Proceedings of the 25th Annual International Conference of the IEEE EMBS
Cancun, Mexico • September 17-21, 2003

Immune-Microassay with Optical Proteinchip for Protein Detection

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Abstract—**An optical proteinchip upon antigen-antibody affinity and the chip analysis system have been used in immune analysis. The chip is processed with chip design, surface patterning, surface modification, ligand immobilization to form a microarray with many immune probes - one kind of antigen (or antibody) occupying one unit in the microarray and many such units in matrix. In micro-reactor of bio-molecule interaction, when ligand met its receptor, they would bind into bio-specific complex, which resulted in the thickness of the probe surface layer (surface concentration of antigen-antibody) increasing. The variation of protein pattern on the chip surface corresponding to the ligand immobilization and protein interactions was screened with optical imaging ellipsometry. A non-labeling micro-assay for protein detection was realized with antigen-antibody affinity and non-disturbance optical sampling, which was available for the direct test of crude samples, such as serum, etc. Some application results in immune analysis were presented also.**

Keywords—**Optical proteinchip, Imaging ellipsometry, Antigen-antibody affinity**

I. Introduction

The optical ProteinChip was developed recently [1, 2]. The immune-microassay with the optical Proteinchip for antigen or antibody detection and its applications are demonstrated here. In order to approach to the immune-microassay, the Proteinchip and its analysis system is simply mentioned at first.

For the optical protein-chip, the substrate of the chip is treated by photolithography or "soft" lithography technique in order to create patterned surface [3, 4]. Each area of the pattern is corresponding to one bioactivity with an antigen (or antibody) immobilization, and the interval between the areas is non-bioactive and optically extinctive to form a microarray. In this case, each area of the protein-chip may function as an immune-probe. When the antigen (or antibody) in the solution interacts with its corresponding antibody (or antigen) on the microarray surface and assembles into complex upon their affinity. The layer on the surface where the interaction takes place becomes thicker (or surface concentration higher). A significant increase of the attached layer thickness (or surface concentration) indicates that the solution contained the antibody (or antigen). The imaging ellipsometry is used to detect the protein layer pattern on the microarray surface. All the lateral thickness (or surface concentration) distribution of protein layer pattern would be detected at the same time, which might indicate the existence of the antibody(s) in the tested solution for immune tests.

The advantages of the proteinchip are: 1) a multi-test parallel performed simultaneously; not only for one kind of antibody or antigen, but also for many kinds of antibody (or antigen) available at the same time. 2) labeling free; no disturbance to bioactivity of analytes, the crude samples, such as serum, tested directly. 3) in-situ and ex-situ test available for immune analysis; the function of real-time analysis for antigen-antibody interaction supplying some important kinetic data such as the interaction rate and conditions, etc. 4) less consume of analytes; 5) quantitative test with calibrations.

II. Methodology

The proteinchip technology includes several sections: 1) the chip design, which depends on the analytes since each protein has its individual properties; 2) the chip substrate is patterned for numbering areas in microarray; 3) the ligand immobilization, chemical or biochemical surface modification, and covalent or non-covalent antigen or antibody adsorption for each area; 4) the reactor for antigen-antibody interaction; 5) the microarray testing as an immune microassay; 6) the database corresponding to antigens and antibodys for further analysis, etc.

The testing system with imaging ellipsometry has been mentioned in details somewhere else [5]. It has the ability to test the sub-monolayer of proteins, and the thickness resolution reaches the order of 0.1 nm.

The evolution of the chip looks like: (A) A solid substrate of the biochip is prepared with the technique of photolithograph, similar to which used for micro-electronics elements, to create patterned areas and each area isolated with shading background, that is non-bioactive and optically extinctive. Each area surface of the pattern is atom-graded flat like a mirror, and its surface is chemically and/or biochemically modified for ligand immobilization. (B) The ligand is immobilized with physical adsorption, self-assembly or covalent binding to form a sensing surface and the specific binding sites of ligand exposing to the upside. Various kinds of ligands are pre-attached on each area individually to form many immune-probes in microarray on the proteinchip surface. (C) After the interaction between antigen-antibodys, the molecule complexes appear in the pattern surface corresponding to analytes in the solution tested. (D) The sampling is performed with the imaging system of in-situ or ex-situ availability. The results are finally grabbed and stored in a computer for further analysis, furthermore communicated with the database of antigen-antibody for immune estimation.

For the in-situ sampling, the interaction process between the analytes in solution and the ligands on the surface of patterned biochip can be visualized in one kind of reaction cell specially designed. The cell has a small volume containing the solution and two optical windows for the probe light beam of imaging in and out. The normal of the two windows is parallel to the incident direction and the reflection direction of the probe light. The windows with

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optical quality are transparent for the incident light. The solution could be poured into the cell and pumped out by a micro-fluidics system.

The chip is inserted into the cell. When the solution containing the analytes is poured into the cell, the analytes in the solution will react with their corresponding ligands on the area surface of the chip according to the affinity of protein interaction, so that the thickness (or the surface concentration) of sensing layer in patterned areas increase. The variation process of the layer thickness (or the surface concentration) can be grabbed in a series of images with time. Furthermore, it is possible to obtain kinetic information for the interactions from a digital image processing.

III. Results and Discussion

A. Micro-immuno-array of proteinchip

A silicon wafer surface was treated with soft photolithography into 12 square areas (0.5 x 1.5 mm^2) separated by the width of 1 mm of shading background. It was modified with dichlordimethylsilane to prepare for ligand immobilization. There were four kinds of proteins in triplicate immobilized in the areas individually, which was human fibrinogen (Fib), monoclonal antibody for hepatitis B surface antigen (antiHBsAg), human immunoglobulin G (IgG), and human serum albumin (HSA). Eight square areas were incubated in a serum mixture containing antiIgG, antiFib, antiHSA and HBsAg, which was pumped by the microfluidic, flowing through each square area in turn. After reaction, the proteinchip was detected with imaging ellipsometry. The result was shown in Fig. 1, some significant increases of the layer thicknesses appeared in the square areas exposed to the serum in the microfluidic in series.

