CD146 detection with real-time total internal reflection imaging ellipsometry

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Abstract — **Biosensor with the total internal reflection imaging ellipsometry (TIRIE) uses an evanescent wave as optical probe to monitor bio-molecular interaction with a high sensitivity due to its property of phase sensitive. Here, the biosensor is applied for a quantitative detection of CD146 with concentrations of 0.1 to 100 ng/mL in order to realize a high sensitive quantitative detection. Moreover, the regression curve between the signal of biosensor (y) and CD146 concentration (x = lnC+2.4) is deduced as a linear y=1.0544x+0.7839.**

Keywords—**TIRIE biosensor, CD146 detection**

I. Introduction

Adhesion molecule CD146 (100-130kDa) belongs to the immunoglobulin super family and it is originally identified as a biomarker for melanoma [1] . Recently, CD146 is found as novel target molecule on endothelial cell and involved in tumor angiogenesis [2] . Also CD146 is considered as critical molecule in cell invasion [3; 4; 5] and anti-CD146 antibody could inhibit tumor metastasis and angiogenesis through its down regulation of NFk [6; 7] . In this paper we attempt to detect the CD146 molecule in positive serum with the TIRIE biosensor.

TIRIE is imaging ellipsometry performed in the total internal reflection mode which is introduced previously [8] .The biosensor with TIRIE using evanescent wave as optical probe to observe bio-molecular interaction has high sensitivity due to its phase sensitive [9] , otherwise avoids the solution disturbance and transparency influence. It is a powerful tool for the visualization and analysis of bio-molecular mono-layers. It can be operated in real time mode for the bio-molecular interaction process detection. Its properties are fast sampling for a large field of view, non-disturbance, qualitative and quantitative detection with label free and low reagent and specimen consumption. Its multi-channel micro-reactor has functions of the solution delivery, the ligand immobilization, the surface blocking, and a high throughput detection.

II. Materials and methods

A. Materials

The SF10 glass slides were purchased from Changchun institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences (China). The 11-mercaptoundecanoic acid (MUA) was purchased from Sigma (USA).1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N- hydroxy-droxysuccinimide (NHS) were purchased from ACROS. All chemicals for preparing Phosphate-buffered Saline (PBS, 10mM phosphate, 0.1M NaCl, pH 7.4) and PBS solution with 1% Tween 20 (PBST) were purchased from sigma. Deionized water (resistivity18.3 MΩcm) was produced by ion exchange demineralization, followed by passing through a Milli-Q plus system from Millipore (Millipore, Bedford, MA). Mouse monoclonal antibody to CD146 AA1 was provided by institute of Biophysics of Chinese Academy of Sciences. All human sera were supplied from Anzhen Hospital (China).

B. Substrate

The SF10 glass slide was used as substrate and prepared by the evaporation of 2nm of chromium on surface then added by the evaporation of 30nm of gold. The gold surface was immersed into a 1mM MUA- ethanol solution for at least 18 Hs, followed by a thorough rinsing with both ethanol and deionized water. The MUA mono-layer with carboxyl group was self-assembled on the gold surface.

C. TIRIE setup

The schematic of the biosensor is shown in Figure 1. The TIRIE biosensor system is based on imaging ellipsometer under the total internal reflection condition equipped with a

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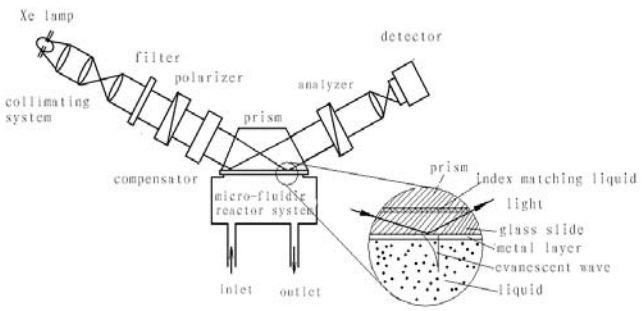

Figure 1.Schematic of the TIRIE biosensor. The zoom shows the details of the environment around the protein chip.

coupling prism and micro-fluidic reactor system. The principle of the TIRIE is briefly described here, and more details can be found elsewhere [10; 11]. Light beam with wavelength 633nm from light source goes through the polarizer and the compensator and then passes a 59° prism. The substrate equipped with the protein micro-array on chip mounted on the flow cell of the micro-fluidic reactor system contacts the prism bottom. For the prism and the glass slides coupling, an index matching liquid is used between them (refractive index n=1.73). After reflection from the bio-chip surface the light beam goes through the analyzer and then focused on a sensing area of the charge coupled device (CCD) camera. The video signal corresponding to the biomolecular mass surface concentration distribution was captured, digitized and stored in gray-scale format (8bits 0-256 grayscale) in a computer.

