Development of protein chips based on self-assembled monolayer and protein A

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Abstract—In order to improve the steric hindrances and less ordered structures in the long-chain SAMs, the SAM of 4,4-Dithiobis(butyric acid) (4,4-DTBA) was investigated to be a thin monolayer for the protein chip. This work presents the feasibility study of 4,4-DTBA as a monolayer of the protein chip based on gold surface. Characterizations of surface topography using atomic force microscopy (AFM), contact angle and fluorescence using protein A-FITC were performed to investigate the binding efficiency for the SAMs of 4,4-DTBA and 11-mercaptoundecanoic acid (11-MUA). The results show that the binding efficiency of 4,4-DTBA is higher than that of 11-MUA. The SAM of 4,4-DTBA can be used as a monolayer for the protein chip and may have a comparable binding efficiency.

I. INTRODUCTION

In recent years, the study of protein chips has dramatically increased because the protein chip plays an important role in immunoassay for applications of clinical medicine and disease diagnosis. The protein microarray can be prepared with small amounts of proteins and reagents by immobilizing hundreds or thousands of different proteins to substrates. Therefore, it accelerates drug discovery and disease diagnosis. When the proteins such as immunoglobulin (IgG) are immobilized on a solid surface, their activity is usually less than that in the aqueous phase. The main reason for activity reduction is due to the random orientation of protein molecules on the solid substrate. To improve and construct the well-defined antibody surface, the well-know protein A is used as a binding material. Protein A, a cell wall component of Staphylococcus aureus, binds with the Fc part of the antibody. Therefore, the protein A leads to highly efficient immunoreaction and enhances detection system performance [1-6].

The SAMs have recently drawn a great amount of concern in a bio-MEMS technology for its versatility in bio-applications. With the demands of SAMs for its functionality, the studies about the surface modification using SAMs are another interesting approach for MEMS technology. SAMs can potentially give a robust method for fabricating protein multilayers [7]. The protein needs much care to keep its three-dimensional structure and function of bioactivity due to the unique properties of protein, especially sensitive to the surface of substrates. To combine the protein A and the solid substrates, the fabrication of nano-scale structure using self-assembled monolayers (SAMs) have attracted much attention because of interest in two-dimensional molecular assembly and their potential applications in molecular devices, sensors, surface engineering, etc. Pure SAMs of thiol and disulfide with different chain lengths and terminal group on gold surface have been studied for several years [8]. However there are some disadvantages of the long-chain SAMs, such as 11-MUA has a high densities of surface function group leading to steric hindrances, and less ordered structures due to bulky terminal group used.

In this work, the feasibility of the SAM of 4,4-DTBA as a monolayer for the protein chip based on gold surfaces was studied. The chain length of SAMs with 11 carbons is usually used to have a good binding with protein. However, the SAMs with 11 carbons easily generate the steric hindrances and less ordered structures which influence the binding of protein [9]. In order to reduce this problem, the 4,4-DTBA (disulfide) with four carbons was used as a monolayer [10] and compared with 11-MUA with eleven carbons. The characterizations of monolayer fabricated by 4,4-DTBA and 11-MUA was investigated using AFM and contact angle. In addition, protein A-FITC was immobilized on self-assembled monolayer [11] using EDC/NHS to study the binding efficiency of reaction between the 4,4-DTBA and 11-MUA.

II. METHOD AND MATERIALS

The experiments are divided into two groups: one is the SAMs of 11-MUA and the other one is the SAMs of 4,4-DTBA. For each SAM, the concentrations of SAMs are 10mM, 20mM, 40mM, 80mM and 160mM. The concentration of protein A-FITC is 0.0167 mg/ml. Moreover, the prevention of protein adsorption by the surface-grafted polymers such as OEG has been well-known [12-14]. They have been used most widely.
for surface modification because of its unique properties such as hydrophilicity, flexibility, high exclusion volume in water, nontoxicity, and nonimmunogenecity. In order to improve the nonspecific binding, the OEG-silanized dissolved into THF was used to prevent protein A-FITC from the adsorption on the substrates in this work.