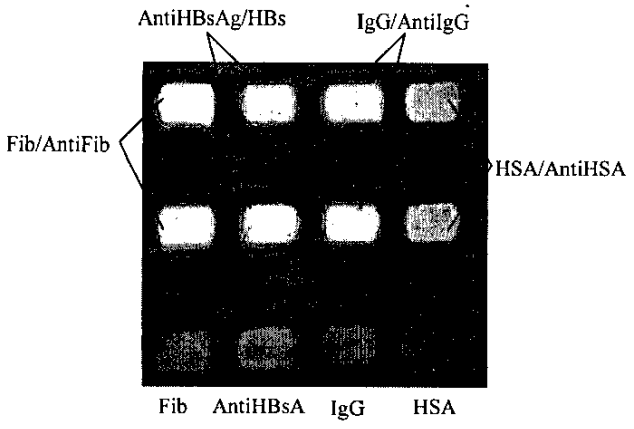

Fig. 1. Micro-immuno-array on proteinchip for detection of antiFib, HBsAg, antiIgG, and antiHSA.

The images sampled are shown in light intensity distribution, which correspond to the sensing layer thickness distribution or the surface concentration of proteins. It is capable of proving that

$$Th(x,y,t) \propto \sqrt{I(x,y,t)} \qquad (1)$$

the thickness Th(x,y.t) of the protein layer is proportional to the square root of the intensity I(x,y,t) [6]. In this way, the thickness distribution can be shown in three dimensions. Further, the surface concentrations were calculated according to the following equation: Surface concentration ($\mu g/cm^2$) $\approx$ K×d (nm), where K≈0.12 [7].

B. Detection for multi-dynamic-process of protein interactions in the microarray of proteinchip

The detection results for protein binding according to their affinities could be obtained on the proteinchip. During the process of the binding, the dynamic processes of protein interactions became observable also. For the purpose of real time observation of multi-protein interaction processes, a similar chip with four kinds of ligand immobilized was prepared on the patterned surface. The chip was inserted into the cell containing solution for in-situ experiments and the chip surface with sensing areas was clearly observed. Then the mixture of antiserum containing antibodies of antiIgG, antiHSA and antiFib, etc. was poured into the cell. Several binding processes between antigens on the surface and antibodies in the solution were observed simultaneously, which corresponded to the affinities of IgG-antiIgG, HSA-antiHSA, and Fib-antiFib, etc. respectively. The binding processes resulted in the layer thickness on the corresponding sensing areas increasing with time. Fig. 2 just shows a series of thickness variation with time for protein binding processes. These were deduced from the average thickness of corresponding sensing areas during the in-situ experiments. We followed the relationship between the intensity I(x, y. t) and the thickness Th(x, y, t) of protein layer, and the thickness is proportional to the protein surface density. So the thickness variation means the protein binding to the surface on their affinity. Further observation showed that the binding rate for different protein couple was different from others, even though in the same experimental condition.

The areas where the antigen and antibody reacted into their complexes were chosen for the quantitative result of the interaction process in real-time visualization. The statistical average thickness (in arbitrary unit) over the IgG, HSA and Fib areas are given in Fig. 2. The vertical is the thickness of the sensing layers assembling into complex layer in arbitrary unit, and the horizontal is the time in logarithmic expression. There are two interaction rates for the three binding processes, below and over 100 s being seen obviously.

The antibody molecules diffused to everywhere of the cell to reach a uniform distribution of molecule concentration owing to a concentration diffusion, when the solution with antiserum was poured into the reaction cell containing the solution without antiserum before. Some antibody molecules diffusing to the sensing surface reacted with the corresponding antigens on the surface binding into their complexes. This was corresponding to the process below 100 s. The diffusion rate that controlled the binding rate is the capital phenomenon. Then the concentration distribution of

antibody in the cell reached uniformity. The interaction with a higher rate occurred on whole the sensing surface over 100 s. It showed that the interaction rate is different for the three binding processes. The binding rate between Fib and antiFib is faster than the others, and the slowest one is between HSA and its antibody. Here, only a sketchy description for the protein interaction processes detected simultaneously is presented as a demonstration. The further theoretical analysis and deduced results will be presented in details in other papers.

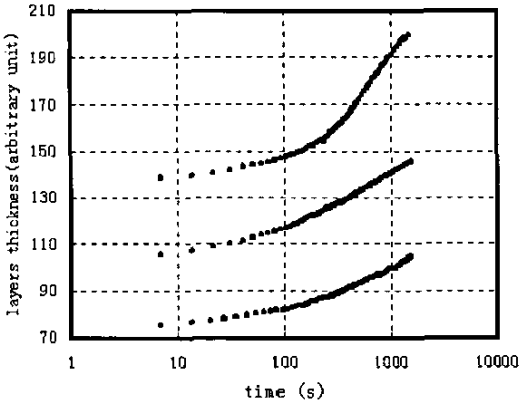

Fig. 2. Fib-antiFib, IgG-antiIgG and HSA-antiHSA binding processes

IV. Conclusion

The above demonstration of micro-immuno-assays of proteinchip for multi-protein detection shows that the proteinchip is a potential way for multi-protein analysis. It has advantages in simple operation, labeling free, and simultaneous multi-channel analysis.

Acknowledgment

The national natural science foundation of China (No. 60178033, No. 90206029) and Chinese Academy of Sciences are acknowledged for their supports.

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