Micro-patterned plasma polymer films for bio-sensing

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ABSTRACT

Bi-functional micro-patterned plasma polymer films were prepared by first depositing a full-area amino-containing plasma polymer film (PPF) onto a substrate, followed by the deposition of a patterned oxygen-containing PPF via a polymeric mesh used as contact mask. As a result, an ultrathin film with alternating areas of amine and oxygen-containing groups is obtained. Such micro-patterns bear potential as templates for multi-sensitive bio-sensor substrates. The chemical surface composition is assessed using XPS and ToF-SIMS. On the masked amino-rich area a lateral chemical micro gradient was observed due to mesh mask shadowing. Stability of the coating was investigated over time in ambient air and in water, showing that the surface properties were maintained after 7 days in air and 1 day in water. To demonstrate functionality, the micro-patterned surface was exposed to a green fluorescent protein (GFP) and lateral resolution and selectivity during adsorption was studied.

KEYWORDS

Micro-patterned polymer films; plasma deposition; surface functionalization; film characterization; protein adsorption.

1. INTRODUCTION

The use of plasma-based coatings for biomedical purposes is a growing field. As these coatings are prepared by a solventless and eco-friendly plasma deposition method, it is possible to provide surfaces able to favor cell cultures [1-3] as well as surfaces that can be used for bio-sensing. [4,5] Optical bio-sensing devices, such as Surface Plasmon Resonance (SPR) sensor [4] or Transmission Interferometric Adsorption Sensor (TInAS) [5], can be used to detect adsorption of biological ligands from complex fluids. These label-free detection devices are based on affinity interactions between biomarkers and immobilized biomolecules, such as antibodies, proteins, lipids or DNA [6,7]. Surface preparation of the biosensor is thus crucial in order to ensure the reproducibility of the quantified results when analyzing biological fluids.

Biomolecules can be covalently immobilized to the surface of the sensor utilizing polymeric films or hydrogels made by molecular assembly as host matrix. More recently, molecular assembly has attracted increased attention as it allows patterning with two or more functional properties: for example, enhancement of the specific recognition of a biomolecule and avoidance of the nonspecific adsorption of other compounds. For example, such surfaces were obtained by molecular assembly at micro-[8] and nanoscale [9], where biomolecules responsible for the affinity of the sensor were immobilized on the terminal functional groups of poly(L-lysine)-graft-poly(ethylene glycol) copolymers, which provide a hydrated brush-type coating that can avoid non-specific adsorption [10]. These methods have in common that organic solvents are required during surface preparation. A more eco-friendly and industry transferable method to prepare micro-patterned polymer films via plasma processing is presented here.

Plasma processing of sensor surfaces is accompanied by a number of key advantages such as *in-situ* pre-cleaning and subsequent polymerization or functionalization steps. Furthermore, plasma deposition readily forms flat and smooth coatings at full surface coverage. Thanks to previous studies on such plasma depositions [11-14], the thickness of the deposited films, number of accessible chemical groups and cross-linking degree (e.g. stability) can be controlled.

For a micro-patterned plasma polymer film (μ p-PPF) produced *in-situ*, be it in a low-pressure plasma reactor [15-17] or in an atmospheric plasma [18,19], a number of sequential approaches have already been investigated [20]. Typically, they consist of either etching a polymeric surface or depositing a polymer film through a mask [15-18,21-23]. Another

established method to obtain micro-patterned chemical functionalization is colloidal lithography or colloidal nanolithography, where the etching or the deposition occurs between adsorbed particles that form a colloidal mask [23-28]. Some plasma reactors are able to produce some degree of micro-patterning thanks to a sophisticated geometry of the used electrode; e.g. a microplasma device exploiting plasma polymerization through asymmetric glow discharge [19,29-31]. Other methods consist of depositing a plasma polymer film onto a substrate, then creating a micro-pattern thanks to lithography [32] or nano-lithography [33].

The aim of this paper is to propose a new type of micro-patterned coating with spatially defined distinct functional groups that is generated in-situ during a plasma process. To achieve this goal, two different plasma polymer films (PPFs), i.e. amino-containing (a-C:H:N) and oxygen-containing (a-C:H:O) PPFs, were partially overlaid at the surface of the substrate. Such PPFs were selected due to their general interest as biointerfaces as well as previous own work [34]. The micro-patterning was obtained in an innovative manner by the use of a monofilament mesh mask during the second a-C:H:O PPF deposition step. The hence obtained μ p-PPFs were then characterized by scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and water contact angle measurements (WCA). The lateral definition and selectivity of the μ p-PPFs was assessed via adsorption of a fluorescent protein, which binds selectively to the amino-rich parts of the surfaces, as followed by microarray scanner analysis.

