

Design and fabrication of polymeric thinfilm and ultra thin film sensor chip for biomolecules capturing by Surface plasmon resonance technique

Thidarat Wangkam and Toemsak Sriksirin

Abstract—

Development of sensor chip to be high specificity and fitted in each application is important. This work, we designed and fabricated the sensor chips to be model of biomedical chip in form of polymethyl metacrylate (PMMA) thin film and Self-assembled monolayer (SAM) thin film which were coated on gold substrate with physisorption and chemisorption method, respectively. The detection of bimolecular interaction such as protein and collagen were tested by Surface Plasmon resonance (SPR) technique. SPR is an optical technique, which is highly sensitive on the change of the optical properties of the biomolecules in nanoscale. The suitable condition for protein and collagen adsorption on these two thin film were studied. It was found that the different types of surfaces on various pH condition has effect on the biomolecule adsorption. The fabricated sensor chips can be used to be the model chip for bimolecular capturing and capable to develop in medical application further.

Keywords— Surface Plasmon resonance, thin film, bimolecular capturing

I. Introduction

The biofunctionalization on thinfilm for biomolecule is an importance for biomaterials and biomedical applications. In each application need different conditions, thus the design of sensor chip to suitable in real application have to be understood including the biological and chemical conditions also the nature of samples which are complexes. A number of techniques have been used for protein adsorption such as atomic force microscopy (AFM) [1], ellipsometry [2], total internal reflection fluorescence [3]. these technique are used to characterize and develop for reaching to real application.

Surface Plasmon resonance (SPR) is the another interesting optical technique which is capable to study the protein-protein interaction, immunosensor and the various applications.[4-6] The high sensitivity of SPR would also monitor the bimolecular adsorption with label free technique[5].

Thidarat Wangkam

¹Department of Industrial and medical Instrumentation,
Faculty of Applied science,

²Biosensors and Bioelectronics Technology center (BIOSE), Science and
Technology Research Institute
King Mongkut's University of technology North Bangkok
Thailand

Toemsak Sriksirin

Department of Physics
Faculty of science, Mahidol University
Thailand

In this work, we designed and fabricated the sensor chip which made from thinfilm and ultra thinfilm to suitable for the biomolecule adsorption such as protein and collagen to expect to be sensor chip model for biomedical sensor chip further.

II. Experimental

A. Chemicals

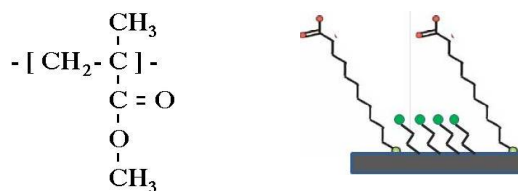
PMMA were obtained from Sigma (Sigma-Aldrich Co., St. Louis, MO, USA). A gold-coated substrate from Ssens (Hengelo, Netherlands), coated with 5 nm of chromium and 45 nm of gold, was used. The gold-coated substrates were cleaned with piranha solution, sonicated in water and methanol for 30 min and blown with nitrogen gas prior to the spin-coat process.

Lyophilized type I collagen (Sigma-Aldrich, St. Louis, MO, USA) was dissolved at 1 mg/ml in Sodium acetate buffer pH4, in PBS pH7 and Tris pH9 for study the optimum pH condition on the thin films surface. Bovine serum albumin (BSA) (Sigma-Aldrich Co., St. Louis, MO, USA) is water-soluble and has a molecular weight of 66 kDa. The pH of buffer was controlled by hydrochloric acid, HCl, (Lab scan, Thailand) and sodium hydroxide (Merck). N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylamino-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.

B. Thinfilm preparation

The gold substrates were cleaned with freshly piranha solution (70:30 v/v of H₂SO₄: H₂O₂) for 15 min, the gold was thoroughly rinsed with DI water then dried with nitrogen gas; afterwards gold was cleaned with ozone cleaner for 10 min. Finally, the cleaned bare gold was prepared to be polymer surface and Mixed SAMs surface.

