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Physical vs. photolithographic patterning of plasma polymers: an investigation by ToF-SSIMS and multivariate analysis

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Abstract

Physical and photolithographic techniques are commonly used to create chemical patterns for a range of technologies including cell culture studies, bioarrays and other biomedical applications. In this paper, we describe the fabrication of chemical micropatterns from commonly used plasma polymers. Atomic force microscopy (AFM) imaging, Time-of-Flight Static Secondary Ion Mass Spectrometry (ToF-SSIMS) imaging and multivariate analysis have been employed to visualize the chemical boundaries created by these patterning techniques and assess the spatial and chemical resolution of the patterns. ToF-SSIMS analysis demonstrated that well defined chemical and spatial boundaries were obtained from photolithographic patterning, while the resolution of physical patterning via a transmission electron microscopy (TEM) grid varied depending on the properties of the plasma system including the substrate material. In general, physical masking allowed diffusion of the plasma species below the mask and bleeding of the surface chemistries. Multivariate analysis techniques including Principal Component Analysis (PCA) and Region of Interest (ROI) assessment were used to investigate the ToF-SSIMS images of a range of different plasma polymer patterns. In the most challenging case, where two strongly reacting polymers, allylamine and acrylic acid were deposited, PCA confirmed the fabrication of micropatterns with defined spatial resolution. ROI analysis allowed for the identification of an interface between the two plasma polymers for patterns fabricated using the photolithographic technique which has been previously overlooked. This study clearly demonstrated the versatility of photolithographic patterning for the production of multichemistry plasma polymer arrays and highlighted the need for complimentary characterization and analytical techniques during the fabrication plasma polymer micropatterns.

Keywords

ToF-SSIMS; Principal component analysis (PCA); plasma polymer; patterning; photolithography

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Supporting Information Available

The supporting information section contains the AFM topographical images corresponding with the line scans presented in Figure 1 (Figure S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

1. Introduction

The field of biological sensing consists of a wide range of areas based on both well-established and emerging technologies. It has become apparent that in order to better understand biology, surfaces that present arrays of different immobilized oligonucleotides/DNA/RNA (genomics), antibodies/proteins/peptides (proteomics) or cells (cellomics) are an indispensable tool.¹⁻⁵ The high-density, high-sensitivity multi-analyte chips required for such sensors can only be successfully produced with a precise and reproducible patterning methodology that allows controlled positioning of chemically distinct bioactive and bio-inert areas

Plasma polymerization has gained significant interest in recent years for the creation of chemically functionalized surfaces for use in biomedical applications.^{6, 7} It is typically a single step process that produces adherent coatings of controllable thickness (between 5 and ~100nm) and thus is a straight forward tool for the chemical modification of many biomedically relevant surfaces. Through the careful control of the deposition parameters (i.e. power, temperature), it is possible to maintain a high degree of the chemical functionality associated with the monomeric precursor.⁸ This technique allows for the polymerization of well established monomers including acrylic acid,⁹ to more unique systems including plant extracts (1-8 cineole).¹⁰ Previous work has involved the fabrication of protein resistant coatings^{11, 12} and coatings to improve the attachment of biomolecules¹³ and cells.^{14, 15}

In general, the chemical patterning techniques commonly used in bioengineering can be divided into four categories (i) lithographic patterning, (ii) scanning probe microscopies (SPM),^{16, 17} (iii) transfer via polymeric stamps (soft lithography),¹⁸ and (iv) physical patterning. The challenge with plasma polymerization lies in developing patterning techniques that address both the specific issues related to the gas phase deposition process and that can be readily integrated into the processing routes without damaging the polymer films. A number of different approaches to the patterning of plasma polymer have been taken by different groups. One of the simplest routes involves the use of physical mask. A solid material or mask is effectively used to block a section of the surface and direct deposition onto defined areas of the substrate. Some examples include the use of polymeric masks¹⁹ and TEM grids.^{20, 21} Alternate approaches involve post processing of the surface to remove or chemically modify specific regions of the surface. This has been achieved via a number of routes including ion beam etching²² and plasma etching through masks²³ as well as electron beam lithography,²⁴ all of which have been shown to be able to produce chemically resolved micro- and in some cases nano- scale plasma polymer patterns.

The use of photolithographic patterning for plasma polymers was first demonstrated by Goessl.²⁵ Since that time a number of groups have utilized this approach to both develop patterned single chemistry plasma polymers, using the resists as masks to etch through a plasma polymer film,²³ or selectively perform a wet chemical step.²⁶ The ability to produce patterns from multiple plasma polymers lay at the heart of the initial studies by Goessl and forms the basis of this study. This approach is particularly attractive in light of the desire to create increasingly complex chemical arrays and the opportunity presented by plasma polymerization to produce thin films from a very wide range of different monomers. As such, patterns with alternating surface charge, hydrophilicity, biospecificity and adhesion properties can all be envisaged using photolithographic patterning of plasma polymers. Of course to achieve many of the specific properties i.e. non-fouling or spontaneously reactive films, very specific deposition conditions are used and the coatings may vary significantly in stability, particularly when exposed to solvents and even aqueous solutions commonly used in photolithography. An added complexity is the possible cross-reactivity of the polymers, particularly when a deposited film is exposed to a second monomer in the gas phase. The challenge then lies in understanding

how the patterning technique may affect the coating chemistries, and which combinations of chemistries are possible with different patterning techniques.