2. EXPERIMENTAL SECTION

2.1. Preparation of the μ p-PPF

Deposition was performed in a cylindrical symmetric plasma reactor where the two plane parallel electrodes (30 cm in diameter) are separated by a glass ring (5 cm in height). The upper electrode contains a gas showerhead, while the chamber is pumped through a central opening below the lower electrode, which is coupled to the RF generator enabling well-defined deposition conditions [14]. A capacitive-coupled field with 13.56 MHz radiofrequency excitation was used to power a plasma of C_2H_4/NH_3 and C_2H_4/CO_2 mixtures to deposit the amino-containing (a-C:H:N) and oxygen-containing (a-C:H:O) films, respectively. After the deposition of nominally 5 nm of a-C:H:N, samples were exposed to ambient air and a precision plain weave textile mesh (SEFAR PET 1500 36/92-90W PW) with $183\ \mu m \times 183\ \mu m$ square openings, and nominal thread diameter of $90\ \mu m$, was placed

onto samples. This mesh was used as mask in direct contact with the a-C:H:N-coated sample during the deposition of a-C:H:O of nominally 5-10nm thickness (Figure 1).

The highly stable and previously established a-C:H:N coating [35], comprising 2 at% of NH₂ groups, was prepared according to the following conditions: NH₃ and C₂H₄ flow rates of 7 sccm (gas ratio 1:1), pressure of 10 Pa, and power of 50 W for 40 s. Onto this stable base layer, an oxygen-containing a-C:H:O coating was deposited according to conditions that were optimized in previous studies [36,37]: CO₂ and C₂H₄ flow rates of 24 and 4 sccm respectively (gas ratio 6:1), pressure of 10 Pa, and power of 30 W for 5 min. Glass slides, Si wafers and Ag-coated glass slides were used as substrates in this study, and were previously cleaned by Ar plasma (10 min, 50 W, 10 Pa).

Figure 1. Schematic illustration of the preparation of the μp-PPFs. The deposition of the second a-C:H:O layer is carried out through a polymeric mesh.

2.2. Surface characterization

2.2.1. SEM analyses

Scanning electron microscopy (Hitachi S-4800) was performed in order to visualize the patterning at the surface of Ag-coated glass slides. Note that a ~ 50 nm silver layer was used to dissipate the surface charge, and may generate secondary electrons to enhance the pattern imaging [15]. Analyses were performed using an acceleration voltage of 2 kV at a current of 11 μA. Pictures were taken with 100x magnification.

2.2.2. XPS analyses

The chemical composition of the surfaces was analyzed by X-ray photoelectron spectroscopy (XPS) measurements using a Scanning XPS Microprobe (PHI VersaProbe II spectrometer, Physical Electronics) with monochromatic Al K α radiation (1486.6 eV). The operating pressure of the XPS analysis chamber was below 5×10^{-7} Pa during the measurements. The spectra were collected at photoemission take

off angles of 45° (with respect to the sample surface) and using a focused X-ray beam with a diameter of 20 µm. Survey scan spectra (0 – 1100 eV) were acquired with an energy step width of 0.8 eV, acquisition time of 160 ms per data point and analyzer pass energies of 187 eV. Region scan spectra were acquired with an energy step width of 0.125 eV, an acquisition time of 1.2 s per data point and analyzer pass energies of 29 eV. X-ray excited secondary electron images were done on 1 mm x 1 mm area, thanks to a focused X-ray beam with a size of 9 µm. In addition, also chemical mapping for carbon and oxygen (with pass energy of 117 eV) was performed using an X-ray beam with a diameter of 20 µm. Obtained spectra were rescaled by shifting the spectra relative to the aliphatic carbon at 285.0 eV to account for charging effects. Curve fitting (least-squares fit routines) with CasaXPS software version 2.3.16 was employed to calculate the atomic concentrations.

