31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Protein A for Human IgG Oriented Immobilization on Silicon Surface for an Imaging Ellipsometry biosensor

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Abstract—**Imaging ellipsometry biosensor was developed on the basement of imaging ellipsometry for thin-film and surface characterization. Using protein A to immobilize Human IgG on silicon surface for the imaging ellipsometry biosensor immunoassay was studied. As a result, The Human IgG immobilized on silicon surface by protein A bound much more polyclonal antibody than that on hydrophobic surface directly, which indicated that protein A could be used to immobilize Human IgG molecules in a highly oriented manner and Human IgG molecule could maintain its native configuration well on the silicon surface. Furthermore, the absorption quantity of Protein A affecting on interaction of Human IgG and antibody molecules was investigated. The surface concentration of Human IgG and antibody molecules was enhanced as well as the increasing of the absorption quantity of Protein A, but there are no obvious increase when the ratio of surface concentration of Protein A layer to that of saturated adsorption is larger than 80%. However, even decrease slightly.**

I. Introduction

IMAGING ellipsometry is an optical, visualized, reflection-based technique for characterizing thin-film and surface [1]. Ellipsometry biosensor, a biosensor technology, is developed in recent years on the basement of imaging ellipsometry technology to investigate protein adsorption and interactions. Actually, a number of techniques have been employed on this purpose, such as immunoflusrescence, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and surface plasma resonance etc. But these solid-phase methods are always disturbed by conjugated markers or handling with radioactive materials. As a contrast, imaging ellipsometry biosensor is capable of investigating the bio-molecular surface concentration on solid-phase surface directly without any label on the reactant. That makes the imaging ellipsometry biosensor have the possibility of being applied widely in different biological systems. At the same time, imaging ellipsometry is a very sensitive surface characterization method and is suitable for investigating super thin film. Another advantage of imaging ellipsometry is that an ellipsometric analysis of a large surface rather than single point measurement can be made by using a CCD camera. Which made it is possible to develop multi-sensing and high throughout systems basing on it.

Antibody molecule binds to its corresponding antigen with extremely high specificity. Utilizing this high specificity, antibodies has been widely immobilized on various solid-phase surfaces using for purification Protein and diagnostic immunoassays etc. [2]. In this paper, the immobilization of antigen on silicon surface has been investigated. Silicon is a favorable substrate in ellipsometry experiments for it's fine optical surface and high refraction index [3]. Usually, the activity of antibody immobilized directly on the solid-phase surface is less than that of soluble antibody. This may due to the steric hindrance and random orientation of the molecules on the solid-phase surface [4]. To overcome this problem, protein A was used to modify silicon surface and immobilize antibody on it. Protein A has been used successfully to orient the antibodies in a variety of immunoassays, such as magnetic beads, ELISA [5]-[7] and ellipsometry biosensor [8], [9]. The first two are achieved by labeling while imaging ellipsometry is a label-free technique. The feasibility of using silicon surface coated with protein A to immobilize antigen instead of immobilizing antigen directly on silicon surface for imaging ellipsometry biosensor has also been investigated in this paper. Moreover, the absorption quantity of Protein A affecting on the binding between antigen and antibody molecules was studied.

II. Materials and Methods

A. Materials:

Human immunoglobulin G (IgG), antibody of Human immunoglobulin G (anti-IgG), 0.5mg/ml bovine serum albumin (BSA), and protein A were purchased from Sigma (USA). Silicon wafers were purchased from General Research Institute for Nonferrous Metals. Phosphate-buffered saline (PBS: 10mM phosphate, 0.1M NaCl pH 7.2) was prepared in deionized water [8], [9].

B. Preparation of Silicon Wafers:

Cut the polished silicon wafer into rectangles about 0.5cm by 2cm, then immersed them in solution (30% H2O2: 98%H2SO4 = 1: 3(v:v)) for 30 minutes. As well as remove contaminants on the silicon surface, the solution can improve the number of silanol groups on the surface thus making the silicon surface hydrophilic. Hydrophobic surface was gotten

Manuscript received June 20, 2009. This work was supported in part by Science Foundation for Young Teachers of Northeast Normal University (N0. 20070205), and experiments were carried out in National Microgravity Laboratory of Institute of Mechanics, Chinese Academy of Sciences.

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by silylanizing the hydrophilic surface. Rinsed the hydrophilic surfaces with deionized water and ethanol, then immersed them in mixture of dichlordimethylsilane (DMS) and C2HCl3 (20% DMS: 80% C2HCl3=1:10(v:v)) for 5 minutes. After that, rinsed in sequence with ethanol and C2HCl3 and repeated this process for 3 times and kept them in ethanol. For the reaction of dichlordimethylsilane and silanol groups of the surface, a layer of densely packed methyl groups formed on silicon dioxide layer and making the surface highly hydrophobic[8]. Due to the fact that natural silicon dioxide layer and the silanization layer have almost the same optical character and keep invariable during the measurement, both of them are always considered as a uniform background.