In this paper, we have examined the physicochemical aspects of two types of patterning techniques for plasma polymers, physical masking and photolithography. Micropatterns were fabricated with plasma polymers derived from glycidyl methacrylate (ppGMA), maleic anhydride (ppMA), acrylic acid (ppAAc), allylamine (ppAAM) and tetraglyme (ppTG). These systems were chosen as they presented an opportunity to examine a wide range of final film properties, deposition conditions and allow for the investigation of cross reactivity between film chemistries. Atomic force microscopy (AFM) was initially employed to investigate the physical boundaries created by the patterning techniques. Time-of-Flight Static Secondary Ion Mass Spectrometry (ToF-SSIMS) analysis and imaging of the patterned surfaces was then undertaken to resolve the different chemistries and assess the chemical and spatial resolution of the patterning processes. The results clearly illustrate that using photolithographic patterning it is possible to create multi-chemistry patterns with both spatial and chemical resolution down to 1 μm . Critically, the application of multivariate analysis techniques to the ToF-SSIMS images demonstrated that there was an identifiable interface between the chemistries produced using photolithography. This interface was found to comprise of residual Na, which varied the ion yields in these regions, in addition to containing a combination of the two plasma polymer chemistries. The results demonstrated that while the patterning process had little effect on the chemical properties of the films from a wide range of different plasma polymers, application of the appropriate characterization techniques are necessary to obtain a true representation of the spatial resolution and chemical specificity of these patterned surfaces.

2. Experimental

2.1. Materials

Acrylic acid (AAc), allylamine (AAM), glycidyl methacrylate (GMA), maleic anhydride (MA), tetraglyme (tetraethylene glycol dimethyl ether, Tg) and were all purchased from Aldrich UK at purity >98%. Silicon wafers (Orientation: <100>, Thickness; 525 microns, Dopants: P.Boron, Resistivity: 1-10 Ohm cm, Polish: one side) were purchased from Compart Technology Ltd UK. AZ 1512 Photoresist and AZ 351B Developer were both purchased from MicroChemicals, GmbH, and copper transmission electron microscopy (TEM) grids from Agar Scientific Ltd., UK. All other chemicals were purchased from Aldrich UK and used as supplied.

2.2 Plasma polymerization

Prior to plasma polymerization, each of the monomers was degassed several times using freeze thaw cycles. Si substrates were placed under a stream of air after dicing to remove any dust or silicon particles. Substrates were then sonicated several times in isopropyl alcohol (IPA), and then stored in IPA prior to use. Substrates were blown dry with high purity nitrogen gas before plasma deposition.

All polymers were fabricated in a stainless steel T-piece reactor with an internal aluminium disc electrode,¹² with the exception of allylamine which was generated in a T-piece glass reactor with an external helical copper coil electrode.²⁷ The experimental setup has been explained in detail previously.²⁸ Briefly, the radio frequency power source (13.56 MHz, Coaxial Power Systems Ltd., UK) was coupled to the reactor via an impedance matching network. The substrates were placed in the reactor and the vessel was pumped down to a base pressure of 1×10^{-3} mbar. Deposition parameters including deposition power, time and monomer flow rate were fixed at the values given in Table 1.

2.3 Surface Patterning

2.3.1 Physical patterning—A TEM grid was carefully placed onto the untreated or plasma polymer coated silicon wafer. A wide range of standard size patterns were used in the study to vary the resultant pattern features from approximately 5 μm to 200 μm . Once the TEM grid was positioned on the surface, the samples were placed carefully in the plasma reactor. No external pressure was applied to the TEM grids in order to force them into contact with the substrate surface. The plasma chamber was evacuated slowly to avoid any displacement of TEM grids. All TEM grids were used as supplied and discarded after one use. All attempts were made to ensure that the TEM grids were flat against the surface of the substrate during deposition.

2.3.2. Photolithographic patterning—The process of patterning plasma polymers using photolithography was adapted from the methods of Goessl et al.²⁵ The positive photoresist (AZ 1512, MicroChemicals GmbH) was spin coated at 4000 r.p.m onto either the bare silicon wafer or the base plasma polymer. The final resist thickness was 1-1.5 μm . Substrates were then heated to 90 °C for 120 s to harden the resist and remove any excess solvent. The substrates were then brought into intimate contact with an optical photomask and exposed to ultraviolet radiation (Electronic Vision A16-2 mask aligner) for 5 s. The light-exposed photoresist was removed from the surface by immersing the sample in an alkaline developer (AZ 351B, MicroChemicals, GmbH) at a dilution ratio of 1:4 (developer: water) for 30 seconds. Samples were rinsed several times with ultra pure water and dried in a stream of nitrogen, ready for analysis or the deposition of the second plasma polymer layer. After deposition of the second polymer, the remaining photoresist was dissolved from between the films by immersing the samples in a solution of acetone and sonicating them for 3×20 s. The samples were then extensively washed with MilliQ grade water and dried under a stream of nitrogen.

2.4 AFM imaging

Atomic force microscopy was conducted using a Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA). Surface modified silicon wafers were attached to steel discs using silicone free double sided-tape and subsequently mounted onto the piezoelectric stage. The measurements were made in tapping mode using a 'J scanner'. Measurements were made in air. Tapping mode phosphorous doped silicon probes purchased from Veeco possessed spring constants and resonant frequencies of 20-80 N/m and 232-264 kHz respectively. AFM surface analysis was carried out at multiple sample sites and the images shown are representative of the results.

