

Enhancing optical biosensing: Comparing two physical treatments for GPTES chemical functionalization of Cyclo-Olefin Copolymer foil

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ABSTRACT

Cyclo-olefin-copolymer (COC) transparent films are currently the best choice for micro-fluidic bio-sensors for point-of-care diagnostic applications using optical signal detection. However, while the optical and mechanical properties of this polymer are extremely good, the adhesion of the bio-probes on this surface is not optimal, due to its chemical structure, that presents only saturated carbon bonds. The deposition of organo-silane molecules on the COC surface is one of the most effective ways to overcome this problem. But, for the surface functionalization, a surface physical treatment is necessary before the chemical modification of the COC surface. In this paper a comparison of the effectiveness of two different physical treatments, oxygen plasma and UV-ozone, is reported. In particular, the exposure time of the UV-Ozone treatment has been selected to avoid the problem of auto-fluorescence of the modified COC surface, that was observed also for relatively short UV exposure (around 10 min). An investigation of the reactive radicals created on the surface after the physical treatments and the following chemical modification with the organo-silane molecule (GPTES) has been performed using X-ray photoemission spectroscopy. The surface energy and morphology of the films have been also measured by contact angle and optical profilometry. Finally, the bio-probes adhesion performances of the COC surfaces obtained with the two physical treatments and the chemical modification were tested in a fluorescence-based assay, using an organic light emission diode to excite the fluorescence. We observed that the UV-ozone treatment allows to obtain a siloxane network with some reactive epoxy radicals on the COC surface, however, their quantity and distribution are less important and homogeneous than in the oxygen plasma treated surfaces.

1. Introduction

Cyclo-olefin-copolymer (COC) are a class of polymers with superior characteristics in terms of optical properties with very high transparency in the visible spectral region, refractive index similar to glass, high Abbe number, low birefringence and high ultraviolet light transmission. These polymers present also a very high glass transition temperature and a good chemical resistance, moreover they can be processed with various standard processing techniques like extrusion, injection molding, etc [1]. All these characteristics make them very attractive for their use in medical devices and, in particular, when used as thin transparent films, in diagnostic devices where their superior optical properties are important, like in fluorescence microscopy or in microfluidic devices.

However, their intrinsic chemical structure, that presents only saturated carbon bonds, makes difficult the covalent bonding of relevant biological molecules on their surface, which is essential for some diagnostic applications. In order to overcome this problem several surface treatments have been used to create reactive radicals, like hydroxyl and carboxyl groups, on the COC surface. In general, polymeric surfaces can be enriched with hydrophilic moieties through chemical reactions with chemically active oxygen species. These can be generated very effectively by means of plasmas [2,3]: the most used physical treatments to create reactive radical on plastic and metallic surfaces are corona discharge [4], cold oxygen plasma [5] and UV-ozone [6]. Nonetheless, plasma generation require specific and non-always available instrumentation, furthermore, the devices used to perform these treatments

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have very different purchase and running costs as well as different reagents and space requirements.

Exposure of the polymeric specimen to an ozone-enriched atmosphere can be an alternative route to oxygen plasma exposure to generate chemically-active hydrophilic moieties on hydrophobic organic surfaces [7,8,9,10,11,12]. Ozone can be generated in-situ by exposing atmospheric oxygen to 185 nm UV radiation. It is known that the hydrophilization of polymeric surfaces is transient [1], owing to a conformational rearrangement of the polymeric chains after exposure, recovering the pristine hydrophobic character of the specimen [4]. The speed and the extent of this recovery can range from a few hours to a few days [4,5], depending on many factors such as the substrate material [6,7], the exposure time [8], and the after-exposure storage [1,7]. In order to obtain a more stable reactive surface a chemical functionalization of the physically treated surface is necessary. Organo-silane molecules, like (3-Aminopropyl) triethoxysilane (APTES) or (3-Glycidyloxypropyl) triethoxysilane (GPTES) (Fig. 1), have been largely used to this purpose, because they present terminal radicals, like ammine or epoxy group, that are capable to form covalent bonds with biological molecules.

In this paper a comparison between two of the most relevant physical surface treatments, the oxygen plasma and the less expensive UV-ozone, is presented. For the UV-ozone treatment a very simple and cheap 20 W lamp has been used to verify its ability to conveniently modify the plastic surface and to be used as a possible substitute of the oxygen plasma treatment. An XPS analysis of the chemical radicals created and their abundance on the COC surfaces treated with these two methods has been performed, as well as a wettability and a surface nano-morphology measure of the plastic surface after the two treatments, to verify possible surface damages. In order to understand the most suitable conditions, different exposure times of both oxygen plasma and UV-ozone have been used and their effects on the COC surface have been measured with the described techniques. Furthermore, the ability of the two treated surfaces to react with GPTES to form a stable chemically modified surface, and the best conditions to obtain this result, were also investigated. Finally, the COC modified and functionalized surfaces have been used to bound specific biological probes in an OLED-based point-of-care device to test their functionality and to understand if the UV-ozone treatment can be successfully used to replace the oxygen plasma to treat the transparent plastic substrate in a real fluorescence-based diagnostic test.

2. Experimental: protocol and materials

2.1. UV-OZONE treatment

A UV-ozone lamp with a total power of 20 W was positioned in a fume hood for the sample treatment. The fume hood was positioned in a cleanroom class 10,000, where temperature and humidity are kept constant at 21 °C and 45 % respectively. Specimens consisted in 2 cm x 2 cm polyolefins (COC) cuts obtained from a polyolefin foil. The protecting sheet covering the foil side to be exposed was removed just before placing the specimen under the UV lamp, to minimize contaminations.

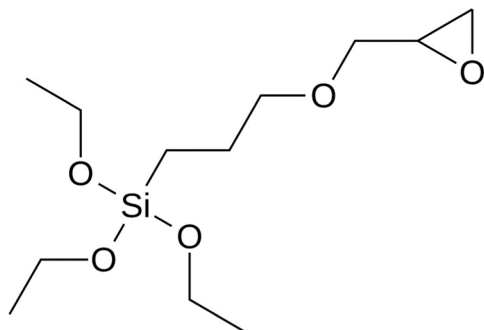


Fig. 1. Molecular structure of GPTES (3-Glycidyloxypropyl) triethoxysilane.

