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Design of sensor chip to repel the non-specific binding for its application in biomedical sensor; effect of human serum samples

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***Abstract*— In biomedical application, it is essential to perform the detection in human serum in most of cases. However, the main difficulty of using serum is high non-specific binding between the sensor surface and serum proteins. The modifications of sensor surfaces were studied in this work. The self assemble monolayer (SAMs) and hydrophilic polymer dextran were coated on gold substrate with chemisorption method and was tested in the property of a repelling the non-specific binding of human serum by monitoring via Surface Plasmon resonance technique (SPR) gtecnique. SPR is an optical technique, which is highly sensitive on the change of the optical properties of the biomolecules in nanoscale. The results were shown that there was effect of serum dilutions on both of the sensors. It was shown the effect of increasing the human serum concentration on the SPR signal increasing. The efficiency of repelling human serum in both SAMs and dextran was shown different. Dextran gave a lower non-specific binding than SAMs surface.**

Keywords— Surface Plasmon resonance, thin film, non-specific binding

I. Introduction

Capability of the measurement of biomolecules substances in real samples is complicating. Because of a complex biological mediums like blood, plasma, or other body liquids, there exist a wide variety of different proteins. As a consequence, these proteins compete among themselves for the adsorption to the exposed surface[1]. The measurement cannot avoid contaminant from these molecules which is a result of a low signal detection or error diagnostic. For decreasing of these contaminants, a method is to decrease of non specific binding on the sensor. Coating of surfaces with non-charged hydrophilic polymers like polyethylene glycol (PEG) and poly(methyl methacrylate) (PMMA) have been found to reduce the protein adsorption due to the reduction of electrostatic forces and the hydrophobic interactions between the surface and the protein in solutions [2].

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A number of techniques have been used for study of some biomolecule adsorption such as atomic force microscopy (AFM) [3], ellipsometry [4], total internal reflection fluorescence [5]. These techniques are used to characterize and show the adsorption evidence. Surface Plasmon resonance (SPR) is an interesting label-free technique [6] which is widely used in biosensor applications because it is capable to monitor the protein-protein interaction, immunosensor and the various applications[8-10].

In this work, we studied the repelling property of thinfilms which is able to repel some biomolecules in human serum.to expect to develop and use for biomedical sensor chip in the future.

II. Experimental

A. *Chemicals*

PDMS for making flow cell 7channel is purchased from Dow corning (Dow Corning Corporation, Midland, Michigan, United States). 3 mercaptopropionic acid (3MPOH) from Sigma (Sigma–Aldrich Co., St. Louis, MO, USA). N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyl amino-Propyl) Carbodiimide (EDC) from Sigma. All the reagents were filtered with this process by filter paper from Whatman No.2. Triton X-100 is used for cleaning solution is purchased from Sigma. The pH of buffer was controlled by hydrochloric acid, HCl, (Lab scan, Thailand) and sodium hydroxide (Merck).

B. *Thinfilm preparation*

The gold substrates were cleaned with freshly piranha solution (70:30 v/v of H_2SO_4: H_2O_2) for 15 min, throughly rinsed with DI water and methanol for 30 min and blown with nitrogen gas prior to the coating process. Afterwards gold was cleaned with ozone cleaner for 10 min. Finally, the cleaned bare gold was prepared to be SAMs and dextran.

The Self-assembled monolayer (SAMs) surface was chemically attached on the gold substrate. The Self-assembled monolayer surfaces preparation begin with immersion of the cleaned gold substrate in a mixture which consisted of a 1:10

ratio of COOH: OH. of 11 mercaptoundecanoic acid (11-MUA) and 3MPOH. After overnight incubation, sensor chips were removed from solution, rinsed with ethanol and dried with nitrogen gas before using

Dextran surface which is hydrophilic-polymer in assumption of much hydrophilicity and flexibility of 3D surface are effect to more probe-analyte binding also prevent non-specific binding of other protein. From previous report method [7], the hydroxyl group on surface from 16 mercaptohexadecane were reacted with epichlorohydrin which introduces epoxy groups then reacted with bromoacetic acid for introduce carboxylic groups on the dextran surface.

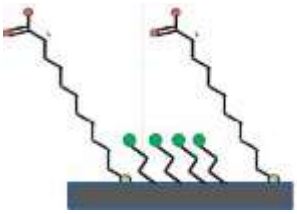

Figure 1. The structure of SAMs

The measurements were performed by the SPR homemade unit (created by NECTEC, Thailand). Gold substrate was placed on the prism with index matching gel which is attached to the flow cell. The sample was pumped with a peristaltic pump passed over the surface in 7 channel flow cell.

The reflected intensity of substrate on prism was reported in Refractive index unit (RIU) while the setup was being various wavelength and fixed angle. The SPR signal was defined as the reflectivity difference before and after sample introduced to the sensor surface. The calibration curve between the reflectivity change, ΔR, and the refractive index unit (RIU) was established just before the experiment by using the glycerol solutions with known refractive indices.

III. Results and Discussions

The sensor surface from Dextran and SAMs film were collected the SPR signal to observation the mass change in each steps of the non-specific binding test.

A. Blocking surface process

Human serum was adsorbed on SAMs and dextran thin film via chemisorption. Due to the tailed chain of SAMs is carboxylic acid, the EDC/NHS was used to be coupling agent via amide linkage to immobilized the analyte.

