Characterization and quantification of biological micropatterns using cluster SIMS

Li-Jung Chen, Sunny S. Shah, Stanislav V. Verkhoturov, Alexander Revzin and Emile A. Schweikert

Introduction

Micropatterning is used widely in biosensor development, tissue engineering and basic biology. Creation of biological micropatterns typically involves multiple sequential steps which may lead to cross-contamination and contribute to suboptimal performance of the surface. Therefore, there is a need to develop novel strategies for characterizing location-specific chemical composition of biological micropatterns. In this paper, C60+ time-of-flight secondary ion mass spectrometry (ToF-SIMS) operating in the event-by-event bombardment/detection mode was used for spatially resolved chemical analysis of micropatterned indium tin oxide (ITO) surfaces. Fabrication of the micropatterns involved multiple steps including self-assembly of poly(ethylene glycol)-silane (PEG-silane), patterning of photoresist, treatment with oxygen plasma and adsorption of collagen (I). The ITO surfaces were analyzed with 26-keV C60+ SIMS run in the event-by-event bombardment/detection mode at different steps of the modification process. We were able to evaluate the extent of cross-contamination between different steps and quantify coverage of the immobilized species. The methodology described here provides a novel means for characterizing the composition of biological micropatterns in a quantitative and spatially resolved manner. Copyright © 2010 John Wiley & Sons, Ltd.

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Experimental

Materials

The surface micropatterning approach employed here was partly based on previously described procedures.[7,8] This method (summarized in Fig. 1) involves functionalization of indium tin oxide (ITO) glass (Delta Technologies, Stillwater, MN) with poly(ethylene glycol)-silane (PEG-silane), resulting in a ∼5-nm-thick self-assembled silane layer. A layer of photoresist (PR) (AZ 5214-E) is lithographically patterned on top of the PEG-modified surfaces and serves as a protective stencil during exposure of the micropatterned surface to an O2 plasma (300 W for 5 min). Thus PEG is removed from the ITO substrate not protected by PR. The removal is followed by adsorption of collagen (I) onto micropatterned surface. Incubation of the surface in acetone results in PR liftoff so that the ITO substrate contains collagen (I) (Sigma Aldrich, St Louis, MO) regions surrounded by PEG-silane. The micropatterned surface is with circular patches of 5–10 nm in diameter, and the distance between two patches is 250 µm. As a proof of concept, we sought to characterize surfaces with 26-keV cluster C60+ ToF-SIMS run in the event-by-event bombardment/detection mode after steps 3 and 6 in Fig. 1.

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The analyzer used in this study is shown in Fig. 2. C₆₀ powder is placed on extraction plate and focused with electrostatic lenses. The primary ions are extracted with voltage applied into a Cu heating reservoir and is heated until it sublimes. The effusion vapor of C₆₀ is ionized by electrons emitted from a heated tungsten wire. The primary ions are extracted with voltage applied on extraction plate and focused with electrostatic lenses. The C₆₀ projectile is then mass-selected with a Wien filter and steered toward an off-center aperture that deflects ions and prevents the neutrals from impacting the target. The total impact energy on the target sample is 26 keV. Electrons emitted from target are deflected by a weak magnetic field to strike the start detector (start signal). Secondary ions experience ToF separation and are detected with a stop detector (stop signal). The stop detector contains a microchannel plate assembly with an 8-anode detector which allows the detection of up to eight isobaric ions per event. The data are acquired in an event-by-event bombardment/detection mode. We obtained secondary ion mass spectra by accumulating approximately 2 million impact emission events. By selecting a specific ion of interest, the coincidence secondary ions can be extracted from an accumulation of secondary ion counts, resulting in a coincidence ion mass spectrum.

**Results and Discussion**

We apply here the concept of coincidence ion emission for testing the chemical homogeneity of patterned surface areas. The quantitative methodology is used to characterize the fabrication quality of micropatterns and the incubated amount of collagen on micropatterned surface. Consider the mass spectrum obtained...
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Figure 2. Schematic of the C$_{60}$ SIMS instrument.[14]

Figure 3. The negative-ion mass spectrum collected from surfaces corresponding to step 3 in Fig. 1. PEG-modified ITO substrate is covered with a PR pattern of 100-µm-diameter holes with center-to-center spacing.