The lamp was positioned at 5 cm from the substrate surface. At this distance the measured total optical power density was 5000 mW m⁻². No additional oxygen was fluxed. Individual samples have been exposed to the UV-ozone atmosphere for 2, 4 and 6 min. The exposure time was limited at 6 min to avoid a problem of autofluorescence of the UV-ozone exposed COC foil when illuminated with a wavelength of 434 nm, which is the wavelength used in the OLED based point of care device. For each exposure time a set consisting of three identical specimens was simultaneously treated: one sample for the XPS analysis, one for the contact angle measurement and one for the functionalization with the GPTES molecules.

To minimize the hydrophobicity recovery effect, the three specimen sets corresponding to 2 and 4 min of exposure were processed simultaneously, with each set removed after the proper exposure time. So, no more than 3 min off-exposure elapse between the first and last specimen. The final 6 min-of-exposure specimens set was exposed to the UV-ozone atmosphere for 6 min in an independent exposure, to reduce the off-exposure time of the previous samples.

After the exposure, each sample was placed on a sample holder with the exposed side being carefully oriented upward. The holders were then sealed with parafilm to reduce contact with air while moving to the next analysis stations. The moving time required to deliver the exposed samples to the XPS station, transfer them on the XPS sample holder and start the vacuum creation was less than 10 min. For the contact angle and GPTES functionalization, the delivery time was less than 4 min and total elapse time between the end of the UV-ozone exposure and the start of the wettability measurements was less than 10 min.

The same procedure has been repeated for two times, on two different sets of samples.

2.2. Oxygen plasma treatment

For oxygen plasma treatment, samples were placed in a vacuum chamber with a constant oxygen flow of 30 sccm. The pressure in the chamber was manually regulated by a throttle valve to maintain 5.6×10^{-1} mbar, and a bias voltage of 50 V was applied to generate 20 W of plasma on the samples for 1, 2, and 3 min. As with UV-ozone treatment, a set consisting of three identical specimens was treated simultaneously for each exposure time: one sample for the XPS analysis, one for the contact angle measurement, and one for the functionalization with the GPTES molecules. Also in this case, the procedure has been repeated for two times, on two different sets of samples.

2.3. GPTES functionalization

Both the oxygen plasma and UV-ozone treated COC substrate samples were immediately immersed into a solution of 95 % anhydrous ethanol and 5 % demineralized water containing 1 wt % of GPTES. After heating the reaction formulation at 60 °C, the COC substrates were left for 2 h and then rinsed three times with anhydrous ethanol. The samples were dried at 120 °C for 60 min in an air-circulating drying oven [13].

2.4. XPS measurements

The XPS spectra were acquired in ultrahigh vacuum (base pressure: $\sim 5 \times 10^{-10}$ mbar) at room temperature in normal emission geometry using a conventional Mg X-ray source ($h\nu = 1253.6$ eV) and a hemispherical electron energy analyzer (120 mm by PSP: total energy resolution ~ 0.8 eV, standard deviation ± 0.2 eV). To compensate for charging effects, all binding energies (BE) were calibrated by fixing the C 1 s binding energy of atmospheric contamination at 284.6 eV. For the core-level spectra fitting a Doniach-Sunjc distribution has been used, after a correction with a Shirley background [14]. All the spectra fittings have been performed using a home-developed IGOR macro running on IGOR-Pro (WaveMetrics ©).

2.5. Contact angle measurements

Contact angle (CA) measurements were carried out using a Data-Physics optical instrument OCA 15Pro (DataPhysics Instruments GmbH, Germany) at room temperature by adding 2 μL of Milli-Q water or diiodomethane to the sample surface. The average CA values were determined by measuring three or four different positions on each sample surface (one sample for each condition). The surface energy, as well as the surface energy of the polar and dispersive parts, was then calculated according to the model of Owens, Wendt, Rabel and Kaelble [15,16].

2.6. Optical profilometer measurements

The profile and roughness of the samples were measured with a 3D Profilim (Filmetrics) using white light interferometry to scan 5 μm in height on the sample surface. After scanning, the profiles were 3-point leveled and the surface roughness was measured by manually selecting a square region-of-interest (ROI) with lateral size $\sim 1.5 \mu\text{m} \pm 0.3 \mu\text{m}$, clear of debris or particles. Six different surface regions were measured for each sample and the mean values reported.

3. Results and discussion

3.1. Surface characterization

3.1.1. XPS measurements: GPTES-free and GPTES-decorated COC surveys

The chemical modifications of the COC UV-ozone and oxygen plasma treated samples were first characterized by XPS to discriminate the surface activation efficacy of the two treatments as a function of exposure time. A survey analysis on all treated samples has been performed and compared with the results obtained on an untreated sample. The results for the GPTES-free specimens are reported in Fig. 2.

The plotted spectra in Fig. 2 have been normalized to the C 1s intensity of the untreated surface. As can be seen, the original sample does not present evidence of oxygen on its surface while in the 4- and 6 min UV-ozone and in all oxygen plasma COC treated foils the oxygen 1s peak is visible. In the case of oxygen plasma treated sample the oxygen peak intensity was independent from the exposure time, meaning a surface

saturation after only 1 minute of oxygen plasma exposure.

The O 1s peak intensity looks much lower for all the UV-ozone than for oxygen plasma treated samples and, in this case, the peak intensity was dependent on the exposure time, being proportional to this one. The integral value of the O 1s peak of the 6 min UV-ozone treated surface is around one tenth of the same oxygen peak in the 1 min oxygen plasma exposed surface, indicating a significant reduction in oxygen polar radical amount on the UV-ozone treated surface.

After the two physical treatments, a chemical modification of the surfaces has been performed, as described in previous sections, to try to deposit a monolayer of an organo-silane molecule (GPTES) which presents a reactive epoxy group. An XPS analysis on these chemically functionalized surfaces has been performed to prove the altered chemical reactivity delivered to the COC by the two surface treatments at different exposure times. The survey spectra of such analysis are reported in Fig. 3.

Comparing the survey plots for the UV-ozone treated samples in Fig. 2 and Fig. 3, it is possible to observe in the latter an increased O 1s peak for all the exposure times, with O 1s intensity increasing proportionally with the exposure time. On the contrary, concerning the oxygen-plasma treated samples, no clear trend is visible and the intensities of the O 1s peaks in Fig. 3 are lower than the corresponding ones in Fig. 2. Importantly, for all the oxygen-plasma treated samples and for the 6 min UV-ozone-treated one, it is also possible to see in Fig. 3 the Si 2s (153 eV) and Si 2p (103 eV) peaks, confirming the successful adhesion of organo-silane molecules on our treated surfaces. Furthermore, it is also interesting to note that the integral value of the Si 2s and Si 2p peaks look similar for the oxygen plasma treated surfaces and for the 6 min exposed UV-ozone samples, indicating a possible comparable presence of this component in these differently treated samples.

