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RESEARCH PAPER

Application of Optical Protein-chip in Detecting Phage M13KO7

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Abstract: Avidin layer was bound on the substrate surface of Silicon wafer modified with aldehyde. The interaction between avidin and biotin was adopted for the immobilization of mouse monoclonal biotin-anti-M13 (antibody GP3)-labeled biotin. The surface was incubated in a solution containing phage M13KO7, which was trapped by the antibody GP3 with the interaction between phage M13KO7 and antibody GP3, resulting in a variation of layer thickness that was detected by imaging ellipsometry. The results showed a saturated layer of antibody GP3 with a thickness about 6.9 nm on the surface of the silicon wafer. The specific interaction between phage M13KO7 and antibody GP3 resulted in a variation of layer thickness. The layer of phage M13KO7 bound with antibody GP3 was 17.5 nm in the concentration of 1.1×10^{10} pfu/mL. Each variation of the layer thickness corresponded to a concentration of phage M13KO7 in the range of 0.1×10^{10} – 2.5×10^{10} pfu/mL, with the sensitivity of 10^9 pfu/mL. Compared with other methods, the optical protein-chip, requiring only short measurement time, label free, is a quantitative test, and can be visualized. This study could be significant on the interactions between the antibody and the virus, showing potential in the early diagnosis of virosis.

Key Words: optical protein chip; imaging ellipsometry; phage M13KO7; antibody GP3

Virus is a noncellular living form that occupies an intermediate position between living and nonliving forms, with considerable harmful effects on animals, plants, and humans. According to statistics, viral diseases in plants have resulted in annual global agricultural losses that amount to 20 billion U.S. dollars. HIV and SARS epidemics have had considerable harmful effects on human health. A deeper understanding of diversity and specific interactions between the antibody and the virus is of significance in the early diagnosis of virosis, the evaluation of drug curative effect, the identification of drug target, the confirmation cure criterion, and the details of biological mechanism. At present, there are three kinds of methods available to detect virus, namely, microbiological culture, polymerase chain reaction (PCR), and

immunology method. The standard laboratory diagnostic tests for viruses are based on their culture. Although the microbiological culture methods are the pioneer, they have disadvantages such as critical operation techniques, increased time-consumption, and high complexity with regard to the equipment. Therefore, this method is not suitable for the detection of virus. The PCR method is very sensitive, but is subject to contamination. Routine immunology methods, such as enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) cannot directly detect virus, and detection of protein occasionally results in the destruction of the sample, thereby giving false positive values. Surface plasmon resonance (SPR) method requires expensive equipment. Quartz crystal microbalance (QCM) can directly

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detect virus; however, it requires critical operation techniques and conditions. In 2003, Saju R *et al* put forth the idea of using atomic force microscopy (AFM) in virus detection. Using AFM an image of the interaction could be obtained; however, it required expensive equipments, the scanning speed was low, and required a complicated sample preparation process^[1]. As an immunological method, the protein chip is a protein analysis technique with properties of parallel, rapid, automatic detection, identification, and purification of protein. At present, it is more widely used; for example, in the interaction between antigen and antibody, identification of antibody, interaction between the cell factor and receptor, detection of hormone, and so on^[2–7]. Optical protein chip is a kind of biomolecular identification and detection technique that combines high spatial resolution imaging ellipsometry with a protein chip that exhibits multiple bioactivities of proteins^[8,9]. After modifying the substrate of Silicon wafer with –CHO, suitable concentration of the compound containing the chemical group is immobilized on substrate. The protein is then covalently and orderly bound on the surface of silicon wafer, which involves a Schiff-base reaction with –CHO. Following this treatment, the surface of silicon wafer becomes a biosensing surface. Corresponding proteins existing in a solution can be detected with the specific interaction between proteins bound on the surface and the target proteins, which results in formation of an image using imaging ellipsometry that is further analyzed using professional software. The technique can directly analyze protein, virus, germ, and even eukaryotic cell. This technology does not require the use of labels, pretreatment, and purification; moreover, it requires a small quantity of the sample, short test time, and can simultaneously analyze multiple proteins^[10]. In 2005, Bae YM *et al* detected *Salmonella typhimurium* using ellipsometry^[11]. The sensitivity was 3.2×10^6 CFU/mL, which showed the advantage of ellipsometry in germ detection. However, detection of virus using optical protein-chip has not yet been reported.

