In vitro protein adsorption on binary mixed self assembled monoalyers studied by surface plasmon resonance

Ching-Hsiung Shen, Zih-Yu Chen, Jui-Che Lin
Department of Chemical Engineering, National Cheng Kung University, Tainan, TAIWAN

Introduction

When biomaterials contact with the biological environment, the thin adsorbed protein layer may cause further biological response such as thrombosis and inflammation. Therefore it was needed to characterize the relationship between the adsorbed protein layer and surface property of biomaterials. Self assembled monolayer (SAM) of the long chain alkanethiols with well-defined structure and variant terminal functionality can serve as a model surface in the study of blood-material interaction.[1] Moreover, recent studies have reported the evaluation of the interaction between proteins and SAM through surface plasmon resonance (SPR).[2]

In this study, we prepared two different mixed SAMs by mixing lab-synthesized sulfonic acid terminated alkanethiol[1] with methyl terminated alkanethiol or hydroxyl terminated one. The adsorption of bovine serum albumin (BSA) and bovine fibrinogen onto these mixed SAMs was measured by SPR. The protein adsorption results were also correlated to the previous platelet adhesion results of these mixed SAMs.

Materials and methods

Preparation of mixed self- assembled monolayers

Fresh prepared gold coated SPR chips were immersed into two different thiol solutions, containing the mixture of the 10-mercaptodecanesulfonic acid with 1-dodecanethiol or 11-mercapto-1 undecanol in absolute ethanol at room temperature for 24 hours. The total concentration was set at 2mM.

Protein adsorption experiment

The SPR biosensors based on wavelength interrogation with four-channel Teflon flow cell[3] was used to measure the protein adsorption on these mixed SAMs. From the standard calibration of this custom-built SPR 1nm wavelength shift was equivalent to 15 ng/cm^2 of adsorbed proteins.[3,4] In this work two different protein adsorption experiments were made: (1) 1 mg/ml BSA was preadsorbed for 60 min, then followed by adsorption of 0.1 mg/ml bovine fibrinogen for 30 min. (2) adsorption of 0.1 mg/ml bovine fibrinogen for 60 min, then followed by 1 mg/ml BSA adsorption for 30 min.

Result and discussion

In figure 1 (a), after flowing 1 mg/ml BSA through SO_3H & $-CH_3$ mixed SAMs, the protein adsorption density was about 30-120 ng/cm^2. Then 0.1 mg/ml bovine fibrinogen was flowed into cell, the amount of protein adsorption were not significant increased except on $X_{SO3H,soln}$=1. When 0.1 mg/ml bovine fibrinogen was pre-adsorbed on $-SO_3H$ & $-CH_3$ mixed SAMs (figure 1 (b)), the surface protein density was all more than 100ng/cm^2. After that, the 1 mg/ml BSA was passed through and there was no significant adsorption occurred.

When 1 mg/ml BSA was firstly adsorbed on $-SO_3H$ & -OH mixed SAMs (figure 2 (a)), the protein adsorption density was less than 2 ng/cm^2. After 0.1 mg/ml bovine fibrinogen was sequentially flowed through, the amount of protein adsorbed significantly increased. When 0.1 mg/ml bovine fibrinogen was pre-adsorbed onto $-SO_3H$ & -OH mixed SAMs (figure 2 (b)), the adsorbed protein density was about more than 120 ng/cm^2. After 1 mg/ml BSA was sequentially flown through, there was no significant protein adsorption occurred. In figure 2 it was shown that the pure –OH SAM adsorbed the least amount of BSA and bovine fibrinogen and this was due to the hydrophilicity characteristic and near neutral charge[2].

From the previous *in vitro* platelet adhesion studies, $-SO_3H$ & $-CH_3$ mixed SAMs and $-SO_3H$ & -OH mixed SAMs exhibited similar platelet adhesion density except pure –OH SAM, in which the least amount of platelets was adhered. Therefore, the protein adsorption was highly correlated to the results of *in vitro* platelet adhesion density.

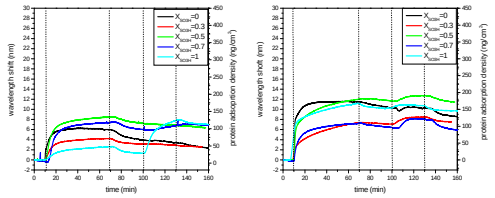

Figure1. SPR sensorgram of (a) pre-adsorption of 1 mg/ml BSA, then followed by 0.1 mg/ml bovine fibrinogen, and (b) pre-adsorption of 0.1 mg/ml bovine fibrinogen, then followed by 1 mg/ml BSA on $-SO_3H$ & $-CH_3$ mixed SAMs.

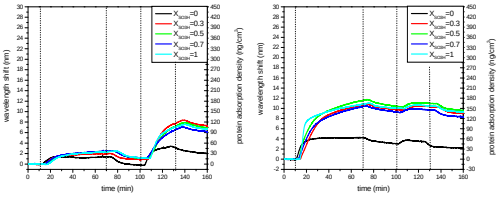

Figure2. SPR sensorgram of (a) pre-adsorption of 1 mg/ml BSA, then followed by 0.1 mg/ml bovine fibrinogen, and (b) pre-adsorption of 0.1 mg/ml bovine fibrinogen, then followed by 1 mg/ml BSA on $-SO_3H$ & -OH mixed SAMs.

Conclusion

From the results above, the addition of $–SO_3H$ significantly increased the BSA and bovine fibrinogen adsorption. The least amount of protein adsorption was noted on the pure –OH SAM and this was highly correlated to the *in vitro* platelet adhesion result.

References

1. Lin JC, Chuang WH. J. Biomed. Mater. Res. 2000; 51: 413-423.
2. Sigal GB, Mrksich M, Whitesides GM J. Am. Chem. Soc. 1998; 120: 3463-3473.
3. Chang Y, Liao SC, Higuchi A, Ruaan RC, Chu CW and Chen WY. Langmuir 2008; 24:5453-5458.
4. Jung, LS, Campbell, CT, Chinowsky, TM, Mar, MN, Yee, SS Langmuir 1998; 14: 5636-5648.