2.2.3. ToF-SIMS imaging

Time of flight secondary ions mass spectrometry (ToF-SIMS.5, Iontof) was performed to analyze the laterally-resolved elemental and molecular composition of the topmost monolayers of the µp-PPFssamples. A 50keV Bi₃⁺⁺ primary ions focused beam was used in high mass resolution mode ($M/\Delta M \sim 7000$). A gentle sputtering with a low energy Cs⁺ beam (250eV, 1000 × 1000 µm²) was simultaneously used to boost the ionization yield in the negative polarity. Secondary ions image data, containing the full compositional spectrum in the mass range 1-500 mu for each pixel, were acquired with ultra-high sensitivity (~ppm) on randomly selected 500 × 500 µm² areas with a 256 × 256 pixels² resolution.

2.2.4. Water Contact Angle measurements

Water contact angle (WCA) was measured on freshly prepared- and aged-samples. For the aging study, samples were stored in air or in distilled water at 20°C. To determine the WCA, at least 3 drops (2 µL) of water (CHROMASOLV®, for HPLC, Sigma Aldrich) were deposited onto the surface and analyzed using the software controlled drop shape analyzer (DSA25, Krüss).

2.3. Spatially-defined protein adsorption

2.3.1. Immobilization of green fluorescent protein

The Y-tagged version of GFP (Y1-GFPuv), engineered to be a substrate for tyrosinase, was produced recombinant in *E. coli* and purified by affinity chromatography as reported. [38] Tyrosinase from *Agaricus bisporus* (T3824, ≥1000 unit per mg) was purchased from Sigma

Aldrich (Buchs, Switzerland). Reaction mixtures that contain 1 mg/mL of the genetically modified green fluorescent protein Y1-GFPuv were prepared in 100 mM potassium phosphate buffer at pH 6.8 and the reaction was started by adding 0.1 mg/mL tyrosinase immediately before the coating process. 200 μ L of the reaction mixture was dispensed on the μ p-PPF in form of a freestanding droplet, in a humid atmosphere to avoid evaporation of the drop, and the exposure was carried out at room temperature for 3h. Contact time in between plasma coating and protein-containing solution was reduced to minimize aging effects. After exposure, samples were thoroughly rinsed three times in ultrapure water for 10 min to remove loosely bound protein molecules. Samples were then air-dried before imaging. The concentration of Y1-GFPuv was determined either by the wavelength shift during dye-protein complexation with the Bradford method [38,39] using bovine serum albumin as standard, or simply using the extinction coefficient $27000 \text{ M}^{-1} \text{ cm}^{-1}$ and a calculated molecular mass of 27862 g mol^{-1} .

2.3.2. Fluorescence imaging of the surface

The presence of green fluorescence on the surfaces was visualized with a LS Reloaded Microarray Scanner (Tecan, Switzerland) equipped with a lasers at 488 nm and 532nm filters). Imaging conditions included a gain value of 160 and a spatial resolution of 4 μ m.

3. RESULTS & DISCUSSION

3.1. Surface characterization

In order to assess the spatial distribution of the different chemical functionalities on the μ p-PPFs, different surface analytic tools were used. To visualize the micropattern, SEM analyses were carried out on a selection of different PPFs: a homogeneous layer of a-C:H:N, a homogeneous layer of a-C:H:O, the pattern-free reference multilayer structure where the a-C:H:O layer was deposited onto the a-C:H:N layer, and the μ p-PPF where the second a-C:H:O layer was deposited through the mask onto the a-C:H:N layer. While the a-C:H:N, a-C:H:O and double-layer PPFs appear to be homogeneous (Figure 2a, 2b and 2c), a regular micropatterning is revealed for the μ p-PPF (Figure 2d), due to a morphological contrast. Moreover, from this SEM analysis, it is possible to give some geometrical information about the prepared pattern: the a-C:H:O squares are of dimension $206 \pm 3 \mu\text{m}$ and are separated by a $58 \pm 2 \mu\text{m}$ wide grid of the masked area that corresponds to the a-C:H:N nano-PPF. In comparison with the nominal mesh opening, the coated area is larger; this is due to the lateral diffusion of the film-forming species created in the plasma chamber while depositing a-C:H:O film before their adsorption at the surface of the a-C:H:N layer.

Figure 2. SEM imaging of samples with (a) a-C:H:N, (b) a-C:H:O, (c) a-C:H:O deposited onto a-C:H:N without any mask and (d) a-C:H:O deposited onto a-C:H:N using a mask to produce the μ p-PPFs.