Phage M13KO7 is a kind of virus that resides in *E. coli*. The virus has no effect on humans and animals. Its size, structure, and immune mechanisms are similar to viruses that affect humans; so the model detecting this kind of phage not only ensures experimenter safety but also helps in researching the feasibility of virus detection using optical protein-chip, which has key significance in exploring new tools in diagnosing virosis at an early stage, and thereby, explain the biological mechanism of virosis. The avidin-biotin system is a routine method that is used with ELISA, RT-PCR, biosensor, and so on, for binding antibody^[12–15]. In this study, the method was initially used with optical protein-chip for binding antibody GP3. Avidin was covalently immobilized on the silicon wafer modified by aldehyde. The antibody GP3 was bound by the interaction between avidin and biotin. If the solution contained the corresponding target (phage M13KO7)

that specifically interacted with the antibody GP3 bound on the silicon wafer, phage M13KO7 and antibody GP3 formed a complex causing variation in the layer thickness. Detection of the variation in layer thickness using imaging ellipsometry could achieve the purpose of phage M13KO7 detection.

1 Materials and methods

1.1 Materials

Silicon wafers were purchased from Luoyang Monocrystalline Silicon Factory, China. 3-aminopropyltriethoxy silane (APTES 99 %, V/V) was purchased from ACROS. H₂O₂ (30 %), H₂SO₄ (98 %), and absolute ethanol were purchased from Beijing Bei Hua Fine Chemicals Company, Ltd. Tween 20, avidin, and Glutaraldehyde (GRA 50 % photographic aqueous solution) were purchased from SIGMA. Phosphate-buffered saline: PBS buffer (10 mmol/L phosphate, 0.1 mol/L NaCl pH 7.2). Deionized water (resistivity 18.3 MΩ·cm) was produced by ion-exchange demineralization. Antibody GP3 was purchased from Exalpha Biologicals. The solution containing phage M13KO7 was provided by the Institute of Biophysics, Chinese Academy of Sciences, China.

1.2 Methods

1.2.1 Polished silicon-wafer surface modification: The silicon wafers were cut into rectangles 20 mm × 5 mm and cleaned in deionized water. The wafer surface was incubated in solution (30 % H₂O₂: 98 % H₂SO₄ = 1:3 V/V) for 30 min on a shaking table. After being rinsed in deionized water and absolute ethanol thrice, the washed surfaces were incubated in a mixture of APTES and absolute ethanol (APTES: absolute ethanol = 1:10 V/V) for 2 h on a shaking table. Following this, they were again rinsed in absolute ethanol thrice. After rinsing in PBS thrice, the washed surface was incubated in a mixture of GRA and PBS (GRA:PBS = 1:15 V/V) for an hour on a shaking table. After being rinsed in PBS thrice, the silicon wafers were stored in PBS.

1.2.2 Immobilization of antibody GP3: Avidin was covalently immobilized on the surface of a modified silicon wafer due to the reaction of the Schiff base with –CHO. Four areas on the chip were chosen to immobilize avidin. Avidin was assembled and formed saturation avidin layer on the surface of the modified silicon wafer. The surface was then blocked with avidin. Avidin concentration was 0.5 mg/mL, and reaction time was about 15 min. The antibody GP3 was bound by the interaction between avidin and biotin. Antibody GP3 concentration was 0.1 mg/mL, and reaction time was 10 min; it was fully bound on the avidin layer as the sensing surface.

1.2.3 Detection of phage M13KO7: Two areas were chosen to detect phage solution, and two were used as control areas. The solution volume of 50 μL was used in each sensing area. Reaction time was 40 min for full-interaction antibody GP3 and phage M13KO7. The chip surface was rinsed with

deionized water to remove the phages that did not bind the antibody. After blowing with nitrogen, the result was read and analyzed using imaging ellipsometry.

1.2.4 Imaging ellipsometry: Imaging ellipsometry is a novel type of display technique for ultra thin film and surface characterization, which was developed several years ago. The technique has the capacity to detect ultra thin and transparent phase objects. A high spatial resolution in the order of microns (laterally) and subnanometers (vertically) can be obtained using this technique. The incident wave of polarized light as the probe beam irradiates the sample was modified, which makes the reflective or transmission beam carry sample information, for example, information such as protein-layer thickness. When imaging ellipsometry detects sample-layer thickness, the value of reflection intensity is presented in gray scale. The variation of protein-layer thickness causes changes in gray-scale value (change in reflection intensity). Assuming that the refractive index was invariable over the range of 0–30 nm of layer thickness, the gray-scale value will be directly proportional to the square of the protein-layer thickness, $I = Kd^2$, where I is the light intensity and d is the layer thickness^[16]. In this manner, the gray-scale value in the image directly reflects the layer thickness (or surface concentration) of protein or virus bound on the silicon wafer.