2.5 ToF-SSIMS Spectroscopy and Imaging

ToF-SSIMS spectra and images were obtained using a ToF SIMS V instrument (ION TOF Inc. Munster, Germany). The analysis chamber was held at $\sim 1 \times 10^{-9}$ mbar during experiments. The primary ion beam was generated using a liquid metal ion gun fitted with a pure bismuth ion source capable of producing Bi_n^{p+} ($n = 1-6$, $p = 1-2$) ions. The kinetic energy of these ions was 25 keV for singly charged ions and 50 keV for doubly charged ions. The angle of incidence of the primary ion beam relative to the sample surface was 45°. Bi^{3++} primary ion source with a kinetic energy of 50keV was used for all image and spectral data acquisition. The primary ion currents, measured using a Faraday cup located on the grounded sample holder, were typically around ~ 0.1 pA (Bi^{3++}) at 10 kHz. Due to the complex chemical nature of the surfaces being analysed, all data was collected in high mass resolution bunched mode to ensure the highest possible mass resolution and to enable identification of the specific fragments from each of the plasma polymers, whilst still retaining good spatial resolution.

Positive and negative ion spectra and images were acquired for each sample. The spectra were acquired from a $100 \times 100 \mu\text{m}^2$ analysis area while the imaging areas were varied (25×25 to

500 × 500 μm^2) according to the experimental requirement. Each positive ion spectra were calibrated using CH_3^+ (m/z 15.023), C_2H_3^+ (m/z 27.023), C_3H_5^+ (m/z 41.039) and C_7H_7^+ (m/z 91.054) peaks. The negative ion spectra were calibrated using CH^- (m/z 13.007), C_2H^- (m/z 25.007) COOH^- (m/z 44.997) and C_4H^- (m/z 49.007) peaks. Calibration errors were kept below 15 ppm in all the cases. Mass resolution ($m/\Delta m$) for typical spectra ranged between 7000 and 9000, calculated at m/z 44.997 (COOH^-) for negative ions and m/z 41.039 (C_3H_5^+) for positive ions respectively. All spectra and images were acquired by setting the primary ion dose density below the static limit (1×10^{13} ions/ cm^2). Spectra and images from multiple points (<3) on each sample were acquired and care was taken to use a new sample area for each analysis. All images contained 128 × 128 pixels. The field of view is given in figure legends.

Multivariate analysis of ToF-SSIMS spectra and images was performed using MatLab 7.4 (R2007a, Mathwork Inc, USA), NBScriptGUI and IonToF-Pak (NESAC/BIO, University of Washington, Seattle, USA), Eigenvector PLS-toolbox Version 3.5, and Eigenvector Solo Version 4.2 (Eigenvector Inc. USA) software. The Ion-TOF spectral data (.dat) files were read directly into IonToF-Pak. Initially, a peak list was created by including peaks across the mass range (usually m/z 0-300) that had raw intensities above 100. The integration limits were checked on each peak individually to ensure the correct area was being measured on each of the spectra. This Ion-ToF-Pak peak list was then used to create a table for all of the spectra from the samples on which multivariate analysis was required. The results of this preparation were in $n \times m$ matrix format where the rows were samples (spectra) and the column were variables (peaks). The sample data in this new matrix were the normalized to the total intensity of the respective spectra for each sample. This normalization helped in compensating for the variations in the instrument over the time of acquisition. Before the PCA analysis, the data were mean centered. Data normalization and mean centering were performed using NBScriptGUI software interface.

PCA—All secondary ion peaks with intensity value above 100 were selected on the image data file (Ion-ToF .RAW file) and exported as one .bif file (binary image file) using Ion-ToF image processing software. The .bif file was read into MatLab and imported into PLS or Solo toolbox workspace. In multivariate image analysis, the samples are the spectra from each pixel and the variables are the peaks in those spectra. Principal component analysis was run on image matrices in their unfolded two-dimensional form; thus, the spectrum from each pixel was treated as a separate sample in the analysis. Spectrum at individual pixel was normalized to the total ion intensity of that individual pixel and the dataset was mean-centered. Principal component score images were constructed by plotting the score value of each pixel in X-Y space, when the dataset was folded back by adding the spatial pixel information.

ROI Analysis—Scatter plots of two uncorrelated images display the intensity values of the image A on the x-axis and the intensity values of the image B on the y-axis. Each pixel of the two original images was assigned to a certain pair of coordinates in the scatter plot. The frequency of the occurrence of an intensity combination in A and B (corresponding to certain coordinates in the scatter plot) is represented in the scatter plot by its color. In context, if the intensities of secondary ion fragments originating from two different chemical regions are displayed on the scatter plot, the pixel distribution can not only be used to investigate details from these unique chemical areas but also to provide information about the polymer – substrate interface or chemical ‘crossover’ regions.