Thanks to XPS mapping, it was possible to image and quantify the chemical composition of the surface. Scanning X-ray imaging (SXI, X-ray excited secondary electron image) showed a higher signal on the grid lines than in the squares between them (Figure 3a). The $[O]/[C]$ and $[N]/[C]$ ratios obtained in the squares are comparable to the ratios obtained for the reference homogeneous a-C:H:O coating (Figure 3b and Table 1), showing that the a-C:H:O coating was successfully deposited through the mask and has a similar surface composition as a monolayer of the same coating. On the contrary, the chemical ratio observed on the masked area does not correspond to any of the monolayer-references. The $[O]/[C]$ ratio is higher (31.7 %) than expected (10.3 % for a-C:H:N) and the $[N]/[C]$ ratio is lower (6.1 % compared to the 18.5 % for a-C:H:N). Thus, the first layer, i.e. the a-C:H:N monolayer, may have been oxidized while depositing the a-C:H:O at the grid position of the textile contact mask. Note that the used mask is made of a woven monofilament mesh allowing for some access of plasma species below the grid lines. Imaging XPS allows visualizing the chemical differentiation of the patterning; in particular, the atomic composition in carbon and oxygen is relevant (supporting information Figures S1 and S2) showing that the less-functionalized

carbon amount increases in the square area and decreases in the grid area, and in analogy, the oxygen amount decreases in the square area and increases in the grid area.

Figure 3. XPS analyses of μ p-PPF: (a) X-ray excited secondary electron image (SXI) and (b) [O]/[C] and [N]/[C] ratios in comparison with the homogeneous reference a-C:H:N and a-C:H:O plasma polymer layers.

Table 1. Quantification by XPS of the elemental composition of a-C:H:N and a-C:H:O homogeneous layers listed as grid- and square- areas of the μ p-PPF.

	%C1s	%O1s	%N1s	%Si2p	[O]/[C]	[N]/[C]	([O]+[N])/[C]
a-C:H:N	76.7	7.9	14.2	1.1	10.3 %	18.5 %	28.8 %
a-C:H:O	79.6	18.9	1.5	-	23.8 %	1.9 %	25.7 %
grid area	72.6	23.0	4.4	-	31.7 %	6.1 %	37.7 %
square area	80.1	18.3	1.5	-	22.8 %	1.9 %	24.7 %

A ToF-SIMS study was carried out to complement the results obtained with XPS, and obtain further molecular information at high lateral resolution. Firstly, secondary ion mass spectra were obtained on the two homogeneous reference layers; a-C:H:N and a-C:H:O, in order to identify the respective characteristic fragments of the two types of plasma polymers. The referenced double layer consisting of a homogeneous a-C:H:O layer on top of a homogeneous a-C:H:N layer was also analyzed. The fragments observed in the bilayer configuration were exactly the same as for a single homogeneous a-C:H:O coating, confirming that the bottom layer has no influence on surface chemistry and that the top layer of the double layer is chemically identical to a single a-C:H:O layer. Then, μ p-PPF was analyzed and secondary ion images corresponding to the respective characteristic fragments as determined

on the homogeneous samples were extracted. Figure 4 shows the spatial distribution of selected fragments at the surface of μ p-PPF (see also supplementary Figure S3). Note that to improve the signal over noise ratio, the contribution of some fragments of similar chemical origin were summed into groups as illustrated in Table 2.

Figure 4: ToF-SIMS negative secondary ions images (Field of view $500 \times 500 \mu\text{m}^2$) of the micro-patterned PPF deposited onto Si-wafer (yellow areas correspond to higher signals, brown areas correspond to lower signals). The RGB-composite image was generated with CHxNO in red, CxNy in green and Cx in blue. The CxHyOz Profile was carried out by extracting RGB information from an area of image CxHyOz using the software ImageJ.

Table 2. Fragments measured by ToF-SIMS during the analysis of the μ p-PPF films. For ToF-SIMS image analysis, only group names will be given, but all of these fragments were considered.

