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Jun Yang [a] , Shujin Zhang [a] & Sen-Fang Sui [a]

[a] State Key Laboratory of Biomembrane; Department of Biological Science & Biotechnology, Tsinghua University, Beijing, 100084

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Polystyrene Based SPR Biosensor Chip for Use in Immunoassay

JUN YANG, SHUJIN ZHANG and SEN-FANG SUI*

State Key Laboratory of Biomembrane; Department of Biological Science & Biotechnology, Tsinghua University, Beijing 100084

Biosensors are widely used in immunoassay. The properties of the biosensor chip has an important influence on the detecting sensitivity of the biosensor. This paper describes a polystyrene-based biosensor chip developed and used on surface plasmon resonance (SPR). The SPR biosensor has a much higher detecting sensitivity than enzyme-linked immmunosorbent assay (ELISA).

Keywords surface plasmon resonance; L-B film; enzyme-Linked immmunosorbent assay

INTRODUCTION

SPR biosensor has been widely used in immunoassay. The

* Corresponding author. E-mail suisf@mail.tsinghua.edu.cn

properties of the biosensor chip greatly influence its sensitivity and usefulness [1]. For example, when a chip with only gold film is used, nonspecific binding occurs and some proteins are denatured. So a suitable chip is needed. Then carboxy-methylated dextran is coupled on a gold film [2], the proteins nearly don't lose activity, and nonspecific binding is negligible, so it can be used well, but the high cost, complex preparation and difficult reserve made it difficult to be widely adopted. Since polystyrene is an effective base for immobilizing proteins in the ELISA, we developed a polystyrene based chip with L-B film method and used it on SPR detection of the interaction between Human immunoglobulin G (H-IgG) and Rabbit anti-Human immunoglobulin G (anti-H-IgG). To investigate its usefulness, we compared the result with that from ELISA experiment.

MATERIALS

H-IgG, Rabbit anti-H-IgG antiserum, gelatin (Sigma Chemical Co.), deionized water (Tsinghua University) and other compounds (analytical pure)

SPR Biosensor and POSTYRENE Based Chip

Figure 1 shows the schematic illustration of an SPR-based setup of Kretschmann comfiguration, and a polystyrene based sensor chip .A thin polystyrene film was transferred with L-B film method onto the gold surface. The polystyrene transferring rate was studied with Surface Plasmon Microscopy (SPM).

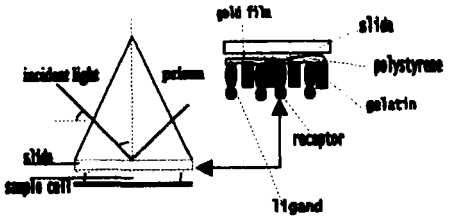


FIGURE 1 Schematic illustrations of the SPR setup and the polystyrene based chip

SPR Detection

To imitate the ELISA, 0.1M $NaHCO_3$ buffer (pH9.6) was added into the sample cell. Then H-IgG was added and washed with $NaHCO_3$ buffer 3 times after the absorbing process reached equilibrium. The influence of nonspecific absorption of the sensor chip was decreased by supersaturated gelatin solution, which strongly interacts with the sensor chip but does not interfere with the immunoassay. After washing the cell 3 times with PBS buffer (pH 7.4), the rabbit anti-H-IgG was then added. When the rabbit anti-H-IgG absorption was in equilibrium, the cell was washed t3 times with PBS buffer. Then the detection was measured.

RESULTS

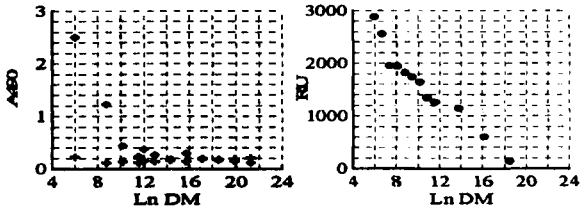
Polystyrene Based Sensor Chip

SPM image indicated that polystyrene covered about 60% of the gold surface; while after transferring polystyrene again, a covering rate of 90% was reached.

Detection Sensitivity

Results from ELISA and SPR on the rabbit anti-H-IgG of

different concentrations are shown in Figure 2. Figure. 2a illustrates when the antiserum is diluted 10^5 times and more, it is difficult to distinguish between the rabbit anti-H-IgG and the control group. While Figure 2b illustrates there is still distinguishable change of θ_{SPR} even when the antiserum is diluted more than 10^7 times. These manifest that SPR biosensor using polystyrene based chip has a sensitivity at least two magnitude higher than ELISA.



(2a) (2b)

FIGURE 2 Comparison of ELISA and SPR results (in 2a ♦: result of the antiserum; +: result of the control group)

CONCLUSIONS

Polystyrene is a good base material for making SPR biosensor chip, and this method is very simple, cheap and quick. SPR biosensor using this chip has a sensitivity about two magnitude higher than ELISA.The POSTYRENE based chip may further trigger the wide-spread use of SPR technology.

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