3. Results

3.1. Physical vs. Photolithographic Patterning – AFM Analysis

AFM data was used to determine the spatial resolution achieved at the interface between the plasma polymer and either the bare substrate or plasma polymer underlayer (Figure 1) (See Figure S1 supplementary data for corresponding topographic images). The resolution of patterns produced using a TEM grid as a physical mask were examined with patterns formed by the deposition of ppGMA directly on the Si wafer and on top of a ppTg thin film respectively (Figure 1 a) and 1 b)). The AFM line scans for this patterning technique demonstrate that there was diffusion under the mask during the deposition process, resulting in a diffused interface rather than a sharp step. For ppGMA on Si, the diffusion distance was approximately 4 μm based on the 80% - 20% resolution definition (See Figure S1). Interestingly, when the same polymer was deposited onto the surface precoated with ppTg the diffusion distance increased to approximately 9 μm .

The same set of samples were then prepared using photolithographic patterning and the resulting AFM line scans are shown in Figure 1 c) and d). The results indicate a sharp interface between the ppGMA and each of the substrates (width approximately 0.1 μm), with no diffusion below the photolithographic mask. While on the silicon substrate there appears to be little residual material on the surface, the line scan for the ppTg coated sample demonstrated a narrow lip at the edge where the plasma polymers intersect. The thickness increase in this region (~4nm) does not correspond to the full thickness of the photoresist (~1 μm) and is ~100nm wide, but may indicate that a small amount of material is retained or trapped at this interface where the two plasma polymers meet. Comparison of AFM step heights with homogeneous film ellipsometric thicknesses measurements carried out on samples prepared under identical plasma conditions show that the processing required for the photolithographic patterning resulted in negligible loss of film thickness (data not shown).

To explore the spatial resolution that could be obtained by photolithographic patterning of plasma polymers, a custom designed optical mask was used to create patterns of ppGMA on ppTTg coated Si wafers. The pattern has 1 μm lines separated by 1 μm in a cross hatch arrangement as shown in Figure 2 c). The AFM topographical images (Figure 2 a) and d)) and line scan (Figure 2 b)) clearly indicated that this complex pattern was not able to be translated using the current photolithography approach. However, it should be noted that at the extremes of the photolithographic region, where 1 μm regions are separated by greater than 1 μm the line scans across the pattern edge (see Figure 2 b), the pattern is reproduced with two regions clearly identifiable with FWHM widths of 1.2 μm . The observation of pattern resolution in the experiment suggests that it is the feature 'spacing' and rather than the feature 'size' which may control the spatial resolution achievable by photolithographic patterning of plasma polymers. The plasma polymer films are <50nm thick, while the photo resist layer is of the order of 1 μm thick, as such, the mechanical properties of the plasma polymer film and its resistance to tearing/deterioration at the extremes of the pattern could be expected to strongly influence the ability to achieve higher resolution patterns. In addition, the adhesion properties of the top plasma polymer layer to the bottom polymer layer have the potential to influence the limits of resolution achievable with this technique.

3.2. ToF-SSIMS characterization of chemical and spatial resolution

The chemical and spatial resolutions of polymer patterns obtained by the two different patterning approaches were explored further using ToF-SSIMS. Figure 3 shows positive secondary ion images of ppTg and ppMA films patterned directly onto a Si wafer using photolithography. Unique secondary ion fragments were used to identify regions of ppMA (CH_3O^+), while ion fragment $\text{C}_3\text{H}_7\text{O}^+$ was used as a marker indicative of the ppTg

regions²⁹. The images show well defined chemical and spatial boundaries between ppMA and ppTg films. An overlay of the line scans for CH_3O^+ and $\text{C}_3\text{H}_7\text{O}^+$ secondary ion fragments (Figure 3 b)) clearly show the changes in the pixel intensities across the two chemistries and indicates distinct boundaries between the chemistries.

Chemical patterns of ppMA were deposited onto ppTg using a TEM grid and investigated by ToFSSIMS imaging (Figure 4 a) and b)). Images constructed from fragments that are indicative of the two plasma polymers showed that physical masking produced a diffuse chemical pattern on the surface. This demonstrates that there is a diffusion of the plasma deposit below the physical mask during the second polymer layer deposition. While there was some evidence in the images for small differences in the ion intensities associated with the patterning, examining the single ion intensity line scans across the CH_3O^+ and $\text{C}_3\text{H}_7\text{O}^+$ images in Figure 4 (d) indicated no significant differences in ion intensities of these specific ions across the sample. A number of other ion fragments that are known to be indicative of the two plasma polymers were also examined with similar results (data not shown).

3.3. Physical vs. Photolithography Patterning – Multivariate analysis of ToF-SSIMS images

3.3.1. Principal Component Analysis (PCA)—Principal component analysis was used in an attempt to identify the specific ions and molecular fragments that cause the highest variations in the image or data set. As ToF-SSIMS data is multivariate in its nature and as plasma polymerization is known to produce films with complex chemical structures, it was hoped that this approach would enable more specific detail on the chemical spatial resolution of the patterned plasma polymers to be revealed.

Figure 6 presents an example of PCA output, where chemical patterns of two strongly interacting plasma polymers, ppAAm on a ppAAc coated Si substrate, have been fabricated using physical masking (Figure 5 a) – c)) and photolithography (Figure 5 d) – f)) strategies.

