

Characterization and quantification of biological micropatterns using cluster SIMS

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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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Introduction

Micropatterned surfaces are used widely in tissue engineering, cell biology, high-throughput screening and biosensors.^[1–3] Processes for fabricating micropatterned surfaces are often complex, involving multiple steps and reagents. Cross-contamination between the steps may lead to suboptimal performance of the surface: for example, lack of specificity of a biosensor or toxicity of a cell culture substrate. Most commonly, characterization of biological micropatterns involves immunofluorescence staining and/or imaging by electron or atomic force microscopy. While informative, these approaches provide limited information about the chemical species present on the micropatterned surface. On the other hand, secondary ion mass spectrometry (SIMS) may be used to analyze chemical composition of the surface without the need for fluorescence tags.^[4–7] Moreover, imaging SIMS provides a view of micropatterned surfaces based on contrasts in ion intensity; however, this method obtains a total mass spectrum of micropatterned surfaces relying on statistical analysis to characterize local surface composition.^[1,7,8] Our laboratory has been developing cluster time-of-flight SIMS (ToF-SIMS) operating in an event-by-event bombardment/detection mode.^[9,10] In this approach, a single projectile impact results in a hemispherical 'crater' of 5–10 nm in diameter and mass spectra of impacts are detected one at a time.^[9,11–13] This mode allows the detection of co-emitted ions from individual projectile impacts, since each single impact emission is resolved in time and space.

In the present study, we demonstrate the application to the characterization of biological micropatterns. This approach offers a novel means of quantitative, location-specific analysis of biological micropatterns.

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 100 μm 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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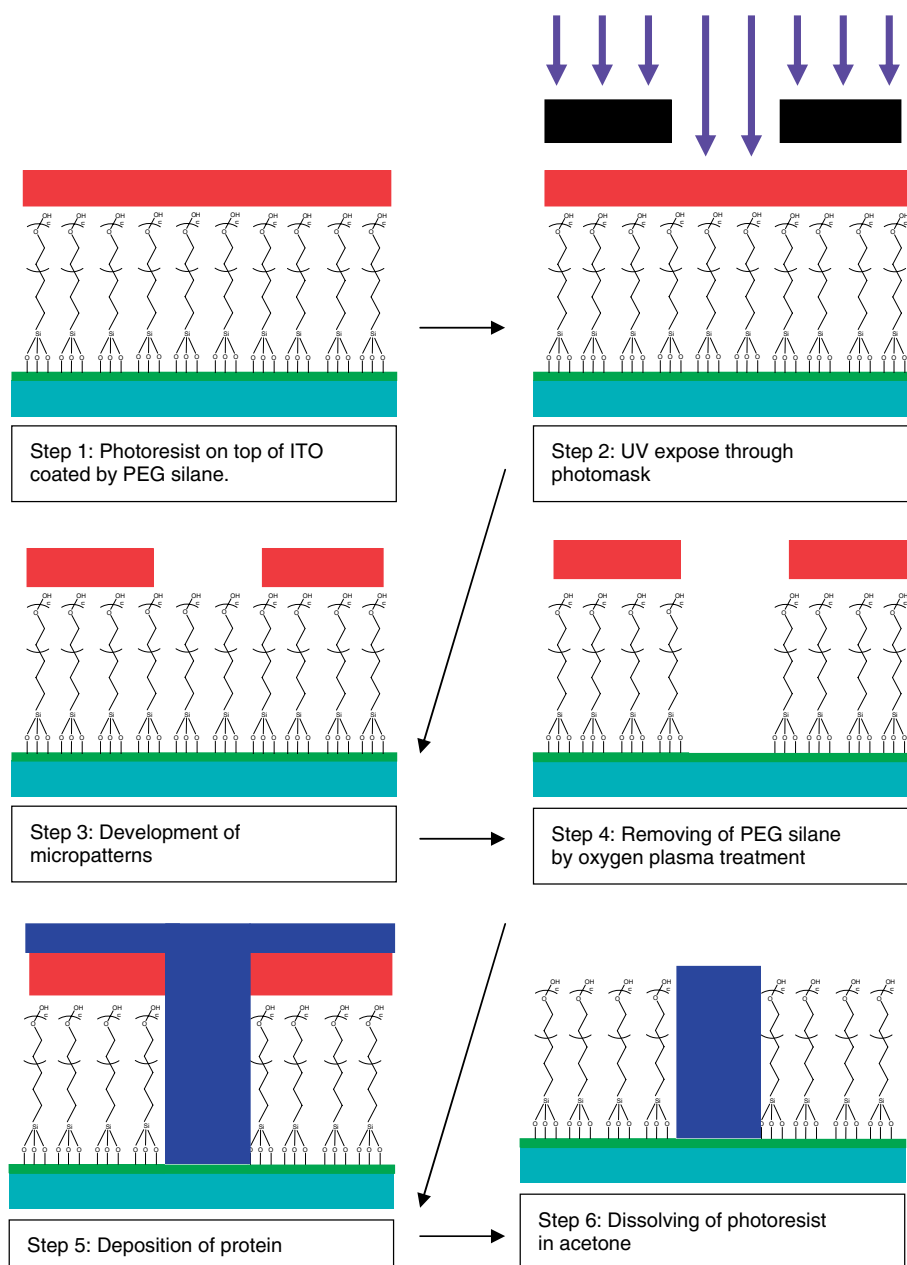