3.1.2. XPS measurements: High resolution core level analysis

To understand the kind and the abundance of the polar reactive radicals formed at the COC surface, higher resolution core level spectra have been recorded and fit for both carbon 1s and oxygen 1s spectral features in the energy range of interest [17,18,19,20,21]. Fig. 4 and Fig. 5 show the fit high-resolution core-level profiles analysis of the GPTES-free and GPTES-decorated COC surfaces treated with 1-min oxygen plasma (left side) and 6-min UV-ozone (right side) for the C 1s and

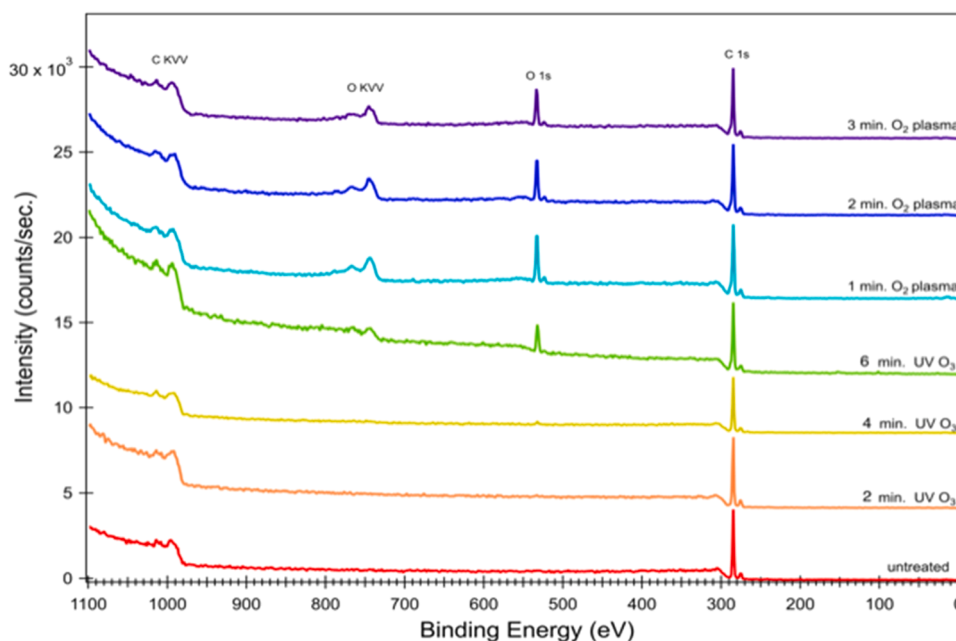


Fig. 2. XPS survey of COC samples treated with UV-ozone and Oxygen plasma for different exposure times (1, 2 and 3 min for oxygen plasma, 2, 4 and 6 min for UV-ozone).

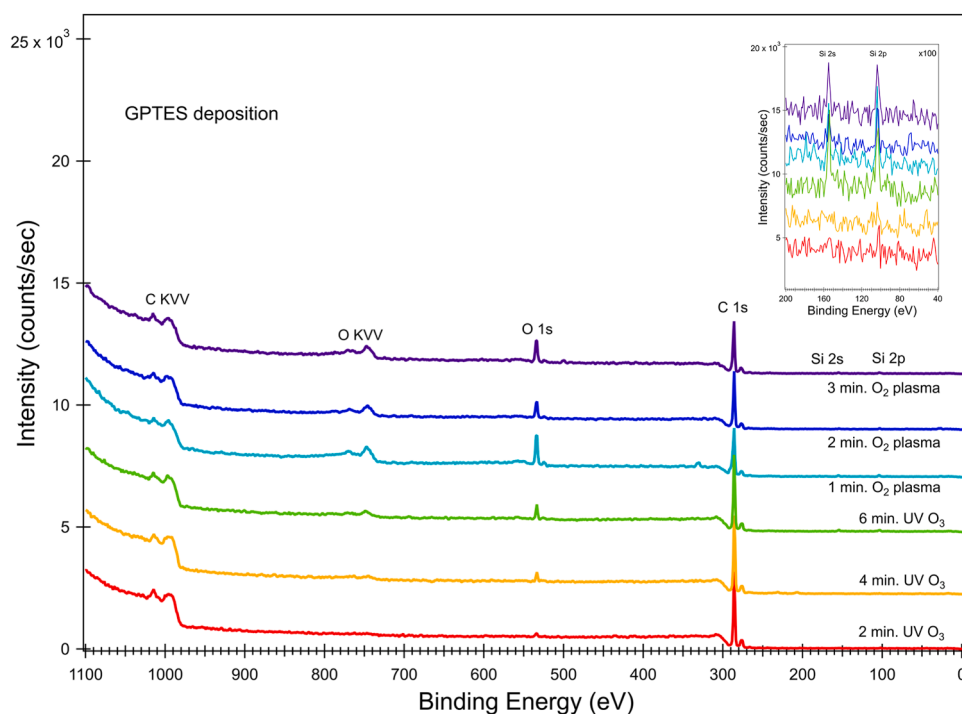


Fig. 3. XPS survey of COC samples treated with UV-ozone and oxygen plasma for different exposure times (1, 2 and 3 min for oxygen plasma, 2, 4 and 6 min for UV-ozone) and functionalized with GPTES. In the inset the expanded binding energy region around the Si emission.

