wafer surface is capable of site-directly coupling antibodies by the aldehydes generated on their carbohydrate side chains. This method emphasizes the utility of the siloxane film for controlling the properties of surfaces in a well-characterized and useful manner that allows the in-depth study of a complex problem: the site-directed immobilization of proteins at solid surfaces without loss of their function.

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