Figure 1. The structure of (a) PMMA and (b) SAMs



For thin polymer films preparation, polymers are deposited on the gold substrate by spin coating (spin coater model P6700D from Specialty Coating Systems, Indianapolis, USA). The spinning solution was prepared by dissolving the PMMA in acetone at 1.0 mg/mL then were stirred overnight to form a clear solution. The structure of PMMA and SAMs were shown in figure 1.

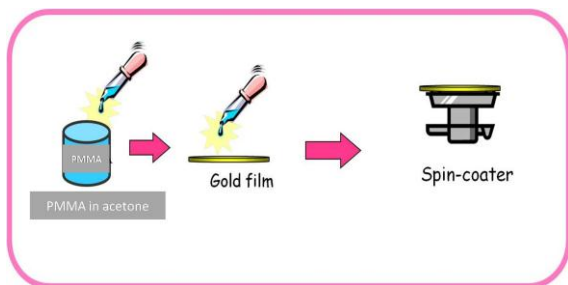


Figure 2. Spin cast method for thin film polymer preparation

The PMMA solution was spun on the substrate at 1500 rpm for 60 s.(Fig 2.) After polymer deposition the samples were vacuum dried overnight at room temperature before using.

C. Surface Plasmon Resonance technique

SPR measurements were performed on surface plasmon resonance spectroscopy (created by NECTEC, Thailand). 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

A. Thin film and Ultra thin film

Demonstrated the measurement the thin film preparation, the sensor surface from PMMA and SAMs film were collected the SPR signal to observation the change of film thickness. The SPR curve of SAMs and PMMA film were compared to the bare gold as shown Fig 3. The change in the reflectivity was measured at different angles. The angle shift to higher angle corresponds to the change of refractive index which results in the film thickness increasing. From the SPR curve was shown a bit increasing angle shift in SAMs and more increasing in PMMA. It means that the sensors which were coated by the PMMA and SAMs can be shown in the SPR signal. Due to the SAMs is an ultra thin film with about 5 angstrom, the SPR angle shift was not different angle. However, it was shown more increasing of SPR angle shift in PMMA film.

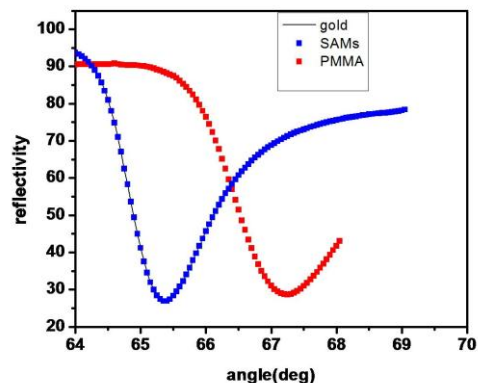


Figure 3. SPR curve of thin polymer and ultra thin polymer film

B. Immobilization process

BSA protein and collagen were adsorbed on SAMs 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.

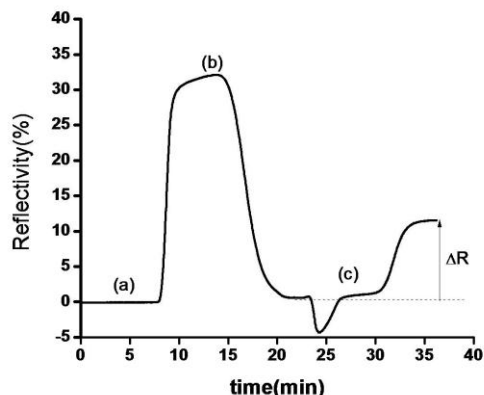


Figure 4. the adsorption process via chemisorption on SAMs ultra thin film sensor surface.