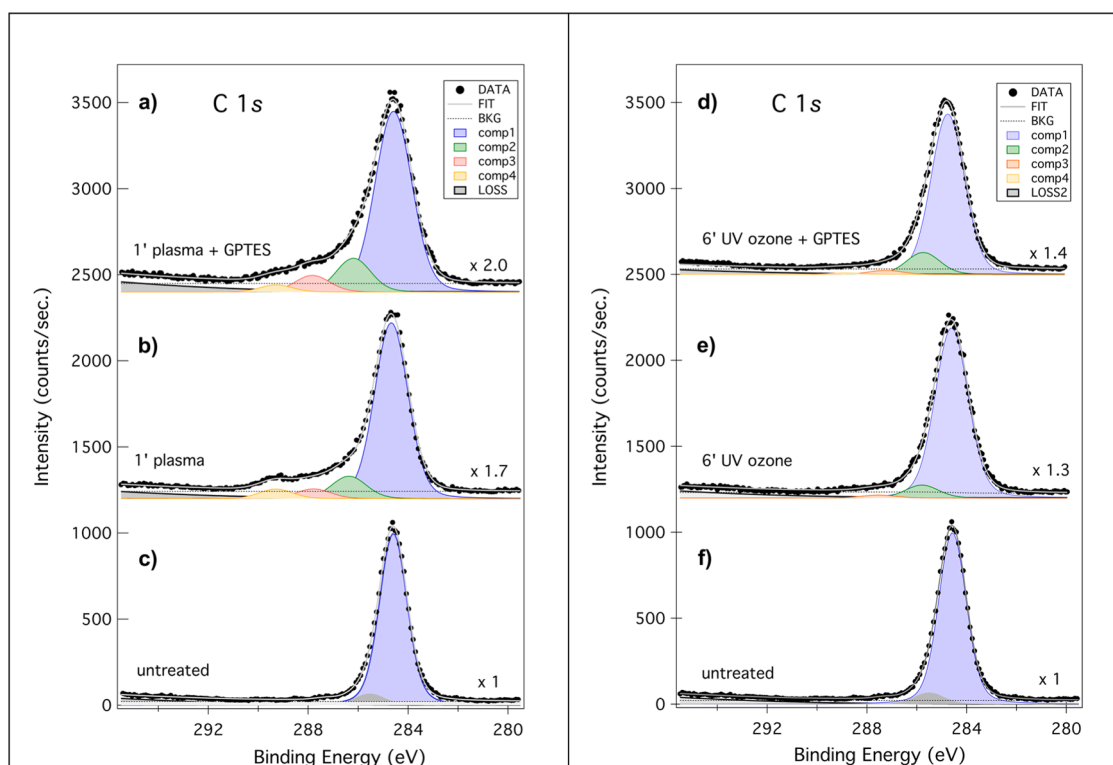


Fig. 4. Carbon 1 s deconvoluted spectra. a) oxygen plasma treated sample (1 minute exposure time) chemically modified with GPTES; b) oxygen plasma treated sample (1 minute exposure time); c) COC untreated sample; d) UV-ozone treated sample (6 min exposure time) chemically modified with GPTES; e) UV-ozone treated sample (6 min exposure time); f) COC untreated sample. Scale factors are reported on the bottom-right corner of each spectrum.

O 1 s background normalized spectra, respectively. For the GPTES-decorated surface, the corresponding relative abundances of the peak components are listed in [Table 1](#) and [Table 2](#).

For the GPTES-free activated surface, the C 1 s core level of the

oxygen plasma treated sample, beyond the carbon sp² component, shows the presence of three different oxygen-carbon bonding, C-O (286.31 eV), C-O-C (287.71 eV) and COOH (289.21 eV) with the percentage of 10.3 %, 4.4 % and 4.4 % respectively. The same kind of

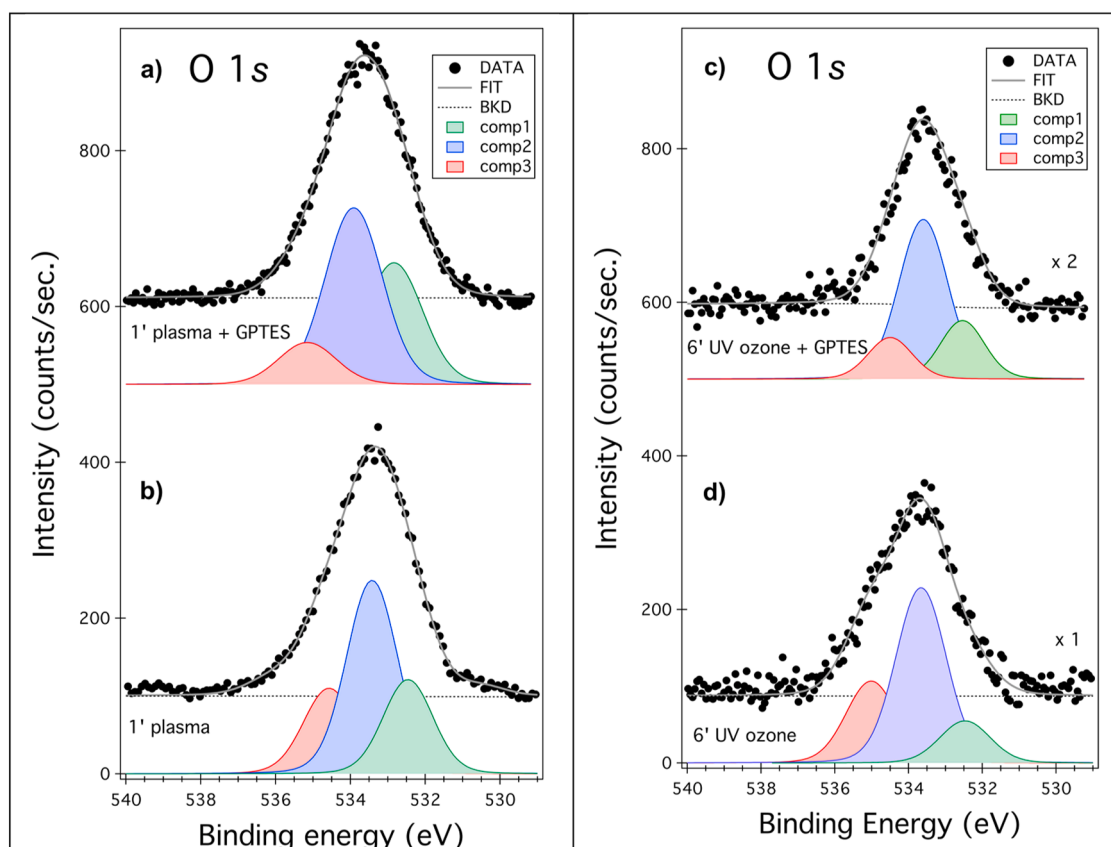


Fig. 5. Oxygen 1 s deconvoluted spectra. a) oxygen plasma treated sample (1 minute exposure time) chemically modified with GPTES; b) oxygen plasma treated sample (1 minute exposure time); c) UV-ozone treated sample (6 min exposure time) chemically modified with GPTES; d) UV-ozone treated sample (6 min exposure time). Scale factors are reported on the bottom-right corner the spectra of UV-ozone treated specimens.

Table 1

Percentage of the different carbon bonds of the two GPTES treated surfaces.