The thicker the protein or virus layer, the higher the gray-scale value will be. The results can transform the variation of gray-scale value to the corresponding thickness distribution in three dimensions to visualize the variation of protein layer.

2 Results and discussion

2.1 Immobilization of antibody GP3

Biotin and avidin can specifically interact, their affinity about 10^{15} L/mol. Each avidin molecule has four interaction sites for biotin. Even when biotin combines with high-molecular-weight compound, it can be still identified by avidin. The interaction between avidin and biotin has been used to immobilize protein in many studies. Fig. 1A shows the scheme for immobilization of antibody GP3 on the silicon surface. Avidin reacts with –CHO on the surface of a modified silicon wafer and forms the avidin layer. The antibody GP3 is immobilized on the avidin layer and forms the antibody layer due to the interaction between avidin and biotin. Fig. 1A shows the antibody GP3 layer on the surface. From the image obtained using imaging ellipsometry, the antibody GP3 formed a saturated layer and covered the entire surface of areas with a layer thickness of about 6.9 nm.

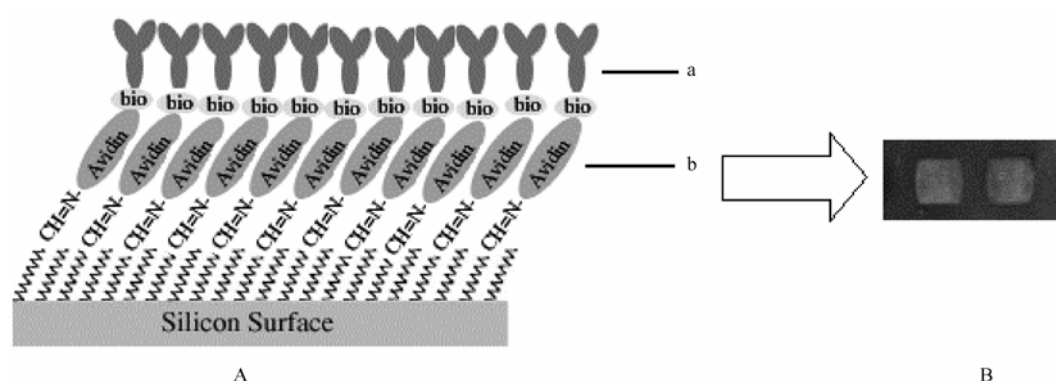


Fig. 1 Schematic illustration and layer of antibody GP3 immobilized on the surface of the silicon wafer

A: schematic illustration of antibody GP3 immobilized on the surface of the silicon wafer. 'a' is antibody GP3 layer. 'b' is avidin layer. B: the image in gray scale of the antibody GP3 layer on the surface of the silicon wafer obtained using imaging ellipsometry.

2.2 Detection of phage M13KO7

When the surface-bound antibody GP3 was incubated in a solution containing phage M13KO7, antibody GP3 could specifically identify minor coat protein pIII on the head of phage M13KO7^[17]. Phage M13KO7 was trapped by the antibody GP3, forming a complex with specific interaction, which resulted in the variation in layer thickness. Imaging ellipsometry could detect the small variation in layer thickness, and the image in gray scale is shown in Fig. 2A. The corresponding thickness distribution in three dimensions is shown in Fig. 2B, which shows the evident increase in protein-layer thickness. The thickness of phage M13KO7

layer bound with antibody GP3 was about 17.5 nm in the concentration of 1.1×10^{10} pfu/mL, which increased by 10.6 nm than the antibody layer.

To verify the specific interaction between antibody GP3 and phage M13KO7, human IgG, HBsAg, solution containing phage M13KO7 (low and high concentration), and normal human serum were simultaneously detected. The result is shown in Fig. 3, which shows that only if a solution containing phage M13KO7 came in contact with the surface of antibody GP3 layer, the thickness was altered.

The result demonstrates that the phage in solution could specifically bind with antibody GP3 although low

concentration and the complex on the surface of antibody GP3 caused increase in layer thickness.