Figure 1. Schematic illustration of the fabrication of protein-attached micropatterns.

Bombardment/detection

A schematic of the C_{60}^+ effusion source coupled to the ToF mass analyzer used in this study is shown in Fig. 2. C_{60} powder is placed into a Cu heating reservoir and is heated until it sublimates. The effusion vapor of C_{60} 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 C_{60}^+ 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.^[14] 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.^[9]

Results and Discussion

We apply here the concept of coincidence ion emission for testing the chemical homogeneity of patterned surface areas.^[9] 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

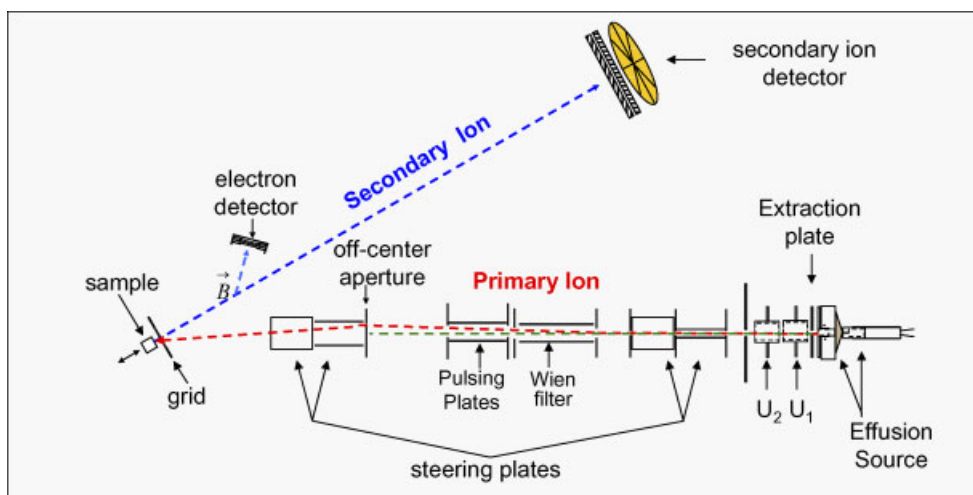


Figure 2. Schematic of the C_{60} SIMS instrument.^[14]