Component	Peak energy [eV]	Bond	OXYGEN PLASMA	UV-OZONE
1	284.6	sp2	77 %	85 %
2	286.31	C-O, Si-O-C	13 %	12 %
3	287.71	C-O-C	8 %	2 %
4	289.21	COOH	2 %	1 %

Table 2

Percentage of the different oxygen bonds of the two GPTES treated surfaces.

Component	Peak energy [eV]	Bond	OXYGEN PLASMA	UV-OZONE
1	532.6	C=O, C-O-C	35.7 %	21.2 %
2	533.6	Si-O-C, Si-O-Si, C-O	51.9 %	63.7 %
3	534.5	H ₂ O adsorbed	12.1 %	15.1 %

oxygen-carbon bonding was observed also for the UV-ozone treated surface but with significantly different percentages, in particular: the C-O is present at 3.5 %, the C-O-C at 2.6 % and the COOH at 0.66 % [22,23,24]. Considering the spectra of the O 1 s core level on the same samples, the analyzed data show again the presence of three components that can be ascribed to the following carbon-oxygen bonding and to the surface adsorbed moisture: C-O (533.6 eV), C-O-C and C=O (532.6/532.4 eV) and surface adsorbed moisture (H₂O) (534.5 eV) [22,23,25].

The relative percentages of the O 1 s components for the treated samples are coherent with the percentages obtained for the carbon high

resolution spectra indicating a much higher presence (around three times) of reactive radicals in the oxygen plasma treated samples. This result is also supported by the contact angle measures, where it is possible to observe a much more pronounced hydrophilic behavior in the oxygen plasma treated samples with respect to those exposed to UV-ozone.

For the GPTES-decorated surfaces, from the high-resolution C 1 s spectra (Fig. 4) it is possible to observe that after the GPTES functionalization the C-O component of both the samples is enhanced, especially for the UV-ozone treated COC surface, where it increased from 3.5 % to 12 %. In this case the component at 286.31 eV can be ascribed also to the bond C-O-Si which is formed after the chemical reaction with the GPTES molecules by condensation with the polar radical present on the physically treated COC surface. It is also possible to observe a different percentage of the C-O-C bond, that can be ascribed to the presence of the epoxy group in the GPTES molecule, in the two differently treated samples. The presence of this group is four times larger in the oxygen plasma treated surface than in the UV-ozone treated one. This consideration is confirmed by the deconvolution of the O 1 s high resolution spectra (Fig. 5), where the oxygen plasma treated samples present a higher percentage of C-O-C groups, as reported in the Table 2.

Finally, Fig. 6 shows the fit core level profiles for Si 2p peaks for the GPTES-decorated samples. The spectra of silicon measured on the two treated COC surfaces present a very different shape that after the deconvolution can be attributed to a different amount of silicon compound on the plastic surface [19]. Table 3 reports the kind of silicon bonds and their relative percentages measured in the two samples.

As reported in Table 3, the oxygen plasma treated COC sample presents a significant percentage of Si-O-C bonds that can be attributed to the presence of organo-silane molecule bonded to the COC surface

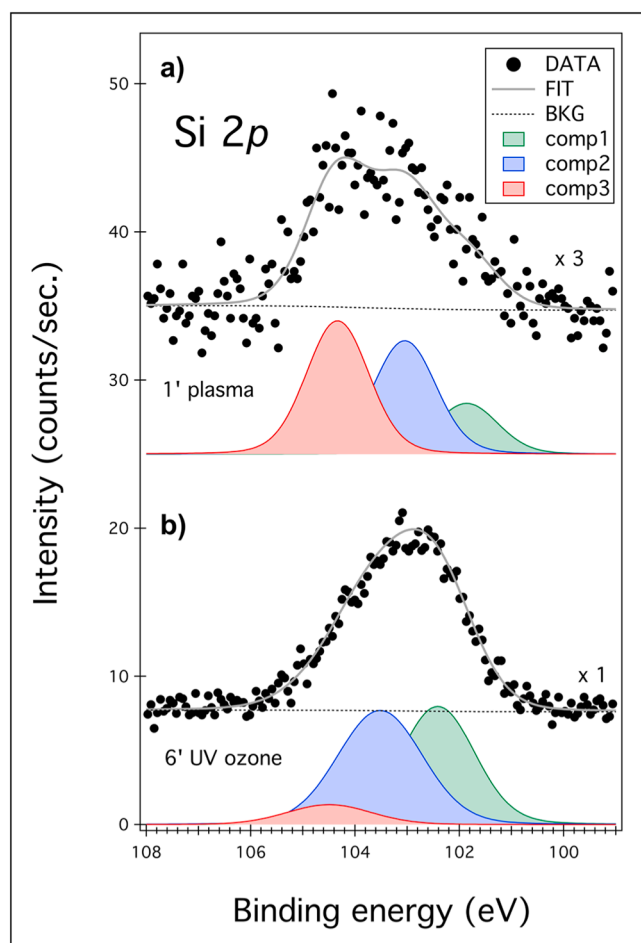


Fig. 6. Silicon 2p deconvoluted spectra of the GPTES functionalized samples. a) oxygen plasma treated sample (1 minute exposure time); b) UV-ozone treated sample (6 min exposure time). Scale factors are reported on the bottom-right corner of each spectrum.

Table 3
Percentage of the different silicon bonds of the two GPTES treated surfaces.

Component	Peak energy [eV]	Bond	OXYGEN PLASMA	UV-OZONE
1	102.41	Si-C/ Si-O-Si	17.03 %	43.72 %
2	103.52	Si-O-C/ Si-O	38.14 %	47.94 %
3	104.50	Si-Ox	44.8 %	8.3 %

thanks to the relevant presence of hydroxyl groups on it. Furthermore, a high percentage of Si-Ox (44.8 %) is measured which can be ascribed to the presence of GPTES molecules in their original configuration, where the Si atom is bond to three oxygen and one carbon atoms. The relatively small percentage of Si-O-Si (and Si-C) bonds can be attributed to the formation of some siloxane networks.

It is known that the alkoxy-silyl groups in an organofunctional silane compound can build oligomers and can react with water molecules to form silanol groups [26,27,28]. These groups are not stable and undergo condensation over time, creating siloxane network that can induce gelation [27,28].