For the patterns formed using a TEM grid, PC1 and PC3 show negligible contrast (Figure 5 a) and b)). The PC1 score image is formed from secondary ion fragments originating from ppAAm as evidenced by the high positive loadings of CH_2N^+ , C_3H_8^+ , $\text{C}_2\text{H}_4\text{N}^+$, $\text{C}_3\text{H}_6\text{N}^+$ and $\text{C}_4\text{H}_6\text{N}^+$ secondary ion fragments (Figure 5 g)). The lack of contrast in the PC scores images along with the low percentage of data captured across all of the PCs examined (1-20) suggests that there are amine containing species deposited across the sample surface, giving rise to very little variation in the spectra across the entire sample area. No evidence of the presence of ppAAc groups (secondary ion fragments with oxygen) was observed in any PC that captured more than 1% of the variance in the data set. The PCA analysis data strongly suggests that the apparent contrast difference observed on the total ion image (Figure 5 c)) are due to differences in ion yield from fragments originating from regions that were directly exposed to the plasma (bright areas) and those that were under the mask, rather than indicating a chemically distinct region associated with the presence of ppAAc. AFM data clearly shows that in the regions exposed to the plasma the ppAAm film thickness was greater than in the areas under the mask, suggesting that the ion yield behaviors may be due to differences in film thickness rather than specific chemistry.

PCA of the photolithographically patterned sample indicated that the 21% variance captured in the PC1 (Figure 5 d)) separated the image into an array comprising two distinct groups of chemistry and replicating the applied photolithographic pattern. From the loadings plot for PC1 (Figure 5 h)), the positive loading secondary ion fragments are rich in nitrogen and they correlate with bright regions on PC1 scores. The contrasting darker regions correspond to negatively loaded secondary ion fragments which are dominated by oxygen containing species. In each instance, the fragments detected from the surface could be directly correlated with the chemistry of the individual plasma polymers. It is interesting to note that the chemical

differences observed on the third principal component (Figure 5 e)), which form the loadings plots corresponding to K^+ and $C_8H_9O^+$, have positive loadings (i.e. bright regions on scores image). $C_8H_9O^+$ is a typical fragment originating from the photoresist used in the patterning of these plasma polymers²⁵. Localized regions of residual photoresist present on the patterned sample (left hand bottom corner – PC3) suggest that the defects are due to experimental errors in the patterning stages, although this type of contamination was not a common observation. The K^+ ions on PC3 were found to be associated with regions where ppAAc films are present. This was further verified by reconstructing individual spectra from ppAAc regions (data not shown).

3.3.2. Pattern Interface Analysis – Region of Interest (ROI) Analysis—Figure 6 shows an example of a scatter plot and ROI analysis performed on photolithographically patterned ppAAm on ppTg. The sample area was analyzed using high mass resolution bunched mode negative ion ToF-SSIMS analysis. Figure 6 a) and b) shows the single ion image of CN^- and $C_2H_3O^-$ secondary ion mass fragments unique to ppAAm and ppTg films respectively, patterned on the Si substrate. The total ion image for the sample is shown in Figure 6 c). Using the CN^- and $C_2H_3O^-$ fragment uncorrelated contrasting intensities, the scatter plot was drawn and the resulting picture is shown in Figure 6 d).

The 128×128 pixel distribution scatter plot has been down-binned to 64×64 pixels to enhance the visual effect without any loss of any spectral resolution. The scatter plot clearly shows that intensity of pixels from CN^- has been assigned to the x-axis while the y-axis is dominated by the signal due to the $C_2H_3O^-$ secondary ion fragment. Also shown on the scatter plot are unassigned pixels present between the two coordinate axis. Three separate ROIs (green, blue and red – see Figure 6 e)) were created to mask pixels distributed on the scatter plot. The masked image (Figure 6 f)) clearly shows that the unassigned pixels located in the middle of the coordinate space originated from the interface between the ppAAm and ppTg regions. Visually it can be determined that the interface between the two chemistries marked by blue color is 2-3 pixel thick which equates to 4-6 μm in length.

The results from the scatter plot analysis of physically patterned ppAAm and ppTg films were used to determine the interface between the chemistries are presented in Figure 7 a) - f). The sample analyzed by high mass resolution bunched negative ion ToF-SSIMS analysis clearly shows near complete coverage by the ppAAm film (Figure 7 a)). There was some evidence for the presence of ppTg-related fragments detected by ToF-SSIMS image analysis, as highlighted by the low counts of $C_2H_3O^-$ secondary ion fragments (see Figure 7 b)). The evidence of ppAAm coverage over the sample analysis area is also evident from the scatter plot (Figure 7 d)), where the CN^- pixels dominate the X-axis of the scatter plot, while the Y axis shows no presence of $C_2H_3O^-$ related pixels, and thus no red pixels are noted on the composite masked image (see Figure 7 f)). It is interesting to note that, visually there is no evidence of the presence of an interface between the patterned regions of the plasma polymers.

3.4. Examination of interface chemistry using ToF-SSIMS images and PCA

PCA was used to investigate the chemistry of the interface between photolithographically patterned plasma polymers. The mass spectrum associated with each pixel in the total ion image was used to reconstruct a dataset defining respectively three distinct regions on the image, (i) ppMA, (ii) ppTg and (iii) the interface respectively. Spectra from each individual region were further added to improve the signal to noise ratio from each chemical class. All secondary ion peaks in the mass range m/z 1-100 with intensities above 100 were used for PCA.

PC1 scores captured 98% variance (Figure 8 a)) in the dataset and effectively separated ppMA (–ve score) and ppTg (+ve score) chemistries. As shown in the PC1 scores plot, the ‘interface chemistry’ area has been classified as an independent group of samples with little evidence the

area being similar to any plasma polymer chemical group (evident from low scores value). Typical secondary ions indicating the presence of the two plasma polymers has been shown in the loadings plot in Figure 9 a).

