SURFACE CHARACTERIZATION AND A LIFT-OFF PROCESS OF A FLUOROCARBON THIN FILM FOR MICRO PROTEIN PATTERNING

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ABSTRACT
This study reports surface characterization and patterning of fluorocarbon (FC) films for micro protein patterning. By means of a contact angle analysis and an atomic force microscope (AFM), we investigated various properties of FC films, such as surface energy, wettability, surface morphology, and surface roughness. The FC films were patterned by a lift-off process. Asahi Glass’s CYTOPTM film was very efficient in preventing nonspecific binding of protein below a concentration of 1 µg/ml of the FITC-labeled BSA.

KEYWORDS: Fluorocarbon film, Protein patterning, Lift-off, Contact angles

INTRODUCTION
Recently, fluorocarbon (FC) film patterning using microfabrication processes has been studied to achieve control of cell or protein adsorption. Since FC films have low surface energy, excellent chemical resistance and thermal stability, they can be good candidates in achieving selective biomaterial adsorption [1,2].

This study introduces a simple and low cost patterning method for FC film using a lift-off process and a spin-coating method. 3M’s Fluorad™ film and Asahi Glass’s CYTOP™ film were tested for FC film patterning. The prepared FC films were analyzed by a contact angle analyzer, an atomic force microscope (AFM) and a confocal microscope. To test the feasibility for protein patterning, a fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (BSA) was selectively immobilized on micro silicon nitride (Si3N4) patterns using the FC film background.

FLUOROCARBON FILM PATTERNING BY A LIFT-OFF PROCESS
To define regions for protein patterning, a 3 µm thick positive photoresist was spin-coated and patterned by photolithography on an Si3N4-coated silicon substrate. Then, an FC solution was spin-coated at 1500 rpm to obtain the FC film. After hard baking at 110 °C for 10 min, unnecessary FC films on the photoresist patterns were removed by a
lift-off process in sonicated acetone solution. The substrate was rinsed in methanol solution and deionized (D. I.) water [3].

SURFACE CHARACTERIZATION

The surfaces of the FC films were characterized by static and dynamic contact angles, surface energy, roughness, and fluorescence intensity. Initially, 3M’s Fluorad™ film showed considerable hydrophobicity (~120°) and low surface energy (8.72 dyne/cm); however, the surface characteristics of the Fluorad™ film were degraded after chemical oxidation using a mixture of CH₃COOH and H₂O₂, showing a remarkable decrease in contact angles (70–80°) and large contact angle hystereses (~60°)

However, the CYTOP™ film preserved surface properties, showing a narrow variation of static and dynamic contact angles, contact angle hysteresis (Δθ) and surface roughness in repeated surface modification steps for the protein patterning for 16 h (3 h chemical oxidation, 12 h aminosilanization, 1 h glutaraldehyde), as shown in Table 1.

Table 1. Surface roughness, dynamic contact angles, and static contact angles of the CYTOP™ films on each step of surface modifications before protein adsorption.

<table>
<thead>
<tr>
<th>Surface modification (Reaction time)</th>
<th>AFM analysis</th>
<th>Dynamic contact angle</th>
<th>Hysteresis</th>
<th>Static contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra / Rrms (Å)</td>
<td>θ advancing / θ receding</td>
<td>Δθ</td>
<td>θ D. I. water</td>
</tr>
<tr>
<td>Sonication (4 min)</td>
<td>6.04 / 7.92</td>
<td>115.39 / 101.90</td>
<td>13.49</td>
<td>112.19</td>
</tr>
<tr>
<td>Chemical oxidation (3 h)</td>
<td>7.80 / 12.3</td>
<td>114.84 / 103.83</td>
<td>11.01</td>
<td>111.63</td>
</tr>
<tr>
<td>Silanization (12 h)</td>
<td>10.9 / 15.0</td>
<td>114.91 / 103.42</td>
<td>11.49</td>
<td>112.98</td>
</tr>
<tr>
<td>Glutaraldehyde (1 h)</td>
<td>10.8 / 14.1</td>
<td>114.03 / 101.67</td>
<td>12.36</td>
<td>109.29</td>
</tr>
</tbody>
</table>