This effect is more evident in the UV-ozone treated sample, where a higher amount of Si-O-Si and Si-C bonds than in the previous sample is measured. Also, from table 1 it is possible to observe that the percentage of C-O-C bonds is much higher in the oxygen plasma treated than in the

UV-ozone treated COC surface, confirming the higher presence of epoxy groups in oxygen plasma treated surface. Moreover, from table 3 we can see that the contribution of the Si-O-C group, which could be attributed to the attachment of the GPTES to the COC via condensation is more important in the UV-Ozone treated surface than in the oxygen plasma treated one. This counterintuitive result can be ascribed to a possible reaction of the GPTES epoxy group in the UV-ozone treated surface with the hydrolyzed Si in the GPTES molecule, after its reaction with water molecules present in the solution used to deposit the GPTES layer on the treated surface. Furthermore, the low Si-Ox percentage (less than 10 %) confirms the reaction of the original GPTES molecule to create a siloxane network.

Then, the XPS observations on our samples confirm that a strong presence of hydroxyl moieties in a physical treated surface is crucial for the formation of an ordered organo-silane layer that can present a relevant number of reactive groups, in our case epoxy groups, available for successive reaction with bio-probes [29].

3.2. Contact angle

The same samples prepared for the XPS measures have also been measured with contact angle using both water and diiodomethane to measure the changes of wettability and surface energy of the substrate after the different physical and chemical modification steps. The results are reported in Tables 4 and 5 and Fig. 7 (graphs).

The pristine COC substrate is not wettable with polar-based solutions due to its intrinsic nonpolar surface characteristics involving a very low polar part of the surface energy (0.01 mN m^{-1}) and a large water contact angle of 99° . After the oxygen plasma treatment of the COC surface, we observed a significant increase in the polar part from 0.01 to more than 36.6 mN m^{-1} (depending on the plasma exposure time), which is mainly attributed to the formation of oxygen containing polar species such as COOH, -COC or -COH groups, as confirmed by XPS analysis. The presence of these polar groups on the surface allows to improve the COC film wettability with a significant decrease of the water contact angle, and they are also used as reactive anchor groups to immobilize organosilanes molecules by a condensation reaction.

As can be seen from Table 4, the water contact angle of the oxygen plasma treated sample is extremely low after only 1 minute of treatment, with a measured contact angle of only 7° . The contact angle is slightly increasing with the oxygen plasma exposure time reaching a maximum value of 19° after three min of exposure [30]. All the samples present a high degree of hydrophilicity confirming the presence of nucleophilic radicals at the COC treated surface, in agreement with the XPS measures. On the contrary the UV-ozone treated samples exposed for 2 and 4 min present a water contact angle very similar to that of the untreated

Table 4
Surface energy (polar and disperse parts) and contact angle of polar and non-polar liquids on UV-ozone and oxygen plasma treated COC surfaces.

Substrate treatment	Surface Energy [mN m ⁻¹]	Polar part [mN m ⁻¹]	Disperse part [mN m ⁻¹]	Theta H ₂ O [°]	Theta CH ₂ I ₂ [°]
Untreated	40.23 ± 0.50	0.01 ± 0.01	40.22 ± 0.50	99 ± 2	41 ± 2
UV-ozone 2 min	40.79 ± 0.89	3.12 ± 0.29	37.67 ± 0.59	96 ± 3	42 ± 3
UV-ozone 4 min	40.60 ± 0.98	0.36 ± 0.08	37.70 ± 0.73	96 ± 6	42 ± 2
UV-ozone 6 min	40.42 ± 0.64	5.07 ± 0.2	35.34 ± 0.37	78 ± 5	42 ± 2
Oxy Plasma 1 min	74.26 ± 2.02	40.35 ± 0.88	33.91 ± 1.14	7 ± 2	26 ± 5
Oxy Plasma 2 min	73.1 ± 1.0	37.86 ± 0.65	35.24 ± 0.38	14 ± 4	23 ± 2
Oxy Plasma 3 min	71.4 ± 0.41	36.64 ± 0.26	34.76 ± 0.14	19 ± 2	25 ± 1

Table 5

Surface energy (polar and disperse parts) and contact angle of polar and non-polar liquids on UV-ozone and oxygen plasma treated COC surfaces after their functionalization with GPTES.

Substrate treatment	Surface Energy [mN m ⁻¹]	Polar part [mN m ⁻¹]	Disperse part [mN m ⁻¹]	Theta H ₂ O [°]	Theta CH ₂ I ₂ [°]
UV-ozone 2 min +GPTES	38.63 ± 0.74	0.3 ± 0.06	38.33 ± 0.68	94 ± 1	41 ± 2
UV-ozone 4 min +GPTES	40.79 ± 0.89	3.12 ± 0.29	37.67 ± 0.59	95 ± 3	42 ± 4
UV-ozone 6 min +GPTES	40.42 ± 0.64	5.07 ± 0.28	35.34 ± 0.37	83 ± 5	39 ± 3
Oxy Plasma 1 min +GPTES	45.98 ± 0.14	7.28 ± 0.10	38.70 ± 0.04	71 ± 1	32 ± 1
Oxy Plasma 2 min +GPTES	46.0 ± 2.19	4.35 ± 0.40	41.65 ± 1.79	76 ± 3	28 ± 7
Oxy Plasma 3 min +GPTES	46.08 ± 0.37	8.29 ± 0.11	37.79 ± 0.27	69 ± 1	33 ± 1

surface being in the range of 96°. A decrease in the water contact angle is measurable only after 6 min of UV-ozone exposure, in this case the contact angle measured was 78°, which show still a relatively high degree of hydrophobicity of the sample. Also, these findings are confirmed by the XPS results showing a poor content of reactive nucleophilic radicals at the treated surface.

The contact angle measures have also been performed on the GPTES chemically functionalized samples after the physical treatments, showing a higher hydrophobicity for the UV-ozone treated surfaces. In general, the increase in hydrophobicity can be ascribed to the presence of alkyl chain that shows hydrophobic properties. Furthermore, in this case, the nonpolar siloxane surfaces, resulting from the siloxane network, have relatively weak interaction with interfacial water molecules. As a result, a nonpolar siloxane surface has a more hydrophobic surface state. For the plastic surfaces with polar sites, such as the surfaces where the epoxy group are more present, as in the case of oxygen plasma treated surfaces, the presence of oxygen can induce hydrogen bonding with the interfacial water molecules, resulting in a more hydrophilic surface [31], and confirming the results obtained with the XPS measures.