C. Immobilization of Human IgG on Hydrophobic Silicon Wafer:

Hydrophobic silicon wafers were immersed into 0.5mg/ml Human IgG solution (diluted by PBS) and incubated for various reaction time, then rinsed with deionized water and dried with nitrogen. Blocked the surface immobilized with Human IgG with 0.5mg/ml BSA solution for 30 minutes, then kept in 0.05mg/ml Human anti-IgG solution(diluted by PBS) for 30 minutes to be totally interacted. Rinsed with deionized water and dried by nitrogen.

D. Oriented Immobilization of Human IgG with Protein A:

Firstly, hydrophobic silicon wafers were immersed in 0.005mg/ml Protein A solution for various reaction time and then rinsed with deionized water. In addition, surface concentration of protein A was measured using imaging ellipsometry. Secondly, the surfaces coated with protein A were blocked with 0.5mg/ml BSA solution for 30 minutes. Thirdly, the wafer was placed into 0.5mg/ml Human IgG solution for 30 minutes and then rinsed with deionized water and dried with nitrogen. The amount of surface-bound Human IgG was also measured using imaging ellipsometry.

To study the binding of immobilized Human IgG, the wafer coated with Human IgG immobilized by protein A was inserted into 0.05mg/ml Human antiIgG in PBS and allowed to incubate for 30 minutes. After rinsed and dried, the amount of surface-bound antiIgG was measured with imaging ellipsometry.

E. Imaging Ellipsometry

Imaging ellipsometry is an optical imaging technology developed on the basement of traditional ellipsometry [10]. Imaging ellipsometry uses a CCD camera to image the ellipsometry response of a larger area on the sample surface while the probe beam of traditional ellipsometer is focused on a small spot on the sample and the reflected light is detected with a single-channel detector, such as a photomultiplier or a photodiode. Thus make it is possible to observe multifunction sample rather than measure single point. Actually, in the process of measuring the thickness distribution of large area layer with imaging ellipsometry, null ellipsometry can't be fulfilled over the entire surface simultaneously for different areas will yield different polarization changes. So both the null and off-null ellipsometric principle should be applied to make the thickness of the layer be proportional to the root square of reflected intensity. Adjust the optical components in the system to fulfill the null conditions on a silicon wafer without adsorbed layers, as well as use the off-null ellipsometric principle to measure the adsorption layer thickness [8], [10], [11]. The absolute thickness of layer could be deduced by a reference layer.

III. RESULTS AND DISCUSSION

A. Human IgG immobilized by the protein A on silicon surface could bind antiIgG effectively

The surface coated with silane was very hydrophobic and Human IgG was immobilized on the surface by physical adsorption. Hydrophobic interaction was major interaction that derived the adsorption of Human IgG. The Human IgG was immobilized on surface coated protein A mainly via the specific binding activity between protein A and Human IgG.

The antiIgG bound with Human IgG immobilized by hydrophobic and protein A surfaces were shown in Fig.1 and Fig.2. [9].

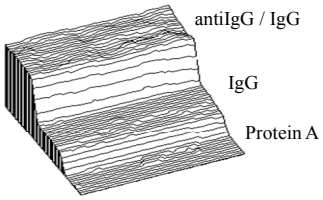

Fig. 1. AntiIgG bound with Human IgG immobilized by protein A on silicon surface

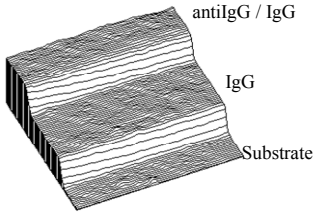

Fig. 2. A antiIgG bound with Human IgG mobilized by physical adsorption on silicon surface.

The Images in three-dimensions were transformed from grayscale format according to the principle that the thin layer thickness is proportional to the root square of reflected intensity in image. As a result, the Human IgG immobilized by protein A on silicon surface bound much more antiIgG than that on hydrophobic surface. That may due to Human IgG molecules immobilized in a highly oriented manner by protein A and thus their functional configuration maintained on the silicon surface. In addition, the reproducibility of Human IgG amount immobilized and the homogeneity of layer adsorbed on silicon surface could meet the requirement of imaging ellipsometry. So it is possible to using protein A immobilizing many kinds of antigens to obtain versatile imaging ellipsometry biosensor.

