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Weiping Qian [a], Bin Xu [b], Zhendong Song [a], Lei Wu [a], Danfeng Yao [a], Fang Yu [a], Zuhong Lu [a] & Yu Weia [a]

[a] National Laboratory of Molecular and Biomolecular Electronics, Southeast University, Nanjing, 210096, P. R. China

[b] Center for Clinical Laboratory Science, Jiangsu Province, Nanjing, 210009, P. R. China

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[a]*National Laboratory of Molecular and Biomolecular Electronics, Southeast University, Nanjing 210096, P. R. China and* [b]*Center for Clinical Laboratory Science, Jiangsu Province, Nanjing 210009, P. R. China*

A convenient and efficient method for site-directed immobilization of antibodies to silicon wafer surfaces modified by 3-aminopropyltriethoxylsilane (APTES) is reported here. X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and enzyme immunoassay (EIA) were used to characterize the immobilized surfaces.

Keywords: APTES; XPS; AFM; EIA

INTRODUCTION

The immobilization of proteins to solid phase materials is important in biosensor and other fields, and frequently used to confer a specific affinity or enzymatic activity to surfaces[1]. Although manifold immobilization methods have been proposed [2] all these years, some disadvantages always exist, that is, random orientation, nonspecific adsorption and poor reproducibility. Therefor, new site-directed immobilization strategies without the above disadvantages are urgently needed. At present, a convenient and efficient method is presented for oriented immobilization of IgG based on covalent binding between chemical bonds. According to our results, the immobilization of IgG molecules

with controlled geometry and without loss of biological functions is testified.

EXPERIMENTAL

The whole procedure is schematically described in Figure 1. Step 1 illustrates the oxidation of anti-HBs IgG. A 3 mg aliquot of the rabbit IgG was dissolved in 1ml sodium acetate buffer (0.15 M, pH5.2), to which 1ml $NaIO_4$ solution (50mM) was added. After reacting for 1h at room temperature with shaking, unreacted $NaIO_4$ was then separated from the reaction mixture by a separate dialysis bag. Step 2 describes the procedure of silicon surfaces silanization and site-directed immobilization. The cleaning technique and amine group derivatization procedures of silicon surfaces were described in other papers[3]. Site-directed immobilization of oxidized IgG (1mg/ml) to APTES modified silicon surfaces was performed in a sodium acetate buffer (pH 5.2) for 3 days at 4°C.

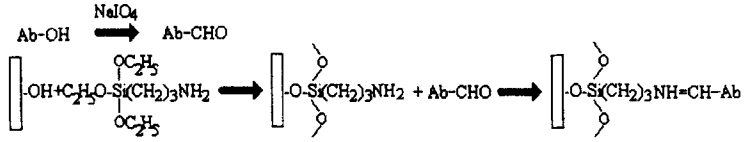

FIGURE 1 The procedure of APTES derivation and site-directed immobilization of rabbit IgG onto the silicon wafer surfaces

The presence of IgG molecules on siloxane films and the retaining of their biology activity were assessed by XPS, AFM, EIA. Samples (4mm×4mm×1mm) were placed in individual compartments of a multiwell plate preincubated overnight at 4°C with 2% bovine serum albumin (BSA) in PBS to block nonspecific adsorption of IgG. 100 μl HBsAg (1ng/ml) was added to each well, and the commercial HRP-IgG test box (monoclone anti-HBsAg antibody attached horseradish peroxidase) was used in a standard detection procedure.

RESULTS AND DISCUSSION

As Figure 1 shows, the site-directed immobilization steps in this work are as follows: first, silanization of a cleaned silicon wafer with APTES results in surface B with terminal amino groups; second, reaction of surface B with oxidized rabbit IgG results in surface C; third, as a contrast, reaction of cleaned surface with oxidized rabbit IgG results in surface A.

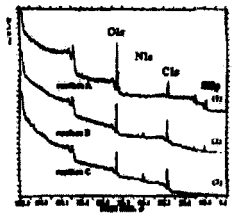

FIGURE 2 XPS spectrum of surface A, B, and C

Figure 2 shows the survey XPS of surface A, B, and C. After the silanization, a N_{1s} peak together with a Si_{2p} peak was detected. When Rabbit IgG was assembled, the intensity of N_{1s} peak (area%) increased from 6.14% to 9.17%, and the Si_{2p} peak disappeared. It is obvious that the enhancement of the content of nitrogen was due to the immobilization of the protein. The depth detected by XPS is limited within 6 nm, so the disappearance of the Si_{2p} peak suggests that the thickness of the organic molecular layers immobilized on silicon wafer surface should exceed 6 nm. For comparison, the spectrum of surface A is also presented. Almost no N_{1s} signals could be detected, while the Si_{2p} signal is obvious. It's sure that to immobilize antibodies on silicon surfaces modified by APTES is much more efficient than just on cleaned surfaces.

AFM images (Figure 3B,C) of surface C reveal that some Y-like structure features are observed to form surface patterns with a geometric size (40-60 nm in fragment diameter) close to that of the fragments of IgG, which are absolutely different from those of the only-APTES-modified silicon surface. As

figure 3A reveals, the siloxane film appears more dense with a smaller thickness (~6 nm). The concentration of freely accessible amino groups on the siloxane film directly effects the next site-directed assembly of antibody molecules.

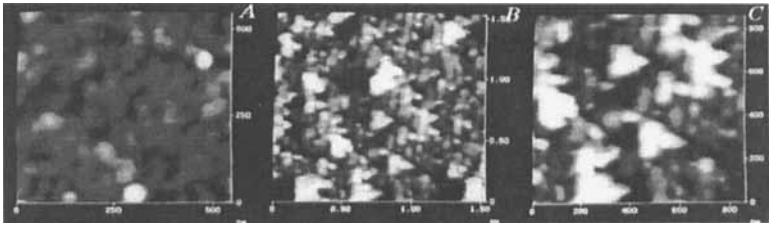

FIGURE 3 AFM images of APTES siloxane film (A) and immobilized IgG [(B) 1.5 μm×1.5 μm and (C) 800 nm×800 nm]

EIA results show that the absorbance (λ=450nm) of aliquots containing site-directed immobilized IgG sample (Surface C) is 0.18±0.02, while the absorbance of control (APTES modified silicon wafer) is zero. This result testifies that not only the IgG molecules were assembled on silicon surfaces, but also their biological functions were kept well.

CONCLUSION

This paper reports a study of the site-directed immobilization of antibodies on silicon wafer surfaces, which is completed by covalent bonds between aldehydes of the oxidized antibody and reactive amine groups of siloxane film. It's found that the antibodies have succeeded to be immoblized on solid surfaces with controlled orientation and without losing biological functions.

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