3.3. Optical profilometry

To measure the surface morphology before and after the physical treatment and the chemical modification, optical profilometer measurements have been performed. For every sample the maximum peak-to-valley value (Rt) as well as the arithmetic mean height (Ra) and the root mean square height (Rq) have been calculated as the mean value of six measures in different location of the same sample. Surface roughness data are reported in Table 6. The UV-ozone plasma treated samples do not show significant variation from the values of the untreated sample for all the different time exposure (2, 4 and 6 min -data not reported-). The oxygen-plasma treated samples show a different behavior, in particular, it is evident an increase in the Rt, and also in Ra and Rq value, while less significant than for Rt, proportional to the exposure time. This roughness increase can be ascribed to the high energy oxygen ions present in the plasma, impinging on the plastic surface and creating, beyond a different number of new polar radical, a surface morphology modification proportional to the exposure time.

After the GPTES chemical modification of the two physically treated surfaces (Fig. 8), it is possible to observe that the UV-ozone treated surface still maintains a very smooth surface morphology with Ra and Rq as low as (1.61±0.4) nm and (2.07±0.517) nm respectively, while the 3 min exposure oxygen plasma treated surfaces show a higher roughness, with values of Ra and Rq of (5.12±5.44) nm and (6.13±4.92) nm respectively. It is evident that the chemical functionalization with the organosilane molecule induced a slight worsening of the roughness in the oxygen plasma treated surface, which contributes to increase the water contact angle on this surface.

4. Bio-molecules adhesion on the two treated surfaces

Finally, the two GPTES treated COC surfaces were used to test their ability to bind a protein bio-probe used in an OLED-based point-of-care device. A bovine serum albumin protein conjugated with aflatoxin B1 (BSA-AFB1), a mycotoxin that can contaminate foodstuffs such as cereals, legumes and dried fruits, was used as bio-probe on both the oxygen plasma and UV-ozone treated surfaces functionalized with GPTES. A drop of 1.5 µL of BSA-AFB1 at a concentration of 100 µg/ml, diluted in carbonate buffer at pH 11, was deposited on both the surfaces and

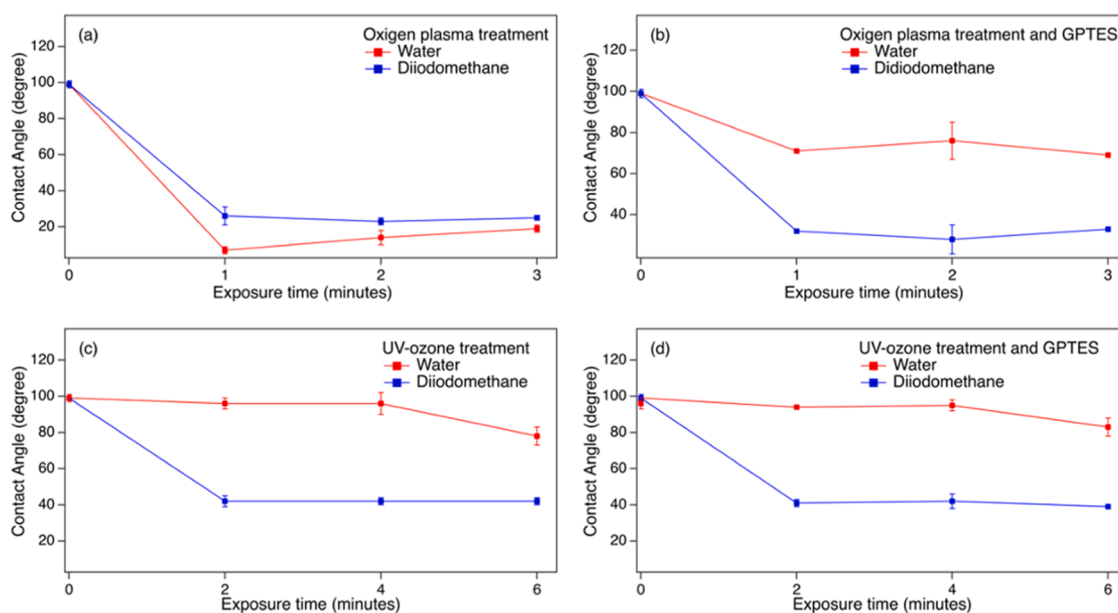


Fig. 7. Graphs of the measured contact angles with water (red squares) and diiodomethane (blue squares) as a function of exposure time to oxygen plasma and UV-Ozone physical treatments. In all the graph the first point (time 0) is the measure of the untreated COC surface (a) on oxygen plasma treated surfaces; (b) on oxygen plasma treated surface silanized with GPTES; (c) on the UV-ozone treated surfaces; (d) on the UV-ozone and GPTES silanized surface.

Table 6

Surface roughness of the UV-ozone and oxygen plasma treated COC surfaces after their functionalization with GPTES.

	Untreated surface	Ox-plasma (1 min)	Ox-plasma (3 min)	GPTES Ox-plasma (1 min)	GPTES UV-OZONE (6 min)
Rt [nm]	13.77±6.74	34.37±12.25	526.9±20.24	288.6±15.3	21.1±4.88
Ra [nm]	1.22±0.30	3.34±0.71	4.26±1.26	5.12±5.44	1.61±0.4
Rq [nm]	1.15±0.34	4.21±0.91	5.85±1.73	6.13±4.92	2.07±0.517

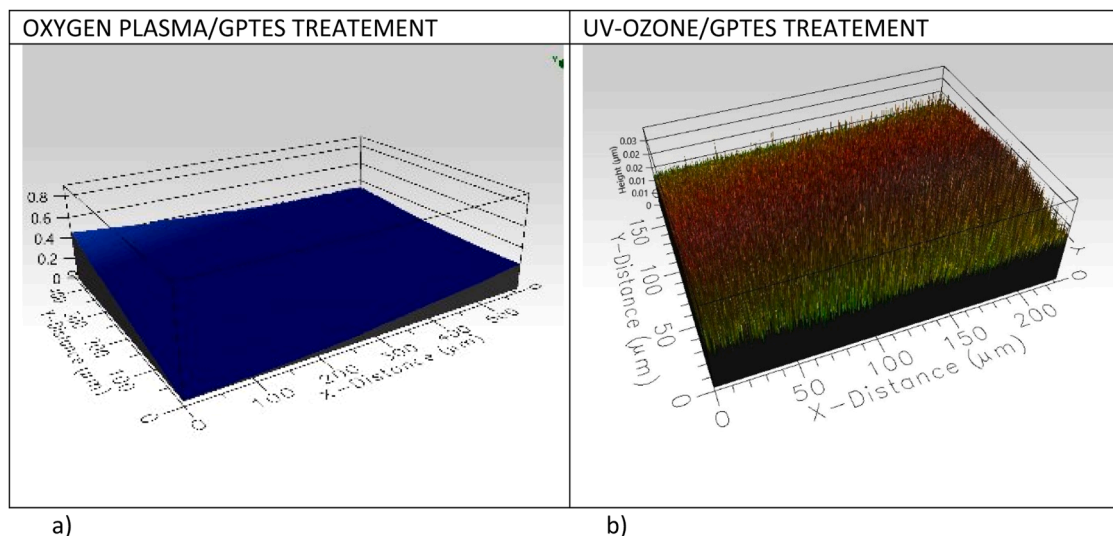


Fig. 8. 3D surface image of the GPTES chemically modified COC surfaces. a) surface treated with oxygen plasma 3 min exposure time, $R_a=(5.12\pm5.44)$ nm, $R_q=(6.13\pm4.92)$ nm. b) surface treated with UV-ozone, $R_a = (1.61\pm0.4)$ nm, $R_q = (2.07\pm0.517)$ nm.