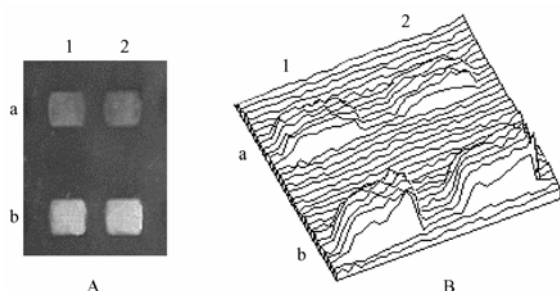


Fig. 2 The image for the detection of phage M13KO7

A: the image in gray scale obtained using imaging ellipsometry. 'a1' and 'a2' areas are antibody GP3 layer as a control. 'b1' and 'b2' areas are phage M13KO7 bound with antibody GP3. B: thickness distribution of the image 'A' in three dimension.

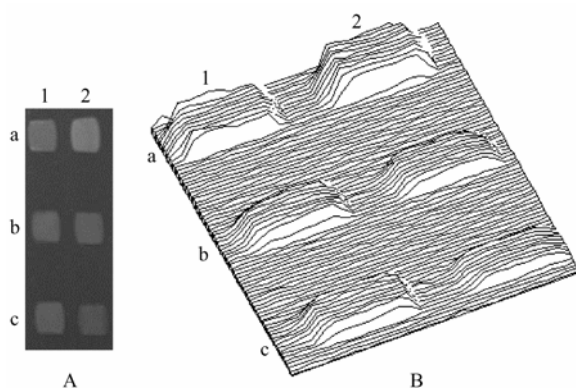


Fig. 3 The image for verification of specific-interaction antibody GP3 with phage M13KO7

A: the image in gray scale obtained using imaging ellipsometry. 'a1' is avidin/antibody GP3/low-concentration phage M13KO7. 'a2' is avidin/antibody GP3/high-concentration phage M13KO7. 'b1' is avidin/biotin-GP3 antibody/human serum. 'b2' is avidin/ antibody GP3 /Human IgG. 'c1' is avidin/ antibody GP3/HBsAg. 'c2' is avidin/ antibody GP3. B: thickness distribution of the image 'A' in three dimension.

2.3 Detection of different concentrations of phage solution

From the result given in section 2.2, the phage in lower concentration solution could be trapped by antibody GP3 on the protein chip. The solution containing phage M13KO7 was diluted to different concentrations to study the sensitivity and the calibration for quantitative detection. The result is shown in Fig. 4. The sensitivity of detection was 10^9 pfu/mL, and resolution was 0.7×10^9 pfu/mL. Each variation of layer thickness corresponds to a concentration of phage M13KO7 in

the range of 0.1×10^{10} – 2.5×10^{10} pfu/mL.

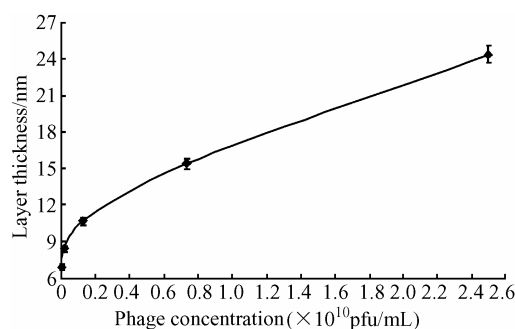


Fig. 4 Detection of different concentrations of phage M13KO7 solution

3 Conclusions

The label-free optical protein-chip prevents destruction of the sample. Detection of phage M13KO7 showed the advantage of the technique in virus detection, which includes use of a small quantity of samples, speedy analysis, low cost, easy operation, multiprotein detection, and visualized parallel analysis. The sensitivity of detection phage M13KO7 is 10^9 pfu/mL. Compared with the other above-mentioned methods, the technique has high sensitivity. The resolution of label-free optical protein-chip depends on the resolution of imaging ellipsometry for layer thickness reaching up to 10^{-10} m, so that the resolution of detecting phage M13KO7 can reach 0.7×10^9 pfu/mL. The increase in layer thickness follows the concentration of the phage M13KO7 in solution in the range of 0.1×10^{10} – 2.5×10^{10} pfu/mL, so the calibration curve can be drawn for quantitative detection. Furthermore, real-time analysis experiments can be used to investigate the interaction between antibody GP3 and phage M13KO7, which can acquire more information pertaining to the interaction. Therefore, the optical protein-chip can conveniently and rapidly detect phage, and it has promising application in the early diagnosis of virosis and in explaining the details of the disease's biological mechanisms.

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