Groups	Fragments	m/z	Groups	Fragments	m/z
CxNy	$^{13}\text{CN}^-$	27.0056	Cx	C^-	12.0019
	C_3N^-	50.0124		C_2^-	24.0026
	C_3N_2^-	64.0189		C_4^-	48.0061
	C_2N_3^-	66.0092		C_6^-	72.0047
	C_5N^-	74.0087		C_7^-	84.0038
CxHyNz	$\text{C}_2\text{H}_2\text{N}^-$	40.0214		C_8^-	96.0044
	$\text{C}_3\text{H}_2\text{N}^-$	52.0222		C_{10}^-	120.0125
	C_4HN^-	63.0157	CxHy	C_3H^-	37.0096
CxHyOz	C_2HO^-	41.0076		C_5H^-	61.0094
	$\text{C}_2\text{H}_3\text{O}^-$	43.0198		C_6H^-	73.0162
	CHO_2^-	44.9929		C_7H^-	85.0089
	$\text{C}_3\text{H}_3\text{O}^-$	55.0234		C_8H^-	97.0175
	$\text{C}_3\text{H}_5\text{O}^-$	57.0373		C_{10}H^-	121.0212
	$\text{C}_2\text{H}_3\text{O}_2^-$	59.0040		C_{12}H^-	145.0251
	$\text{C}_4\text{H}_3\text{O}^-$	67.0237		C_{14}H^-	169.0261
	C_3HO_2^-	68.9884	CHxNO	CNO^-	42.0012
	$\text{C}_3\text{H}_3\text{O}_2^-$	71.0076		CH_2NO^-	44.0142

A composite false color image (Figure 4) was generated where the Cx contribution is given in blue, the CxNy contribution in green and CHxNO contribution in red, visualizing the chemistries at the surfaces of the μ p-PPF.

Cx and CxHy fragments were found to be more abundant in the square areas, which is in agreement with the carbon coverage obtained by XPS. Thus, the a-C:H:O coating released more hydrocarbon secondary ions than the a-C:H:N coating. Other fragments, particularly, CxNy, CxHyNz, CxHyOz and CHxNO are mainly observed in the masked area, as expected. Surprisingly though, Figure 4 (and Figure S3) also reveals that signals are inhomogeneous across the masked area, namely CxNy species seem to be more concentrated in the crossing area of the fiber mesh, whereas CxHyNz, CxHyOz and CHxNO are mainly present in the connecting linear part of the pattern, leading to a lateral chemical gradient inside

the masked area. This result can be explained by the fact that the mask was a polymeric fiber mesh with a weaving structure providing a better mechanical contact to the substrate at locations where the filaments cross, and therefore less lateral diffusion during deposition of a-C:H:O. In the free lying part of the mesh fibers, a limited amount of plasma species can still interact with the a-C:H:N base layer. The chemical gradient is visible in the imaging scans. We can plot an oxygen profile along the gradient provided the $C_xH_yO_z$ fragments, showing that the variation in chemical composition is congruent with the textile binding pattern used in the fiber mesh mask.

For plasma polymer coatings, stability in air and in water is an important factor to be considered. First investigation on evolution of wetting properties over time was carried out by measuring the static water contact angle (WCA) in regular intervals after deposition of the μ p-PPF. As references, the homogeneous layers of a-C:H:N and a-C:H:O yielded an initial WCA of $\sim 55^\circ$ and $\sim 46^\circ$ respectively. Samples have been stored in air or in water for one week. Samples stored in air were directly measured after their storage process, whereas samples stored in water were first blow dried with compressed air at their surface and then measured immediately. Figure 5 shows the static water contact angles measured for the μ p-PPFs stored in air or in water. Note that the drop size was much bigger (2-3 mm diameter) than the pattern size ($264 \pm 5 \mu\text{m}$ width), and thus, both square and grid areas are simultaneously measured as an effective averaged value. This effective WCA shows a similar variation statistics as the WCA measured on plain reference coatings and can thus be used as a measure for the effective aging of the μ p-PPF.

When stored in air the WCA of the a-C:H:N reference remains unchanged for the duration of one week, and the WCA of the a-C:H:O reference is approximately the same within error of the measurement indicating the stability of these coatings. The μ p-PPF curve may be interpreted as a small trend to increase the WCA over one week (starting from 45° to reach $\sim 50^\circ$). This observation might be explained by the fact that some restructuring may occur at the surface of the film.

The effective water contact angle of the μ p-PPF during the first few days are globally close to the wetting properties of the a-C:H:O reference. This result could be explained by the fact that the a-C:H:O coating covers the majority of the surface of μ p-PPF (square-to-grid area of ~ 1.6), and was added at the end of the coating process, and thus, can be considered as a top layer.

