Application of Supported Phospholipids Bilayers for Biosensor based on Imaging Ellipsometry

Y.Y.Chen[1,2], Y.B. Zhang[1,3] , C.X. Wang[1], Z.J. Ding[1], C.H. Huang[1,3] , W.R.Chang[2]*, G. Jin [1,4]*

[1] Institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, Suzhou 215125, P.R. China
[2]Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, P.R. China
[3] Graduate University of CAS, Beijing, 100049 P.R. China
[4] Institute of Mechanics, Chinese Academy of Sciences, Beijing 100080, P.R. China

Abstract—**Application of supported phospholipids bilayers (SPB) is presented for biosensor based on imaging ellipsometry. One SPB is prepared by covalent immobilization of phospholipids monolayer on silicon surface, and followed by adsorption of PEG functionalized phospholipids layer. Another is prepared by adsorption of the mixture of phospholipids and PEG functionalized phospholipids on the hydrophilic silicon surface. Results show that obvious resistance to non-specific protein adsorption on SPB is obtained, and proteins covalently immobilized on the bilayers by the terminal carboxyl group of PEG remain higher bioactivity than that immobilized on silicon surface directly.**

Keywords—**imaging ellipsometry, supported phospholipid bilayers, biosensor, microfluidic array.**

I. Introduction

The concept of biosensor based on imaging ellipsometry is reported in 1995 [1]. As the visualization method of biosensor, imaging ellipsometry which combines the power of ellipsometry with microscopy [2] provides a larger field of view for the biosensor (several square centimeters) with a high spatial resolution in the order of micron (laterally) and sub-nanometer (vertically), which makes the biosensor convenient to detect multiple analytes in a microarray simultaneously. Another interesting feature of the biosensor is label free for biomolecule detection, which avoids problems caused by the use of labeling like radioactive isotopes or fluorophores [3]. However, one drawback of the biosensor is that it can not distinguish specific binding and non-specific adsorption. It is necessary to develop a special surface modification to minimize the non-specific binding.

Supported phospholipids bilayers (SPB) at interfaces have actually been shown a good validity in preventing protein adsorption, and have the potential to make the biosensor be developed into highly selective devices for a variety of biological analytes [4-5]. Here the application of two SPB on silicon surface is studied for the biosensor based on imaging ellipsometry. One SPB is prepared by covalent immobilization of a supported phospholipids monolayer on silicon surface, and followed by incubation with PEG functionalized phospholipids in aqueous solution. Another SPB is prepared by adsorption of the mixture of phospholipids and PEG functionalized phospholipids in aqueous solution on the hydrophilic silicon surface.

II. Experiment and discussion

A. Experimental section

Materials: Chemicals used for the buffer preparation were all of analytical grade. Human immunoglobulin G (hIgG), goat anti-hIgG serum, fibrinogen (Fib), anti-Fib, Human serum albumin (HSA) and Bovine serum albumin (BSA) were obtained from Sigma. Fetal bovine serum (FBS) was purchased from Hyclone (USA). L-α-phosphatidylethanolamine (PE) and Tween 20 were purchased from Sigma-Aldrich. 1,2-Diacyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy (Polyethylene glycol)-2000] (CH_3-PEG-PE) and 1,2-Distear oyl-sn-Glycero-3-Phosphoethanolamine-N-[Carboxyl (Polyethylene Glycol)-2000] (COOH-PEG-PE) were purchased from Avanti Polar Lipids. 1,2-Dimyristoylphophatidylcholine (PC) was purchased from Lipiod. All lipids were stored at -20 ℃ until use. Dichlordimethylsilane, N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-diethyl-aminopropyl) carbodiimide (EDC) were from Acros. Milli-Q ultrapure water with a resistivity ~ 18 MΩcm (Millipore, Molsheim, France) was used for all the solutions. Other chemicals used were analytical grade or better.

Imaging ellipsometry: The experiments were carried out with an imaging ellipsometer developed in our laboratory. As an enhancement of traditional ellipsometry, imaging ellipsometry used a CCD camera to image the ellipsometry response of a larger area sample with lateral distribution in different layer thickness, and the result was grabbed as a digital image and stored in a computer with a grayscale format (8 bits, 0–255 grayscale) for further evaluation by an

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image-processing program. Once imaging ellipsometer was fixed, the detected intensity "I" (grayscale) was the function of the layer thickness (d), namely I=f(d), where f() denoted the function relationship which was determined by layers with known intensity and thicknesses, then the unknown thickness of protein layer could be deduced from the detected intensity according to the function.