A nonspecific binding problem appeared at greater than 2 µg/ml in investigating the nonspecific binding on an FC film surface as a function of FITC-labeled BSA concentration. Surface roughness, contact angle hysteresis, and the fluorescence intensity increased at a concentration above 2 µg/ml (Table 2, Figure 1). In particular, the receding angle was confirmed to be more sensitive to surface degradation by the nonspecific binding of the FITC-labeled BSA than other contact angles. Another noticeable finding was that the static contact angles could hardly discriminate the surface degradation of the FC films.
Table 2. Surface roughness, dynamic contact angles, and static contact angles of the CYTOP™ films as a function of FITC-labeled BSA concentration.

<table>
<thead>
<tr>
<th>BSA adsorption concentration (1 h)</th>
<th>AFM analysis</th>
<th>Dynamic contact angle</th>
<th>Hysteresis</th>
<th>Static contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra / Rms (Å)</td>
<td>θ_{advancing}</td>
<td>θ_{receding}</td>
<td>Δθ</td>
</tr>
<tr>
<td>1 μg/ml</td>
<td>7.27 / 11.81</td>
<td>114.07</td>
<td>99.37</td>
<td>14.70</td>
</tr>
<tr>
<td>2 μg/ml</td>
<td>10.70 / 19.09</td>
<td>113.51</td>
<td>97.64</td>
<td>15.87</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>9.81 / 16.40</td>
<td>111.50</td>
<td>93.30</td>
<td>18.20</td>
</tr>
<tr>
<td>10 μg/ml</td>
<td>16.11 / 23.21</td>
<td>111.82</td>
<td>92.72</td>
<td>19.10</td>
</tr>
</tbody>
</table>

Figure 1. Fluorescence intensity of FITC-labeled BSA adsorbed on the CYTOP™ film surface after surface modifications as a function of FITC-labeled BSA concentration.

PROTEIN PATTERNING USING A PATTERNED CYTOP™ FILM

After surface modification according to the steps described in Table 1, protein patterning using the FITC-labeled BSA was performed on an Si$_3$N$_4$ micro pattern with the CYTOP™ film background. A significant reduction in background signal on the CYTOP™ film was observed in the concentration range below 1 μg/ml FITC-labeled BSA, as expected from the surface characterization of the FC films as a function of FITC-BSA concentration. Figure 2 shows the patterning result for a concentration of 1 μg/ml FITC-labeled BSA. When the concentration of the FITC-labeled BSA was varied from 10 ng/ml to 600 ng/ml, the signal to noise ratio (SNR) linearly increased in the range from 2.27 ± 0.08 to 22.99 ± 2.32 [3].
CONCLUSIONS
A fluorocarbon (FC) thin film patterning method using a lift-off process was developed to be applicable to hydrophobic surface control of micro protein patterning.

The CYTOP™ film was shown to preserve the surface properties, having a narrow variation of static and dynamic contact angles, contact angle hysteresis and surface roughness after repeated surface modification steps for protein patterning for 17 h (3 h chemical oxidation, 12 h aminosilanization, 1 h glutaraldehyde, 1 h 1 μg/ml-BSA adsorption).

It was observed that a nonspecific binding problem appeared at greater than 2 μg/ml when investigating the nonspecific binding on the FC films with FITC-labeled BSA. In particular, the receding angle was sensitive in observing surface degradation due to the nonspecific binding of FITC-labeled BSA protein.

We could make 100 μm diameter protein patterns with high signal to noise ratio in the range from 10 ng/ml to 600 ng/ml.

ACKNOWLEDGEMENT
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REFERENCES