A. Preparation of gold substrates

The silicon (75mm x 25mm) were cleaned by piranha \((\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2=1:3)\) and sequentially rinsed using DI water, acetone, isopropanol and DI water. Then photoresist was patterned on the substrates using the photolithography process. Titanium (10nm) and gold (100nm) films were deposited onto the slides by E-beam evaporator. Finally the gold patterns formed on the slides using the lift-off technique. The fabricated gold substrate is shown in Fig. 1.

![Figure 1. The silicon substrate with gold patterns](image)

B. Formation of monolayers

The gold substrates were cleaned using the hot solution of \(\text{HCl} / \text{H}_2\text{O}_2 / \text{DI water (1:1:6, v/v)}\) for 15 minutes and rinsed with DI water. The clean substrates were immersed into the silanized OEG / tetrahydrofuran (THF) (1:10, v/v) solution \([15]\) for 12 hours. Then the substrates were immersed into 11- MUA (450561-5G, Sigma-Aldrich) and 4,4- DTBA (C15605-10G, Sigma-Aldrich) ethanolic solution with 10mM, 20mM, 40mM, 80mM and 160mM for 24 hours \([16]\). Followed by the removal of self-assembled monolayers, the substrates were washed with degassed ethanol, and dried in a stream of nitrogen.

C. Immobilization of protein A-FITC

In addition, the treated samples were immersed into 1% EDC (03450, Fluka, USA) / NHS (56480, Fluka, USA) which had been dissolved in PBS buffer (pH 7.4, 0.2g KCl, 1.44g NaHPO\(_4\), 8g NaCl and 0.24g KH\(_2\)PO\(_4\) in one liter DI water) for 4 hours, and dried in a stream of nitrogen again. A drop of protein A-FITC with the concentration of 0.0167 mg/ml (P3838, Sigma, USA) was placed on the treated substrates. Then the samples were incubated at 4°C for 24 hours to immobilize protein A-FITC onto the substrates \([17]\).

![Figure 2. The schematic illustration of immobilization of protein A-FITC](image)

D. Characterization of topography

The topography analysis of fabricated thin films was investigated by atomic force microscopy (AFM) (P7L, NT-MDT, USA). AFM was operated in tapping mode in air and at room temperature. Images were acquired at a scan rate of 1.5 Hz with a silicon cantilever, and the scan size used was 1µm x 1µm. In addition, the contact angles of the modified surface with the SAMs of 11- MUA and 4,4- DTBA were measured to investigate the hydrophobicity using Contact Angle Goniometer (MigicoDrop, Future Digital Scientific Corp, USA).

E. Characterization of fluorescence

The characterization of fluorescence on images was carried out to investigate the binding efficiency of protein A-FITC and SAMs by the ratio of fluorescent area to total area. The fluorescent images were taken by the CCD camera (Evoluting VF, Q-imaging, USA) and analyzed by Image-Pro plus 5.0 (Media Cybernetics, USA).

III. RESULTS AND DISCUSSION

A. Topography analysis using AFM

The surface topography of clean Au substrate is shown in Fig. 3. The gold surface is smooth and the surface roughness is 0.37nm.

![Figure 3. Topography analysis using AFM](image)
The surface topography was increased by immersed the two kinds of SAMs (11-MUA and 4,4-DTBA). According to Table 1, the surface roughness of 11-MUA increases with the concentrations. However, the surface roughness of 4,4-DTBA does not increase with the concentrations, and is about 0.914–1.115 nm. Because the SAM of 11-MUA has a high densities of surface function group, it leads to steric hindrances and less ordered structures due to bulky terminal group. It results that the surface topography of 4,4-DTBA is more uniform than 11-MUA.