PC2 scores plot (Figure 8 b)) captures 0.7% variance in the dataset and separates the two plasma polymer film chemistries from the interface chemistry. PC2 loadings plot (Figure 9 b)) shows the presence of typical ppMA and ppTg related secondary ion peaks. Na^+ was found to be loading negatively and correlated with sample classed as 'interface' on the scores plot. The Na^+ is a by-product of the photolithographic patterning procedure, specifically from the Alkaline developer.³⁰ In addition, the presence of negatively loading secondary ions on PC2 corresponding to both ppMA (eg. $\text{C}_3\text{H}_5\text{O}^+$, $\text{C}_4\text{H}_5\text{O}^+$) and ppTg ($\text{C}_3\text{H}_7\text{O}^+$) related peak alongside Na^+ secondary ion fragment were observed. The positive loading secondary ion fragments on the loadings plot also correlates with both plasma polymer chemistries, as well as with some hydrocarbon fragment (eg. C_2H_3^+ , C_3H_3^+ etc.). High variance captured on PC1 (98%) suggests that the differences observed on the samples are mainly due to two distinct chemistries patterned on the surface. PC2 captures less than 1% variance in the dataset; it however effectively distinguishes the plasma polymer chemistry from the interface chemistry. The PC2 loadings plot suggests that a mixture of secondary ion fragments from both the plasma polymer film (ppMA and ppTg) contribute towards the chemical composition of interface.

4. Discussion

The results presented in this paper give an insight into a variety of different physiochemical interactions that occur during the chemical patterning of plasma polymers. The challenge with surface modification of microarrays and chemically patterned materials in general lies in not only achieving reproducible spatial distribution of chemistries but also to retaining the specific functional properties of the thin films. With such a wide range of techniques available for patterning plasma polymers, the challenge lies in understanding the advantages and limitations of the different processes.

The use of physical masks is an attractive one-step technique.²¹ Often overlooked and rarely reported are results which highlight the issues with both the spatial resolution and the specificity of surface chemistry that can be achieved by using this type patterning technique with plasma polymers. For example, Dai et. al.³¹ plasma patterned methanol and n-hexane using TEM grids. The features were examined using scanning electron microscopy (SEM), but no information was obtained concerning the chemical specificity of the patterns. Bullett *et. al.*²¹ attempted to expand on this work by examining the physical patterning of plasma polymerized 1,7-octadiene, acrylic acid and allylamine using TOF-SSIMS and XPS imaging. Using a limited number of spectral components, chemical patterned bars were demonstrated. The spatial resolution and chemical specificity of these patterns were however overlooked by the authors. The line scan from the ToF-SSIMS image presented strongly suggests gas ingress below the mask when considering the axis resolution (100 μm per tick) and lack of a sharp interface. In addition, the ToF-SSIMS image corresponding to oxygenated species from the acrylic acid polymer deposited onto the hydrocarbon 1,7-octadiene clearly shows bleeding of the oxygen species. In a more recent study, pulsed-plasma-induced micropatterns were fabricated using TEM grids,³² and the authors reported high-fidelity micrometer-scale patterns. Examination of the XPS images of the plasma polymerized N-vinylformamide (NVFA) on C_3F_8 coated substrates however suggested bleeding of nitrogen species across the surface. ToF-SSIMS images for negative ions of oxygen and fluorine for plasma polymerized 2-hydroxyethyl methacrylate (HEMA), N-vinyl-2-pyrrolidinone (NVP), and NVFA on C_3F_8 coated substrates were also presented. From these images, a clear distinction between the oxygen and fluorine domains can be made, which infer successful patterning of these two basic chemistries. However, what is overlooked by the authors are the presence of shadows in both O and F

images on either side of the micro patterned squares. Without characterization of these interfaces with ToF-SSIMS multivariate analysis and AFM, it is difficult to examine the true spatial resolution and chemical specificity of these patterns.

The AFM step height measurements carried out on physically masked samples within this study show the presence of a diffuse interface between the plasma polymer film and substrate. The diffusion of radical-rich and ion-rich depositing plasma species was calculated to be approximately 4 – 9 μm inward from the edge of the TEM mask. The extent of diffusion of plasma species in itself thus becomes a limiting factor in both the spatial and chemical resolution that can be achieved using this technique. The AFM step height profile results show an interesting difference in the plasma species diffusion when Si wafer was pre-coated with a plasma polymer. The diffusing gases appear to interact with the substrate $\sim 5 \mu\text{m}$ further when a two layer patterning (ppGMA on ppTTg) was attempted using this technique. The true extent of plasma species diffusion below a physical mask and its effect on surface chemistry has been explored by ToF-SSIMS spectral and image analysis (Figure 4 and 5). The uniform coverage of ppAAm related chemistry over ppAAc, in addition to ppMA related chemistry over ppTg, clearly highlights the complex interactions that can occur in physical masking. The contact of the grid or mask with the surface is one obvious variable in the process that could be monitored and used to improve the resolution of the patterns and thus limiting gas ingress. Nevertheless, the results of this study suggest that differences in ion and radical energies, their path length, life time and velocity, cross reactivity as well as variations in electrical properties of surfaces may all have an influence on deposition profiles of the plasma polymers. In a recent study by Zelzer *et. al.*,³³ deposition profiles of plasma polymerized hexane (ppHex) down small channels were examined using water contact angle mapping and ToF-SSIMS imaging, in addition to characterization of the plasma phase using mass spectrometry. The purpose of this work was to study the penetration of plasma species through small openings, where the influence of the opening cross sectional area was explored. The authors were able to demonstrate using mass spectrometry that the deposition species were seen to deplete down the channels. While the plasma polymerization systems are different, this result reinforces the observations made within this study in regards to physical patterning systems. It also assists in understanding the shadows observed in the ToF-SSIMS images of ref 32 when the considerable large line spacing of the presented patterns is taken into account.