from step 3 in the procedure diagramed in Fig. 1. The spectrum presented in Fig. 3 shows multiple peaks corresponding to PR and PEG. The spectrum of ions in coincidence with $m/z = 107$ ($\text{CH}_2\text{C}_6\text{H}_4\text{OH}^-$) from PR (not shown) contains other peaks due to the same compound. Conversely, a spectrum of ions in coincidence with $m/z = 223$ (PEG-$\text{C}_3\text{H}_7\text{SiO}_2^-)$ from PEG resembles the spectrum from PEG alone (not shown). Figure 3 summarizes the data obtained from probing ∼2 million nanovolumes on the micropatterned surface. The question at hand is, what percentages of the overall area probed (∼250 × 250 µm) are covered by PR and by PEG, respectively? The methodology for a quantitative estimate of the coverage of a given species has been described previously.[15] Briefly, we assume that two co-emitted ions, A and B, originating from the same compound (in the present case from emitted PR or PEG) have a correlation coefficient $Q_{A,B}$ of unity. $Q_{A,B}$ is computed as follows:

$$Q_{A,B} = \frac{Y_{A,B}}{Y_A Y_B} = 1$$

where $Y_{A,B}$ is the coincidental yield of simultaneously detected ions A and B. $Y_A$ and $Y_B$ are the secondary ion yields of detected ions A and B, respectively. The coincidental yield $Y_{A,B}$ is

$$Y_{A,B} = \frac{I_{A,B}}{N_e}$$

where $N_e$ is the effective number of impacts on a specific specimen and $I_{A,B}$ is the number of co-emitted ions A and B, recorded in the coincidental mass spectrum. The secondary ion yields of ion A and
The fractional coverage \( K \) for PR is obtained from the fragment ions at \( m/z \) 107 (CH\(_2\)C\(_6\)H\(_4\)OH\(^-\)) and 227 (C\(_{18}\)H\(_{13}\)(OH)\(_2\)^-). For the sample shown in step 3 of Fig. 1, the value of \( K \) was 83 ± 1%. A similar calculation of \( K \) as the fractional coverage of PEG yielded a value of ~17%. The mask applied for producing the micropattern had a circular patch of 100 \( \mu \)m in diameter (the area in which PEG can be detected) set in a square of 250 × 250 \( \mu \)m covered by PR, except for the nominal 100-\( \mu \)m-diameter patch of PEG in the square’s center. Based on the mask dimensions, approximately 87% of the square still has been covered by PR. The difference between the nominal coverage and the experimentally determined value may be due to imperfect transfer between the photomask and the PR layer.

The validity of culturing cells on ITO surfaces depends on the clean removal of PR from the cell-adhesive, collagen-containing patches because PR residues may be toxic to cells. We tested the presence of collagen in the cell culturing patches via detection of CN\(^-\), CNO\(^-\) and C\(_3\)N\(^-\). The fractional coverage in the ‘collagen islands’ of the total micropatterned surface was determined to be 19 ± 1%. This value was obtained using the co-emission of CN\(^-\) and CNO\(^-\) (Fig. 4). The fractional coverage of collagen should be the same as that of PEG obtained earlier. There is reasonable agreement between the values of 19 and 17% for collagen and PEG, respectively, which validates the collagen adsorption procedure. Another important observation is that virtually no PR ions were co-emitted with CNO\(^-\) (data not shown). This test confirms the efficiency of the PR removal from the micropatterned surface.

**Figure 4.** The negative-ion mass spectrum of collagen-containing micropatterns after the removal of the photoresist (corresponding to step 6, Fig. 1).

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>Ion</th>
<th>Intensity</th>
</tr>
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<tbody>
<tr>
<td>107</td>
<td>CH(_2)C(_6)H(_4)OH(^-)</td>
<td>0.001</td>
</tr>
<tr>
<td>227</td>
<td>C(<em>{18})H(</em>{13})(OH)(_2)^-</td>
<td>0.002</td>
</tr>
<tr>
<td>17</td>
<td>CN(^-)</td>
<td>0.003</td>
</tr>
<tr>
<td>19</td>
<td>CNO(^-)</td>
<td>0.004</td>
</tr>
<tr>
<td>22</td>
<td>C(_3)N(^-)</td>
<td>0.005</td>
</tr>
<tr>
<td>80</td>
<td>SiO(_2)OH(^-)</td>
<td>0.006</td>
</tr>
<tr>
<td>100</td>
<td>HSO(_4)^-</td>
<td>0.007</td>
</tr>
</tbody>
</table>

**Conclusion**

The coincidence mass spectrometry methodology is well suited for testing micropatterned surfaces. It provides a qualitative test of the chemical integrity of surface patches. It can further determine the fractional coverage of surface components. The test case presented here illustrates the key role of event-by-event bombardment/detection SIMS for validating surface engineering procedures.

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**References**