B. Adsorption Quantity of Protein A Affect on Human IgG Oriented Immobilization on Silicon Surfacer

To investigate the adsorption quantity of Protein A affect on the binding between Human IgG and antiIgG, hydrophobic silicon wafers (Hydrophobic angle 70 degree) were reacted with protein A by immersion in 0.005mg/ml Protein A solution for various lengths of time, and adsorption of Protein A got saturated after 30 minutes. The surface concentration of protein A was obtained by imaging ellipsometry, and results were show in Fig. 3.

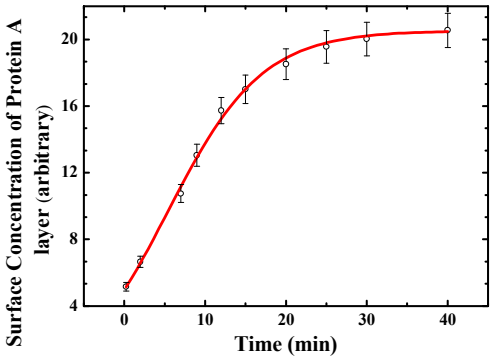

Fig. 3. Adsorption Quantity of Protein A varied with reaction time

After blocked with 1% bovine serum albumin solution (PBS) for 30 minutes, the wafers were placed into 0.5mg/ml Human IgG solution and allowed to incubate for 30 minutes. The amount of surface-bound Human IgG was determined by imaging ellipsometry, results were shown in Fig. 4.

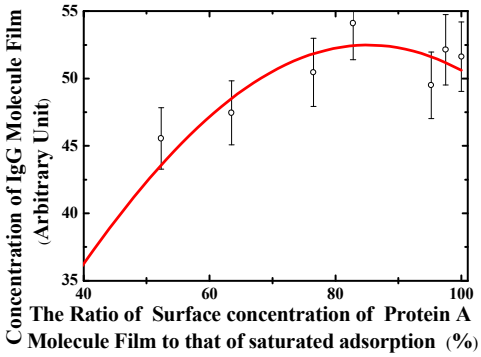

Fig.4. Concentration of Human IgG layer varied with adsorption quantity of protein A

The concentration of Human IgG didn't increase while the ratio of surface concentration of Protein A layer to that of saturated adsorption is larger than 80% and even decrease slightly. That's also the same to the concentration of antiIgG molecule layers bound with antigen as shown in Fig. 5.

Protein A could block the surface to prevent the human IgG adsorb on surface directly, so the human IgG molecule could maintain its native configuration well to bind much more than that on hydrophobic surfaces. But too many Human IgG bound with Protein A may make the space is too crowd to bind tightly and then tended to lose during rinsing. However, the biological activity of the Protein A should also be considered in case of long term experiments.

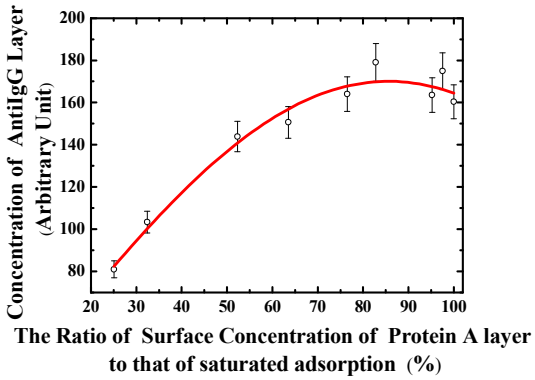

Fig. 5. Concentration of antiIgG layer varied with adsorption quantity of protein A

IV. Conclusions

Using protein A to immobilize Human IgG on silicon surface has been demonstrated. As a result, the Human IgG immobilized by the protein A on silicon surface could bind more antiIgG than antigen physically adsorbed on silicon surface, which indicated that protein A could also be used to immobilize Human IgG molecules in an oriented manner and maintain antigen molecular functional configuration on the silicon surface.

As mentioned above, oriented immobilization of Human IgG by Protein A could increase the sensitivity of the imaging ellipsometry biosensor. Actually, the sensitivity didn't increase while the ratio of surface concentration of Protein A layer to that of saturated adsorption is larger than 80%, however, even decrease slightly. That's to say, there was an optimization region according to the adsorption quantity of Protein A. Carry out experiments in this region is more preferred. On the other hand, the reproducibility of the immobilization and the homogeneity of adsorbed antibody or antigen layer on silicon surface are also very important to obtain higher sensitivity, so that must be considered in the experiments.

However, imaging ellipsometry is a label free detection method so samples could be measured directly without any special treatment, which makes it more reliable, convenient and easy to manipulate. At the same time, it's fast and sensitive enough to detect changes of the layer at a molecular level. Modification surface with protein A for an imaging ellipsometry biosensor has the potential to be further developed for multifunction immunoassay protein chips with high sensitivity.

Acknowledgment

Y. L. Meng thanks G. Jin and Z. H. Wang for their instruction of the experiments.

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