In terms of photolithographic patterning, Bretagnol *et. al.*³⁴ previously demonstrated patterning of plasma polymers from ethylene oxide and allylamine monomers employing ToF-SSIMS images. Line widths of 30 – 160 μm were obtained, however no detailed analysis of the patterns were undertaken using multivariate analysis, nor were the interfaces between the two chemistries examined. Within the current study, photolithographic patterning of plasma polymers has been shown to be capable of producing films with defined chemical and spatial resolution even with complex and reactive chemical species. The AFM step height images (see Figure 1) show the presence of a sharp physical interface between each of the patterned plasma polymer chemistries. Critically, ToF-SSIMS spectra and images obtained from materials before and after patterning indicate that the patterning process had little impact on film chemistry and infers that specific properties including protein resistance (ppTG) and reactivity (ppGMA) were retained in the final patterned surfaces (Figure 3). Recent work undertaken within our laboratory has demonstrated that specific properties including protein resistance are maintained after performing the photolithographic patterning technique.³⁰

Multivariate analysis of the ToF-SSIMS image effectively highlighted the differences between the two patterning approaches (see Figure 5). Analysis of the photolithographic patterning technique clearly demonstrated the successful patterning of two strongly interacting polymers, ppAAm and ppAAc.³⁵ PCA enabled the investigation of apparent contrast observed in the total ion ToF-SSIMS image for the physical patterned substrate and highlighted a difference in the

yield of secondary ion fragments related to the film thickness in different regions of the sample, rather than the presences of a chemically different regions on the patterned surface. ROI analysis was employed to further analyze the ToF-SSIMS data. For the physical patterned substrate, there was no evidence of the presence of an interface between the two polymers. However for the photolithographically patterned system, a clear interface between the two polymers of approximately 3 – 6 μm thickness was observed, where the chemical functionality could not be totally assigned to either polymer. Further examination of the interface using PCA clearly separated the polymer chemistries from the interface chemistry. The PC data suggested that the chemical composition of the interface comprised of fragments from both plasma polymers suggesting that this region was formed by the overlap of the two polymers.

Evidence of incomplete resist removal due to experimental errors and surface defects was also found. Incomplete photoresist removal is often quoted as a drawback of photolithographic patterning of plasma polymers.³⁶ However, the evidence provided in the current study suggests that with careful experimental control and appropriate data analysis tools, the photolithographic patterning approach can be optimized and used for a wide variety of applications without undue chemical contamination. These results also clearly demonstrate that the multivariate data analysis approach employed in the study can be effectively used to assess the final surface chemistry achieved by various patterning techniques. Only by using multivariate data analysis approaches, was it possible to bring forward some interesting edge effects observed at the interface between the two chemistries (see Figure 6 to Figure 8), a factor that has not previously been investigated.

The results presented here not only highlight the challenges in patterning of plasma polymers but also emphasize the need for the use of complementary analytical techniques required for such surfaces. AFM analysis has proved to be an effective tool to interrogate the physical interface created between plasma polymer by physical and photolithographic patterning. A study of the limits of spatial resolution of photolithographic patterning demonstrated that a pattern edge of $\sim 1 \mu\text{m}$ could be obtained and that the feature spacing and not the feature size was the limiting factor. Previous work in this area has involved feature sizes greater than 5 μm .²⁵ This study focuses on only one type of positive photoresist, so it is possible, and in fact probable, that other photoresists may yield different results. In addition, the type of resist could be critical to coating chemical retention. UV exposure of some plasma polymer films has been shown previously to degrade the film properties.³⁷ It is also envisaged that resist thickness could play a role in pattern resolution due to mechanical interactions.

The AFM results also highlight the physical effects of patterning process on polymer films. Incomplete resist removal, multilayer film adhesion and plasma species diffusion were some of the relevant physical properties which AFM analysis was able to address. The lack of any direct chemical information derived from AFM analysis highlight the need for other complementary analytical techniques capable of chemically mapping the surface. Often the complex chemical structures inherent of plasma polymer films coupled with the wealth of information that ToF-SSIMS provides can be problematic, and hence some of the information is effectively lost. Multivariate data analysis approaches used in this study highlights some important issues with residue photoresist, salt contamination and their effects on ion yields, which are rarely reported in the literature but can complicate result interpretation.