The adsorption experiment via chemisorption as shown in fig. 4 was carried out by flowing analyte solution (either collagen or BSA) over the sensor surface. The real time monitoring of the adsorption was shown the change of the SPR signal. PBS pH 7.4 at flow rate 10 $\mu\text{L}/\text{min}$ was flown on the sensor surface before the adsorption process to be baseline (point a of Fig. 4). The activated SAM surface were done by injection the mixing of 1-ethyl-3-[3 (dimethylamino) propyl] carbodiimide (EDC) 0.1M and N-hydroxysuccinimide (NHS) 0.4M for 10 min with flow rate 7 $\mu\text{L}/\text{min}$ for producing NHS ester as shown the signal in point (b) of Fig. 4. The analyte was flown on the sensor at point (c) of Fig 4. The rinsing of PBS was carried out until there were no more changes of the SPR signal. The total change in the SPR signal (ΔR) (the difference between the final time ($t=35$ min) and point a($t=0$))

min)) represented the amount of adsorbed the analyte on the surface. The cleaning solution was flowed over the surface to wash off the adsorption process.

For the physisorption of the protein and collagen on the PMMA sensor surface was begun by the flowing PBS through polymer surface to be baseline, then the analyte with different condition was flown on the sensor surface. The BSA and the collagen adsorbed onto surface was determined by measuring the SPR signal of PBS before and after adding the analytes.

C. The effect of pH on both of the analytes

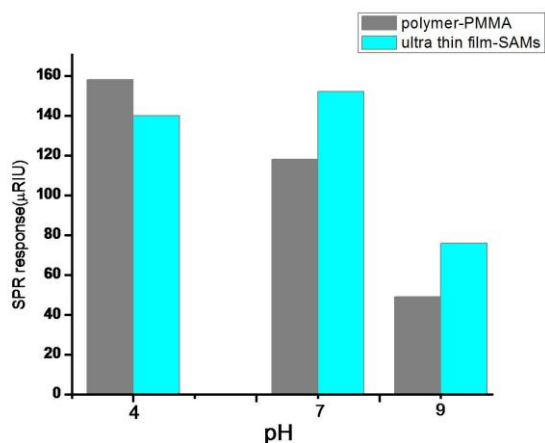


Figure 5. effect of pH on BSA adsorption

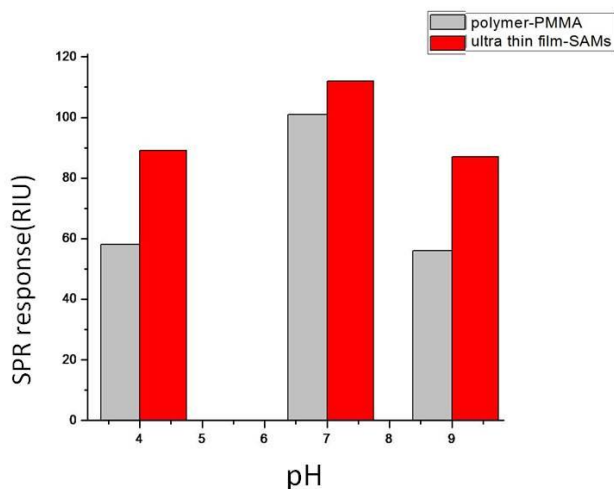


Figure 6. effect of pH on collagen adsorption

The effect of pH on BSA adsorption (fig 5) and on collagen (fig.6) were studied in the pH range from 4-9 with comparing the efficiency of adsorption of PMMA and SAMs thin film sensors. pH buffer was prepared in pH4 with sodium

acetate buffer, pH 7 in PBS buffer and pH 9 was prepared in Tris buffer. In fig 5, BSA was flown on sensor surface while fixing the concentration at 1 mg/ml. The highest SPR signal was shown in pH 4 on the PMMA polymer film, however in case of the SAMs thin film was given the highest adsorption at pH 7. It means that there is effect of to the electrostatic interaction between surface and BSA molecule which has isoelectric point at 4.7 in both thin film but the SAMs surface which is chemisorptions method is shown less effect of pH than PMMA surface.