Microfluidic system: For the fabrication of SPB and the reaction of SPB with protein, a micro-fluidic system [6] in PDMS was used, which includes an 8×6 cell array. When the cell array was attached to the silicon slide surface, 48 individual chambers were formed independently and 48 elliptic spots in the array were patterned onto the silicon surface. Each chamber had two access holes where solution could pass in and out of the chamber through them. By such a micro-fluidic system, different molecules were delivered individually to each spot of the array and immobilized on silicon surface simultaneously. Here it was used for patterning SPB or proteins on silicon surface, transporting of sample solution, reaction of molecular and rinsing.

B. RESULTS AND DISCUSSION

SPB by covalently immobilized on silicon surface:

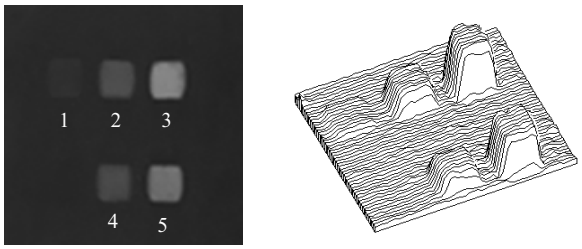
Here SPB consisted of two different phospholipids. One was PE covalently immobilized on silicon surface to form a monolayer, another was PEG functionalized PE adsorbed on the monolayer by hydrophobic interaction to form a SPB. With procedures in Ref.[6], aldehyde groups were introduced on the silicon surface that reacted with amino groups of lipids.

SPB was prepared in the microfluidic system. PE solution(3 mg/ml with the addition of 0.5% Tween 20) was delivered to the silicon surface through the microfluidic system and PE was covalently attached on surface by an imine linkage formed between the aldehyde group on surface and the amine group of PE. After 30 min incubation, buffer was used to rinse the surface, then PEG functionalized PE solution (3 mg/ml)was delivered and incubated with PE monolayer for 30 min to form SPB. Finally SPB was successively rinsed with buffer and purified water.

The ability of SPB on silicon surface to resist non-specific protein adsorption from the aqueous solutions was determined. The PEG grafted on PE is inert, which can avoid protein attaching covalently on bilayer. Fib(1mg/ml), HSA(1mg/ml) and hIgG(1mg/ml) solutions were injected across SPB, respectively. After 30 min incubation, none of the proteins explored caused non-specific adsorption on the bilayer measured with imaging ellipsometry. The adsorption of biomolecules on the bilayer from two more complex solutions, anti-hIgG(10%) and FBS(10%), were studied with imaging ellipsometry, also no adsorption occurred on the bilayers. These results were in agreement with that in other work [7], which indicated that SPB was quite resistant to nonspecific protein adsorption.

hIgG/anti-hIgG binding was employed as a model of antigen-antibody interaction on SPB (the first layer was also PE monolayer and the second layer was COOH-PEG-PE). hIgG solution (0.1mg/ml) was pumped across the bilayers, and then hIgG would be immobilized covalently on surface by stable carbamate bond formed between carboxyl group of PEG chain's end and amino group of hIgG. After incubation for 30 min, BSA solution (10mg/ml) was injected across the bilayers for 30 min. For comparison, hIgG was immobilized covalently on the silicon surface with aldehyde groups directly. Furthermore 10% anti-hIgG in PBS buffer was pumped across hIgG layers for 30 min and rinsed with PBS buffer and purified water. All experiments mentioned above were done on one single silicon slide with the microfluidic system. After dried with a nitrogen blow, the silicon slide was measured with imaging ellipsometry and the result was shown in Fig. 1

The thicknesses of protein layers were calibrated by a traditional ellipsometer (SE400, SENTECH, Germany) equipped with a He-Ne laser (λ=632.8nm, the angle of incidence was 70°). The thickness of SPB-hIgG layers (4.4 nm) was lower than that of hIgG layer (5.0 nm) on silicon slide, but more anti-hIgG (5.7 nm) bound on SPB-hIgG layers than that (4.6 nm) on silicon slide. We repeated the experiment many times and almost same results were obtained, which showed that hIgG molecule attached on phospholipids bilayers can maintain higher bioactivity.