incubated overnight at room temperature. After a washing with a solution of PBS (phosphate saline bath) and 0.05 % Tween 20 (PBS-T), the bonded BSA-AFB1 were incubated for 30 min at 37 °C with 35 μ L of a solution of monoclonal antibodies anti-AFB1 conjugated with the fluorophore ATTO 430 LS (with peak absorption wavelength at 434 nm and peak emission wavelength at 545 nm), at a concentration of 1.5 μ g/ml. All the tests using the two treated substrates were run in triplicate. After two washing steps with PBS-T, the fluorescence of the spots was measured switching on the OLED, emitting with a peak wavelength of 434 nm, and measuring the excited fluorescence in transmission. The images were recorded with a CCD camera with a 12-bit digitalization and a band-pass filter to allow the transmission of only the fluorescence radiation at 545 nm, with an integration time of 30 seconds. The fluorescence values were measured as the mean value of the illuminated pixels in the bright spot, after the background subtraction, as described in [32,33,34]. The results of the fluorescence obtained using the two differently treated surfaces are shown in Fig. 9.

As can be seen, the fluorescence signals recorded on the oxygen plasma treated substrate are more intense (mean 728 counts) than that obtained using the UV-ozone treated substrate (mean 324 counts) with more than the double of counts number. Furthermore, the repeatability of the fluorescence intensity signal was better using the oxygen plasma treated substrate, with a standard deviation of only 18 counts respect to that obtained with the UV-ozone treated substrate with 107 counts. These results indicate that a higher number of epoxy groups are available for the protein bonding in the oxygen plasma treated substrate and that their distribution is more uniform on the substrate surface, allowing a higher sensitivity and a better repeatability of the assay results than in the UV-ozone treated COC surface. It is then evident that, while also the UV-ozone surface physical treatment allows to create a number of reactive radicals at the COC surface, their number and surface distribution are less favorable to the following chemical functionalization with GPTES and then to the creation of significant amount of epoxy groups available for the formation of stable covalent bonding with

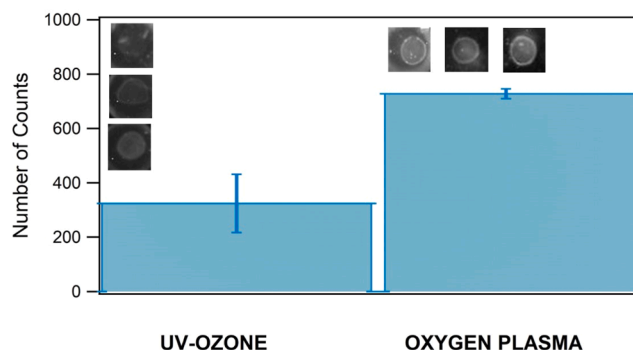


Fig. 9. Fluorescence intensity measured (number of counts) as a mean of the illuminated pixel in the fluorescent spot (BSA-AFB1 plus mAb anti-AFB1 conjugated with the fluorophore ATTO 430 LS) in triplicate. The fluorescent spots were obtained using the same assay procedure but using the COC substrates treated respectively with UV-ozone and oxygen plasma, and chemically functionalized with GPTES.

proteins or other bio-probes.

5. Conclusions

Two physical treatments on a Cyclo-Olefin-Copolymer surface, namely oxygen plasma and UV-ozone exposure, have been investigated to understand their ability to be successfully chemically modified with the organo-silane molecule GPTES; this surface chemical modification being useful for the creation of reactive epoxy groups on the COC film surface to promote the covalent bonding of bio-probes. The investigation of the treated surfaces with X-rays photoelectron spectroscopy (XPS) on the physically and chemically modified surfaces has shown a more significant presence of reactive radicals, like COOH, -COC or -COH groups, on the oxygen plasma treated surface as well as a higher

ability to react with GPTES to create free reactive epoxy groups on the plastic surface. On the contrary, the UV-ozone treated surface has shown a lower abundance of reactive carboxy radicals and the following chemical reaction with GPTES has promoted the creation of a siloxane network, reducing the number of free reactive epoxy group available for the covalent bonding of bio-probes. The surface energy of the different treated surface was also measured with a contact angle technique confirming the XPS analysis results and showing a higher hydrophilicity of the oxygen plasma treated samples in respect of the ones treated with UV-ozone. Also, the hydrophobicity recovering of the GPTES chemically modified surfaces was more pronounced in the UV-ozone treated surface. Moreover, an optical profilometry analysis of the morphology of the same surfaces has evidenced an increase in the surface roughness of the oxygen plasma treated surface proportional to the exposition time, coherently with the slight water contact angle increase measured on the sample exposed to plasma for three minutes. Considering that the XPS analysis has shown an oxygen saturation in the COC surface after only one minute of the oxygen plasma exposure time, and that a better surface morphology is obtained, it is possible to conclude that the sample exposed to oxygen plasma for one minute and then functionalized with GPTES gives the best result in term of possible bio-probe adhesion. These conclusions have been confirmed by the last experiment where the two optimized substrates treated with UV-ozone and oxygen plasma were used to bound a protein antigen (BSA-AFB1) in an assay using fluorophore conjugated monoclonal antibodies excited with a custom-made OLED. The assay results showed an intensity of the fluorescence signal double in the case of the oxygen plasma treated surface which also presents a better reproducibility in repeated tests.

CRediT authorship contribution statement

Barbara Ressel: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jurij Urbančič:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Marco Beltrami:** Writing – review & editing, Investigation, Data curation. **Erik Betz-Güttner:** Writing – review & editing, Investigation, Data curation. **Cinzia Cepek:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Martina Conti:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Ayesha Farooq:** Investigation. **Patrizia Melpignano:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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