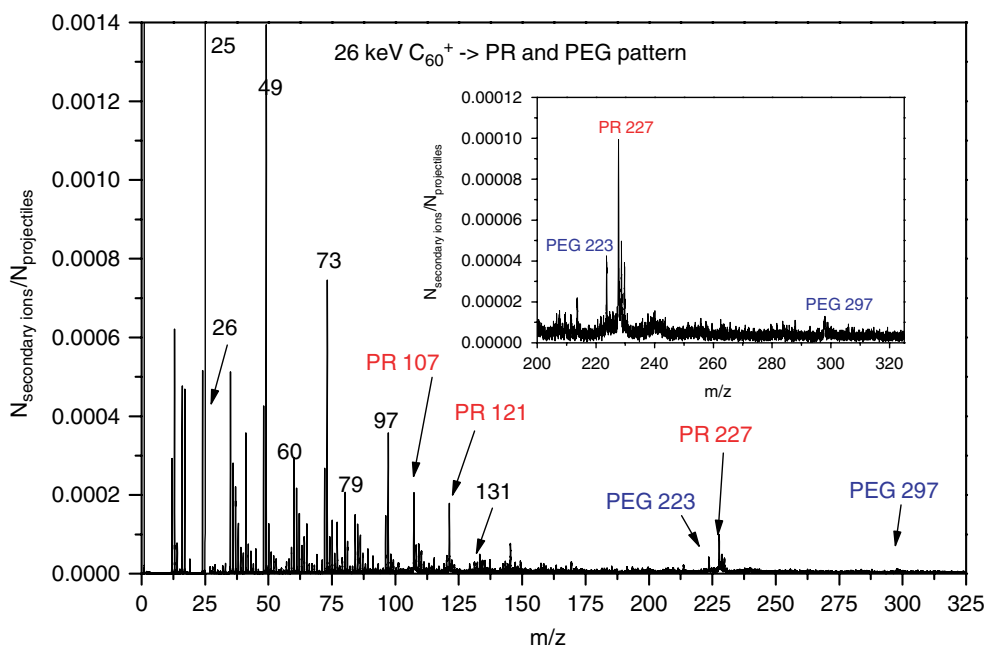


Figure 3. The negative-ion mass spectrum collected from surfaces corresponding to step 3 in Fig. 1. PEG-modified ITO substrate is covered with a PR pattern of 100- μm -diameter holes with center-to-center spacing.

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 $m/z = 223$ ($\text{PEG-C}_3\text{H}_7\text{SiO}_2^-$) 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 ($\sim 250 \times 250 \mu\text{m}$) 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:

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

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

$$Y_{A,B} = \frac{I_{A,B}}{N_e} \quad (2)$$

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

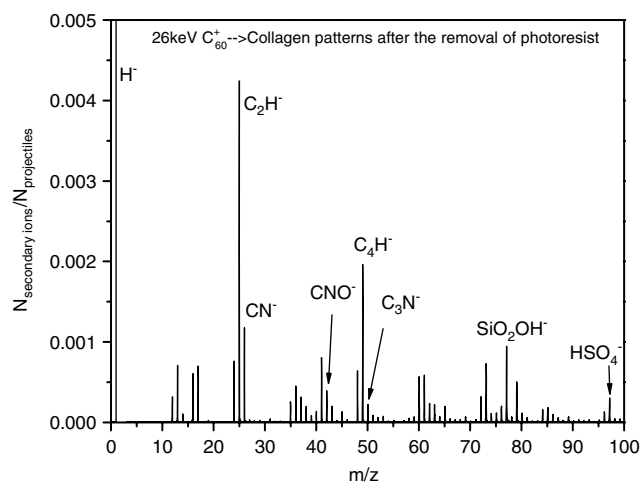


Figure 4. The negative-ion mass spectrum of collagen-containing micropatterns after the removal of the photoresist (corresponding to step 6, Fig. 1).

B are computed as follows:

$$Y_A = \frac{I_A}{N_e} \quad (3)$$

$$Y_B = \frac{I_B}{N_e} \quad (4)$$

where I_A and I_B are the peak areas of ions A and B, respectively. Using Eqns (1)–(4) one can calculate N_e :

$$N_e = \frac{I_A I_B}{I_{A,B}} \quad (5)$$

For a practical application, we use the coverage coefficient K to express the fractional coverage of specimens on the micropatterned surface:

$$K = \frac{N_e}{N_0} \times 100\% \quad (6)$$

where N_0 is the total number of impacts. The effective number of impacts on a specific specimen, N_e (given in Eqn. 5), does not depend on ionization probabilities and detection efficiencies of ions A and B. Thus, for surface objects that are larger than the size of the emission volume,^[15] the fractional coverage can be calculated using the coincidental method. Knowledge of the ionization probabilities of the co-emitted ions is not required.

The fractional coverage K for PR is obtained from the fragment ions at m/z 107 ($\text{CH}_2\text{C}_6\text{H}_4\text{OH}^-$) and 227 ($\text{C}_{15}\text{H}_{13}(\text{OH})_2^-$). For the sample shown in step 3 of Fig. 1, the value of K was $83 \pm 1\%$. A similar calculation of K as the fractional coverage of PEG yielded a value of $\sim 17\%$. The mask applied for producing the micropattern had a circular patch of 100 μm in diameter (the area in which PEG can be detected) set in a square of $250 \times 250 \mu\text{m}$ covered by PR, except for the nominal 100- μm -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_3N^- . The fractional coverage in the 'collagen islands' of the total micropatterned surface was determined to be $19 \pm 1\%$. 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.

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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