5. Conclusion

AFM, ToF-SSIMS imaging and multivariate analysis have been successfully employed to compare two commonly used patterning techniques for plasma polymers. These characterization techniques provided the necessary spatial chemical resolution and enabled the quantitative assessment of the quality of these patterns. ToF-SSIMS images clearly indicated

that well defined chemical and spatial boundaries can be obtained from photolithographic procedures, while physical patterning resulted in diffusion of plasma species below the mask and subsequent loss of chemical resolution within the patterns. These results were verified using multivariate analysis techniques including PCA and ROI analysis. The use of PCA enabled the apparent contrast observed in the total ion positive image for the physically patterned substrate to be determined. It was found to result from a difference in yield of secondary ion fragments originating from the top polymer layer rather than the presence of a chemically different bottom layer. ROI analysis allowed for the identification of an interface between the two polymers patterned using the photolithographic technique of approximately 3 – 6 μm . Further examination of the interface using PCA clearly separated the polymer chemistries from the interface chemistry. The results obtained in this study clearly highlight the necessity for complimentary characterization and analytical techniques in the fabrication of micropatterns from plasma polymers and have identified key issues often overlooked in the formation of these patterns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

AFM line scan image data obtained for chemical patterns of a) ppGMA on Si wafer using TEM grid, b) ppGMA on ppTg using TEM grid (Substrate – Si wafer), c) ppGMA on Si wafer using photolithographic patterning, and d) ppGMA on ppTg using photolithographic patterning (Substrate – Si wafer).

Figure 2.

Tapping mode AFM height image of ppGMA patterned onto ppTg background. Inset (c) shows the schematic of optical mask used for patterning the plasma polymers while inset (d) presents an overlay of optical mask and resultant pattern resolution obtained after photolithographic patterning.

Figure 3.

Positive single ion ToF-SSIMS images of ppMA patterned photolithographically on Si wafer precoated with ppTTg ($500 \times 500 \mu\text{m}^2$). Unique secondary molecular fragments are presented in insert a). The pixel intensity plot for secondary ions $\text{C}_3\text{H}_7\text{O}^+$ (ppTTg) and CH_3O^+ (ppMA) shown in insert b). The image resolution was calculated to be $\sim 10 \mu\text{m}$, based on 80:20 definition.

Figure 4.

Positive single ion images of ppMA patterned using a physical mask on Si wafer coated with ppTg acquired by ToF-SSIMS ($500 \times 500 \mu\text{m}^2$). Unique secondary molecular fragments are presented in insert a). The pixel intensity plot for secondary ions $\text{C}_3\text{H}_7\text{O}^+$ (ppTTg) and CH_3O^+ (ppMA) shown in inset b).

Figure 5.

PCA of ToF-SSIMS images ($500 \times 500 \mu\text{m}^2$) acquired from ppAAm patterned onto ppAAc. Insert images a) -f) shows the data sets PC1 and PC3, and the total positive ion image acquired for the sample for physical and photolithographic patterning respectively. Insert g) and h) present the loadings for PC1 for physical and photolithographic patterning respectively.

Figure 6.

ROI analysis of ToF-SSIMS images ($250 \times 250 \mu\text{m}^2$) from photolithographically patterned ppAAm on ppTg coated Si wafer. Images a) and b) are single ion images reconstructed from secondary ion fragments CN^- and $\text{C}_2\text{H}_3\text{O}^-$ based on the total ion image (c). Image d) shows the pixel distribution of CN^- and $\text{C}_2\text{H}_3\text{O}^-$ fragments on the X and Y axis respectively. Image e) presents the three separate ROIs used to mask the pixel distribution on the scatter plot. A reconstructed masked image of pixels is shown in image f). The green region corresponds to ppAAm while red regions show the presence of ppAAc. Blue region represent the interface between the ppAAc and ppAAm films.

Figure 7.

ROI analysis of patterns of ppAAm on ppTg coated Si wafer deposited by physical masking technique. Images a) and b) are single ion images reconstructed from secondary ion fragments CN^- and $\text{C}_2\text{H}_3\text{O}^-$ based on the total ion image (c). Image d) shows the pixel distribution of CN^- and $\text{C}_2\text{H}_3\text{O}^-$ fragments on X and Y axis respectively. Image e) presents the three separate ROIs used to mask the pixel distribution on the scatter plot. A reconstructed masked image is shown in image f). The green region corresponds to ppAAm while red regions show the presence of ppAAc. Blue region represent the interface between the ppAAc and ppAAm films. Each image represents $500 \times 500 \mu\text{m}^2$.

Figure 8. PCA scores plot from the ToF-SSIMS image of ppMA photolithographically patterned on ppTTg showing the sample distribution on PC1 a) and PC2 b) respectively.

Figure 9.

PC1 (a) and PC2 (b) loading plot for positive ion ToF-SSIMS spectrum reconstructed from photolithographic patterns of ppMA and ppTg.

Table 1

Plasma polymerization settings employed in this study

Precursor	Monomer Temp (°C)	Chamber Temp (°C)	P _{pk} (W)	t _{on} (μs)	t _{off} (μs)	P _{Effective} (W)	Flow Rate (sccm)	Deposition time (min)
Tg	95	55	10	-	-	10	2.7	25
GMA	RT	RT	10	80	800	0.09	2.5	20
MA	RT	30	10	80	800	0.09	2.5	20
AAc	RT	RT	10	-	-	10	2.2	15
AAm	RT	RT	10	-	-	10	2.2	10
Oxygen	RT	RT	50	-	-	50	2.7	120 - 240

(Tg – Tetraglyme, GMA – Glycidyl methacrylate, MA – Maleic anhydride, AAc – Acrylic acid, AAm – Allylamine, RT – Room temperature)