After storage in water (and subsequent brief blow drying), a decrease of the WCA was observed for references a-C:H:N and a-C:H:O. This might be related to a slight hydration of the surface, and some water molecules can be still bound onto polar chemical groups, such as carboxylic acid, hydroxyl or amino groups, through hydrogen bonding. Considering the μ -PPF, a slightly higher WCA was measured compared to the reference a-C:H:O, but WCA decreased with the same trends as observed for the a-C:H:O reference, showing that the wetting properties of the bilayer coating essentially follows the top layer.

Finally, the small lagging effect of μ -PPF and both reference coatings when immersed in water for 1 day suggest that it is safe to use these coatings for the GFP adsorption test requiring less than four hours water exposure.

Figure 5. Static water contact angles of μ p-PPFs stored in air and in water.

3.2. Spatially-defined protein deposition

Finally, the functionality in view of future bio-sensing applications of the micro-patterned surface has been tested by immersing the μ p-PPF in a solution that contains GFP, and, tyrosinase to covalently bind GFP onto amino groups. Indeed, a similar approach could be used later to adsorb enzymes or antibodies at the surface of the μ p-PPF, leading to new biosensors. GFP was chosen because of its fluorescence that can be readily measured after immersion and a cleaning step.

Fluorescence imaging of the μ p-PPF surface before and after immersion in GFP shows the preferential spatial distribution of the GFP (Figure 6). The proteins are specifically adsorbing on the masked amino-containing areas of the μ p-PPF (see supplementary Figure S4).

A first possible explanation for this selective adsorption may be related to the surface charge. Indeed, GFP has an isoelectric point of ~ 5 and thus carries a net negative charge when incubated in phosphate buffer at pH 6.8. GFP will thus preferentially adsorb to positively

charged surfaces such as the amino-functionalized regions of the μ p-PPF. Moreover, in this study, as we used a-C:H:N and a-C:H:O coating, it is possible to sense the different chemical affinities, i.e. $-\text{NH}_2$ vs. $-\text{CO}(\text{X})$, where X can be $-\text{OH}$, $-\text{O-Alkyl}$ or $-\text{Alkyl}$ for example, by using such enzyme. Namely, tyrosinase enhances the oxidation of the tyrosine of the Y-tag of the engineered proteins, enhancing a chemical coupling with the amino groups present at the surface of the substrate [38,40]. Thus, a combination of adsorption and enzymatic immobilization occurs in the masked a-C:H:N area [38,39].

Furthermore, it appears that the fluorescence intensity is much larger at the grid crossing areas, demonstrating an even stronger adsorption of the proteins at these specific locations of the well masked a-C:H:N surface. Moreover, due to the use of tyrosinase, a certain amount of GFP would have been covalently bound onto the amine groups [40]. As revealed by ToF-SIMS, these regions correspond to a pure non-oxidized a-C:H:N film, whereas the rest of the grid is composed of $\text{C}_x\text{H}_y\text{O}_z$ and CH_xNO . This demonstrates that GFP not only adsorbs on pure a-C:H:N but can also adsorb on the oxidized regions, although to a lower degree. By creating smooth oxidation gradients within μ p-PPF the interaction of proteins with surfaces can be specially controlled and might be of interest for bio-sensing applications.

Figure 6. Microarray scanner imaging of the μ p-PPF (a) before and (b) after GFP adsorption.

4. CONCLUSION

In this study, the feasibility of an all-plasma process to design a micro-patterned plasma polymer film(μ p-PPF) with both oxygen-rich and amine-rich regions was demonstrated. SEM, XPS and ToF-SIMS surface characterization of the μ p-PPF revealed the lateral distribution of the surface chemical groups: amine groups were located in the grid area, whereas the oxygen-containing groups provided by the second plasma deposition were in the square area. Moreover, the existence of a lateral chemical gradient within the masked amine-based area was found congruent with the fiber mesh mask mechanical contact. As a simple technique, a textile web mesh was used as a mask allowing variable contact geometries with the surface and therefore locally controlled lateral diffusion of plasma species during plasma treatment. Over micrometer distances, local lateral chemical gradients were observed.

In summary, the here presented μ p-PPFs combine vertical chemical gradients from amino-containing to oxygen-containing PPF, and lateral chemical gradients from amino-containing PPF to oxidized PPF. This provides an interesting template surface for the development of future multi-functional affinity bio-sensors.

The stability of the μ p-PPF can be inferred from a nearly constant water contact angle over several days. In addition, the selectivity for protein adsorption onto the μ p-PPF was successfully tested by exposure to a solution that contains a fluorescent protein GFP, which was observed to adsorb solely on the nitrogen-containing areas.

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