The collagen adsorption was shown in fig 6, the result was shown the optimally adsorbed in the at nearly neutral pH give the highest SPR signal. This result is similar to previously resulted with collagen study into microribbon [7-8]. They suggest that there is effected by the electrostatic interactions between collagen molecules and its isoelectric point is an important parameter (pI of collagen is 7.8)[9]. The behavior and morphology of collagen adsorption on thinfilm should be studied further.

Conclusion

In this study, the PMMA thinfilm and SAMs ultra thin film were investigated by SPR technique. The results can be concluded that both of thin films can be used for capturing the BSA protein and collagen via physisorption and chemisorptions. The pH conditions are also studied in the adsorption of these biomolecules. It means that the sensor chip can be designed by controlling the pH condition. For development to the real application, the characteristics of the thin films need the investigation further.

Acknowledgment

This work was supported by Science and technology research institute, King Monkut's University of technology North Bangkok, Bangkok, Thailand. T.W. would like to thank Thailand National Electronics and Computer Technology Center(NECTEC), National Science and Technology Development Agency for SPR instruments and would like to thank CIMs research group, Mahidol University, Bangkok, Thailand for supporting some instruments.

References

- [1] Dupont-Gillain and Rouxhet, "AFM study of the interaction of collagen with polystyrene and plasma-oxidized polystyrene", *Langmuir*, Vol 17, pp. 7261-7266, 2001.
- [2] M. Wahlgren, T. Arnebrant, I. Lundström, "The adsorption of lysozyme to hydrophilic silicon oxide surfaces: Comparison between experimental data and models for adsorption kinetics", *J. Colloid Interface Sci*, Vol 175, pp.506-514, 1995.

- [3] B.K. Lok, Y.-L. Cheng, C.R. Robertson, "A total internal-reflection technique for the examination of protein adsorption", J. Biomed. Mater. Res., Vol 11 , pp. 915–938, 1997.
- [4] R.J. Green, J. Davies, M.C. Davies, C.J. Roberts, S.J.B. Tendler, "Surface plasmon resonance for real time in situ analysis of protein adsorption to polymer surfaces", Biomaterials, 18 ,pp. 405–413, 1997.
- [5] Bo Liedberg and Knut Johansen, "Affinity Biosensing Based on Surface Plasmon Resonance Detection" Methods in Biotechnology(1998), 7: 31-53.
- [6] Navina Mehan, Vinay Gupta, K Sreenivas, and Abhai Mansingh, "Surface Plasmon resonance based refractive index sensor for liquids", Ind. J. Pure& Applied Phys, 43:854-858, 2005
- [7] Jiang F, Horber H, Howard J, Muller DJ "Assembly of collagen into microribbons: effects of pH and electrolytes" Struct Biol, Vol 148, pp 268–278, 2004
- [8] Liang Z, Zhou C, Zeng R, Cai H and Guo Z, "Visualization of the Nanoscale Assembly of Type I Collagen on PLA by AFM", Scanning, Vol. 32, pp.104–111, 2010.
- [9] Arthur W. Thomas ,Margaret W. Kelly, "The iso-electric point of collagen", J. Am. Chem. Soc., , Vol 44 (1), pp 195–201, 1922
- [10] K. Norrman, A. Ghanbari-Siahkali and N. B. Larsen. " Studies of spin-coated polymer films, Annu. Rep. Prog. Chem., Sect. C: Phys. Chem, Vol 101,pp.174-201,2005.
- [11] Nanxia Rao, Xin Xie, David Wieliczka, and Da-Ming Zhu, "Quartz crystal microbalance sample stage for *in situ* characterization of thickness and surface morphology of spin coated polymer film", Rev. Sci. Instrum, vol 77, pp.116111, 2006.
- [12] Abraham Ulman, "Formation and Structure of Self-Assembled Monolayers", Chem. Rev. vol 96, pp.1533-1554, 1996
- [13] Albano Cossaroet al., "Functionalization of Gold Surfaces: Ultra High Vacuum Deposition of Cysteamine on Au(111), J. Phys. Chem. C ,vol 114,pp.15011–