Table 1. The surface roughness of SAMs

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>10mM</th>
<th>20mM</th>
<th>40mM</th>
<th>80mM</th>
<th>160mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface roughness (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-MUA</td>
<td>0.692</td>
<td>0.858</td>
<td>0.987</td>
<td>1.059</td>
<td>1.055</td>
</tr>
<tr>
<td>4,4-DTBA</td>
<td>1.031</td>
<td>0.914</td>
<td>0.943</td>
<td>1.115</td>
<td>0.987</td>
</tr>
</tbody>
</table>

B. Contact angle analysis

Figure 4 shows the contact angle of un-cleaned and cleaned gold surface. The contact angle of un-cleaned surface is 82.4°. After the washed step by the hot solution of HCl / H2O2 / DI water (1:1:6, v/v), the contact angle reduced to 71.8°.

C. Characterization of Immobilization with protein A-FITC

After the samples were immersed into the solutions of the SAMs with different concentrations of SAMs for 24 hours, the samples were coated by protein A-FITC. Figure 5 and Figure 6 show the fluorescent images for 11-MUA and 4,4-DTBA, respectively. According to the results, the fluorescent area increases when the concentrations of SAMs increase.
Figure 7 shows the concentrations of SAMs versus the ratio of fluorescent area to total area. The ratio of fluorescent area for both kinds of SAMs increases when the concentration of SAMs increases. In addition, the ratio for 4,4-DTBA dramatically increases after the concentration of 80 nm.

Figure 5. Protein A-FITC immobilized on 11-MUA with the concentration of (a) 10 mM (b) 20 mM (c) 40 mM (d) 80 mM (e) 160 mM

Figure 6. Protein A-FITC immobilized on 4,4-DTBA with the concentration of (a) 10 mM (b) 20 mM (c) 40 mM (d) 80 mM (e) 160 mM

Figure 7. The ratio of fluorescent area to total area for 11-MUA and 4,4-DTBA at different concentrations.

Figure 8 and Figure 9 show the fluorescent images of 11-MUA and 4,4-DTBA, respectively, at different concentrations on gold patterns with the diameter of 500 um. We can see that the intensity of fluorescence increases when the concentration of SAMs increases. The ratio of fluorescent area to total area versus the concentration of SAMs is shown in Figure 10. At higher concentrations of SAMs, the ratio of 4,4-DTBA is higher than that of 11-MUA. According to Fig. 7 and Fig. 10, the SAM of 4,4-DTBA has better binding ability with protein A-FITC than 11-MUA.

Figure 8 and Figure 9 show the fluorescent images of 11-MUA and 4,4-DTBA, respectively, at different concentrations on gold patterns with the diameter of 500 um. We can see that the intensity of fluorescence increases when the concentration of SAMs increases. The ratio of fluorescent area to total area versus the concentration of SAMs is shown in Figure 10. At higher concentrations of SAMs, the ratio of 4,4-DTBA is higher than that of 11-MUA. According to Fig. 7 and Fig. 10, the SAM of 4,4-DTBA has better binding ability with protein A-FITC than 11-MUA.
IV. CONCLUSION

The feasibility of 4,4-DTBA as a monolayer for the protein chip was studied. Characterizations of surface topography, contact angle and fluorescence were conducted to investigate the binding efficiency for the SAMs of 4,4-DTBA and 11-MUA. The surface roughness measured by AFM increases with the concentrations of 11-MUA. The increase in surface roughness may result from steric hindrances and less ordered structures generated by 11-MUA due to high densities of surface function group. In addition, the results of contact angle show that the monolayer of 4,4-DTBA is more hydrophobic than 11-MUA. It may be helpful for adsorption of protein. Moreover, characterization of fluorescence resulting from protein A-FITC illustrates that the ratio of fluorescent area to total area increases with the concentrations of SAMs, especially for 4,4-DTBA. It indicates that the SAM of 4,4-DTBA has a better binding ability compared to 11-MUA. It is concluded that the SAM of 4,4-DTBA can be used as a monolayer for the protein chip and may have a comparable binding efficiency.

References

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