A B

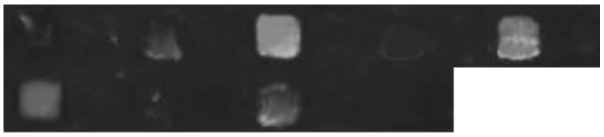
Fig.1. Silicon slide with 5 spots patterned different layers shown in grayscale image (A) and the corresponding thickness distribution image (B). Spot 1 SPB layers, spot 2 SPB-hIgG layers, spot 3 SPB-hIgG/anti-hIgG complex layers, spot 4 hIgG layer, and spot 5 hIgG/anti-hIgG complex layers.

SPB by adsorption on the hydrophilic silicon surface:

In this section, SPB was formed with small unilamellar vesicles (SUVs) fusion on hydrophilic silicon surface. The brief procedure of SUVs preparation was as follows. COOH-PEG-PE was mixed with PC in chloroform. The solvent was then evaporated under a stream of nitrogen followed by desiccation under vacuum at least for 2 hours.

The thin lipid film on the wall of vial was rehydrated in PBS (pH 7.4). The solution was clarified by water bath ultrasonic and extruded at least 11 times through a polycarbonate filter, which had an average pore size of 50 nm.

HSA was chosen as a model ligand to couple to PC/COOH-PEG-PE membrane by activating free carboxyl termini of PEG with NHS/EDC method. With the same procedure of the above section, the non-specific adsorption inhibition and protein bioactivity were studied. Also, the relation between ligand coupling efficiency and the molar content of COOH-PEG-PE was examined, as shown in Fig.2.



A: 1 2 3 4 5
B: 1 2 3 4

Fig. 2 Silicon slide with 9 spots patterned different layers shown in grayscale image. Spot A: 1 Vesicles control (containing 1.5 mol% PE-PEG-COOH). 2 Vesicles containing 0.5 mol% PE-PEG-COOH without NHS/EDC activating. 3 Vesicles containing 0.5 mol% PE-PEG-COOH with NHS/EDC activating. 4 Vesicles containing 1.5 mol% PE-PEG-COOH without NHS/EDC activating. 5 Vesicles containing 1.5 mol% PE-PEG-COOH with NHS/EDC activating. Spot B: 1 negative control. 2 Vesicles containing 4.5 mol%PE-PEG-COOH without NHS/EDC activating. 3 Vesicles containing 4.5 mol%PE-PEG-COOH with NHS/EDC activating. 4 blank control. HSA and anti-HSA were introduced into each spots after forming phospholipid membrane on silica surface (A3, 5 and B3 activating with NHS/EDC, and others were not).

After bumping HSA and anti-HSA across the lipid membrane, there was no obvious grays increase for those lacks of carboxyl activation (as shown in A2, 4 and B2), which illustrated the resistance of SPB to nonspecific adsorption of protein. Moreover, the higher the molar concentration of PEG is, the better the inhibition of SPB to nonspecific adsorption. For those with NHS/EDC activating (as shown in A3, 5 and B3), the amount of protein coupled on SPB was decreasing with the increase of the molar fraction of COOH-PEG-PE. Obviously, there is a trade-off between the better inhibition to nonspecific adsorption of protein and the higher protein bioactivity for the molar concentration of PEG. Here, the protein could be well immobilized and the nonspecific adsorption of protein was reduced below the detection limit when the molar concentration of PEG was as high as 1.5 mol%, which meant it could be used for the biosensor based on imaging ellipsometry.

III. Conclusions

SPB was fabricated on silicon surface for biosensor based on imaging ellipsometry, which was highly protein resistant. Moreover, proteins could be immobilized covalently on the bilayers by the terminal carboxyl group of PEG chain's end, which its bioactivity was higher than that immobilized on silicon surface directly. It showed the biosensor with the potential to be further developed into a sensitive immunoassay technique.

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Corresponding author: G. Jin

- Institute: Institute of Mechanics,
- Street: Bei-si-huan west road
- City: Beijing 100190
- Country: P. R. China
- Email: gajin@imech.ac.cn

Co-corresponding author: W.R. Chang

- Institute: Institute of Biophysics

- Street: 15 Datun road, Chaoyang district
- City: Beijing
- Country: P. R. China
- Email: wrchang@sun5.ibp.ac.c