III. Results and discussion

The goal of the experiment is to detect the target CD146 molecule with low concentrations in normal serum and obtain a regression curve of biosensor signal versus the concentration of target molecule. The ligand of target molecule is AA1 the monoclonal antibody of CD146. The immobilization condition of the ligand is determined by an optimization result of the concentration (mouse ascites diluted 200 times with PBS buffer) and the immobilization time is10 min. In our experiment, the concentration of CD146 in sera is 0.1, 1, 10, 20, 40, 100 ng/mL, respectively. All results of CD146 real-time detection processes are shown in Figure 2. The zoom in Figure 2 is the analysis of regression curve of Region 7 and Region 8 which show the dynamic process of CD146 with different concentrations reacting with its specific antibody on surface. Curve markers from g to a correspond to different concentrations of CD146 from low to high. The signal in grayscale before and after the interaction is shown in Table 1. The data in the region 8 is averaged over the last 300 S indicated in the Figure 2.

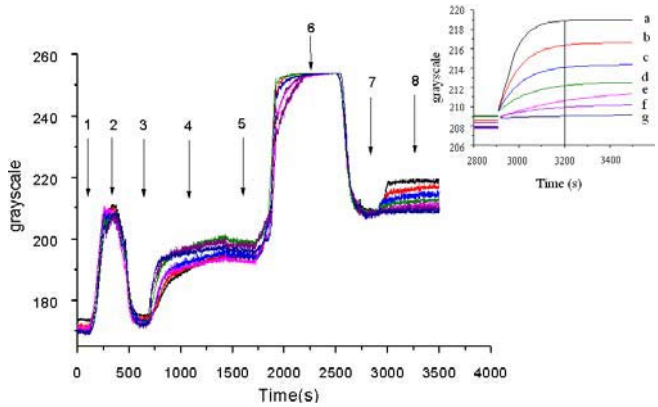

Figure 2. the real-time curves of the dynamic process for the CD146 binding with its antibody AA1 in various concentrations. The 1st region was the baseline. The 2nd region was corresponding to the NHS-EDC activation of carboxyl group assembled on the gold substrate; 3rd -PBS rising; 4th - AA1(1:200) immobilization on gold-coated substrate surface; 5th -PBS rinsing, 6th - blocking , 7th -PBS rinsing and 8th -CD146 binding with its antibody. Curve a-f was related to the several CD146 concentrations of 100, 40, 20, 10, 1, 0.1 ng/ml. The curve g was the reference.

Table 1: the surface concentration in grayscale before and after CD146 binding with its antibody

Concentration(C) (ng/ml)	7th Region	8th Region	Difference between ligand and interaction region
100	209.1±0.7	218.4±0.4	9.3±1.1
40	208.8±0.4	216.5±0.5	7.7±0.9
20	208.0±0.5	214.4±0.6	6.4±1.1
10	209.0±0.4	212.4±0.4	3.4±0.8
1	208.1±0.5	211.6±0.5	3.5±1.0
0.1	208.4±0.3	209.9±0.3	1.5±0.6
0	208.2±0.2	209.1±0.3	0.9±0.5

Though the signal of the biosensor is quite low at the concentration 0.1ng/ml but significant compared with the reference. It confirms that the sensitivity of the biosensor reaches the order of ng/mL. For quantification, the regression curve is established between the signal of biosensor and the concentration of CD146(C). The relationship between the signal in grayscale difference (y) and the concentration appears a logarithm formulation (x=lnC+2.4) especially in lower concentration showed in Figure 3. The signal variation is proportional to the concentration as expected in the regressive fitting curve and the relationship between the signal in grayscale difference(y) and concentration of CD146 (x) is y=1.0544x+0.7839.

The signal corresponds to 10ng/mL appears abnormal in regression curve. The signals of 10ng/mL and 20ng/mL

are so quite close that it's hard to distinguish. Maybe it's impacted by the ununiformity among independent channels or the stochastic noise, so that the improvement of the uniformity among independent channels and the ratio of signal and ratio is required. The work for the improvement is on the way, and some improved results could be foreseen.

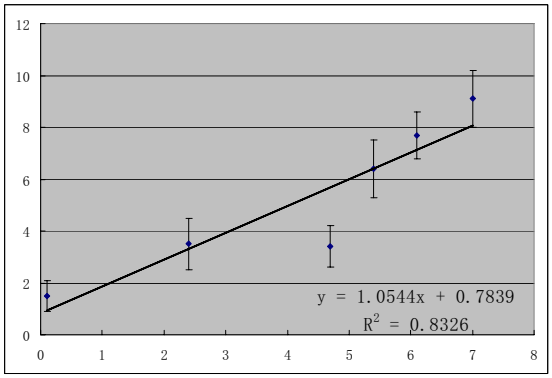

Figure 3. the regressive fitting curve between the signal in grayscale difference(y) in Table 3 and the concentration (C) of CD146 (x).. Here, the relationship between x and the concentration of CD146 is x = ln C+2.4.

IV. Conclusion

CD146 with concentration of 0.1-100 ng/ml range in serum has been detected with the TIRIE biosensor dynamically and quantitatively. The concentration of the detected sample is lower than the standard sample 243ng/mL which confirmed the biosensor could be applied in CD146 of low concentration detection. A linear relationship y=1.0544x+0.7839 between the biosensor signal (y) and the concentration of CD146 with logarithm formulation (x=lnC+2.4) has been obtained by regression curve which would be used as a reference of calibration for further CD146 detection. The total internal reflection biosensor shows a potential for clinic application.

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