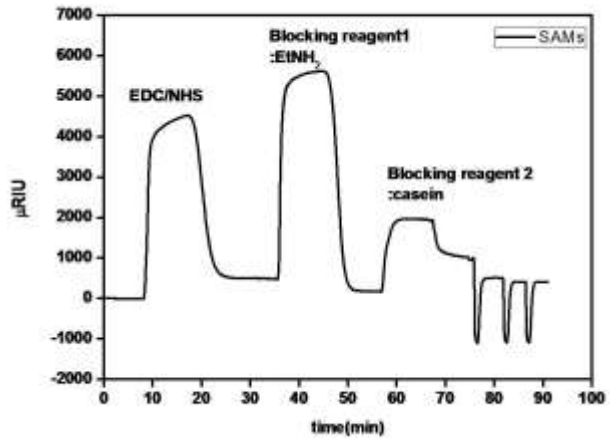

Figure 2. the blocking process via chemisorption on SAMs ultra thin film sensor surface.

The adsorption experiment via chemisorption as shown in fig. 2 was carried out by flowing PBS buffer pH 7.4 over the sensor surface at flow rate 10 μL/min before the blocking process. The activated SAM surface were done by injection the mixing of1-ethyl-3-[3 (dimethylamino) propyl] carbodiimide (EDC) 0.1M and N-hydroxysuccinimide (NHS) 0.4M for 10 min with flow rate 7 μl/min for producing NHS ester . The ethanolamine which is used to be blocking reagent 1 and casein is reagent 2were flown through the surface for blocking, respectively.

While the sensor surface was completely covered with inactive area, the human sera were flown to test the non-specific binding on this blocked surface. The pulse of glycine used to wash off at the end of the process.

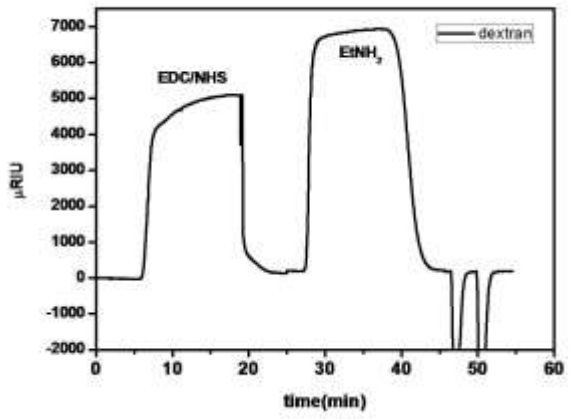

Figure 3. the adsorption process via chemisorption on SAMs ultra thin film sensor surface.

The adsorption experiment on dextran sensor surface via chemisorption as shown in fig. 3. The process was carried out by flowing PBS pH 7.4 at flow rate 10 μL/min to be baseline in initial time. The activated dextran surface were done by injection the mixing of EDC 0.1M and NHS 0.4M for

10 min with flow rate 7 µl/min.The ethanolamine which is used to be blocking reagent flowed throught the surface for blocking the active area. While the sensor surface were completely cover with inactive area, the human sera were flown to test the non-specific binding on this blocked surface then pulse glycine for washing surface as same as the SAMs surface.

B. *The effect of serum dilutions on sensor surfaces*

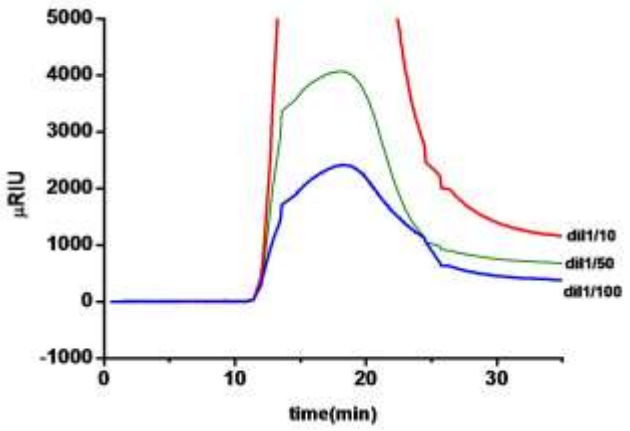

Figure 4. effect of serum dilutions on SAMs surfaces

Effect of increasing human serum concentration on the sensor signal was shown in Fig 4. Human serum was diluted in matrix buffer at varying concentrations.Various of serum dilutions (dilution1/100 –dilution 1/10) were flown on both of SAMs and dextran surface to observe the non-specific binding. Fig4., the adsorption of human serum on the inactive surface which had been prepared in previous process were shown the increase of signal correlated to a higher concentration (low dilution condition). This result was also shown in the same trend in dextran surface.

The efficiency of repelling human serum in both SAMs and dextran is different. The higher prevention of human serum was shown in dextran surface in any dilution conditions.

Conclusion

In order to satisfy the conditions of repelling properties on human serum samples, the sensor surface needs to be modified. In this study, the SAMs and dextran thin film were investigated the repelling property after these sensor were blocked the active group on the sensor surface. The results were shown the SPR signal which can be concluded that there are different efficiency of the prevention the non-specific. Dextran was shown the lower binding the serum in each dilution condition. However, both of thin films can be used in real samples testing. Nevertheless, the modification sensor has been shown to be strongly dependent on the composition and biologic fluid nature the biomolecule and its physicochemical properties which we have to investigate further.

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