

Patterned Surface Activation of Cyclo-Olefin Polymers for Biochip Applications

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Abstract

Two different surface activation methods (UV/ozone and oxygen plasma treatment) were applied for patterned surface activation of cyclo-olefin polymer (COP) surfaces combined with different masking techniques (metal shadow mask and protective tape). Surface properties were characterized by various methods such as contact angle measurement, ATR-IR, XPS and Surface enhanced ellipsometric contrast (SEEC) microscopy. UV/ozone and oxygen plasma allowed for patterned surface modification of COP leading to the formation of carboxylic and hydroxyl groups on the activated part of the surface. Stability against organic solvents was determined by rinsing the activated substrates with 2-propanol. For UV/ozone treatment it was found that a thin film of degradation products remains on the COP surface and is at least partly removed in the following washing or rinsing steps.

Keywords

Biofunctionalization · surface modification · plasma modification · polymer photochemistry

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1 Introduction

In the last decade the use of microsystems technology for biotechnological applications has seen a tremendous development. Especially in the field of bioanalytical methods; miniaturized systems like microfluidic devices and biochips have enabled important advances in genomics, proteomics, and diagnostics. In the early days such devices were mainly fabricated using glass or silicon substrates and adapting established fabrication technologies from the electronics and semiconductor industry. Later, fabrication of microfluidics in silicone elastomers using soft-lithography became very popular as it enabled rapid prototyping and widespread out-of-cleanroom applications [1]. Currently, the development focuses on the use of thermoplastic polymers as they allow for low-cost high volume fabrication by e.g. micro-injection molding and hot-embossing. One of the most promising thermoplastic materials for the development of low-cost microfluidic devices and lab-on-a-chip systems are cyclo-olefin polymers and copolymers (COP/COC) [2]. They are especially suited for miniaturized biosensor systems comprising optical detection schemes due to their easy processing and excellent optical properties (high transparency and low autofluorescence). While a huge number of well-developed surface modification techniques is available for many standard materials used in microfluidic technology (such as glass, silicon or PDMS) [3], such methods are still relatively scarce for thermoplastic polymers like COP.

For the fabrication of biochips and microfluidic devices the surface of thermoplastic polymers like COP has to be modified, basically for two different reasons: a) to facilitate bonding (the sealing of channels with a cover plate or foil) of microfluidic devices and b) to allow for biofunctionalization of the surface with specific (bio-)receptors or biological components (e.g. antibodies, nucleic acids, enzymes, cells, etc.). Among the most convenient surface treatment methods to enable low temperature bonding are plasma and UV-photochemical treatments [4,5]. Similar concepts have been described for direct biofunctionalization with proteins and oligonucleotides after UV-ozone treatment [6,7] and cell seeding after plasma

treatment [8]. However, the direct immobilization of biomolecules to the surface of polymer biochips or microfluidic channels might suffer from drawbacks like low surface stability, high degrees of unspecific binding, etc. Therefore, functional coatings, which allow the specific binding of functional (bio)molecules while showing low unspecific binding during the assay, are gaining more and more importance in the field of biosensor development. Many different materials have already been used to create coatings for the immobilization of biomolecules on polymer surfaces among them are e.g. polyelectrolyte multilayers [9], silane coatings [10], polysaccharide coatings [11], or protein coatings [12]. Coating the surface of non-polar, inert polymers like COP/COC with such materials usually requires a so called surface pretreatment step in order to activate the surface and introduce polar groups that can act as anchoring points for subsequently applied functional coatings.

Two different approaches for surface activation, UV/ozone, and oxygen plasma treatment, of COP material have been investigated in this work. Chemical surface properties of pristine, as well as UV/O₃, and plasma treated polymer surfaces were characterized using contact angle measurements, attenuated total reflection infrared (ATR-IR) spectroscopy, and X-ray photoelectron spectroscopy (XPS). Etching depth and patterning were investigated using optical thickness measurement and imaging on COP model films by surface enhanced ellipsometric contrast (SEEC) microscopy.

2 Materials and Methods

2.1 Substrate materials and sample preparation

COP (Zeonor 1060R) pellets were obtained from Zeon Chemicals and injection molded Zeonor 1060R slides were obtained from SonyDADC (Austria) and microfluidic chip-shop (Germany).

Thin model films of COP were prepared by spincoating onto special SiO₂ coated silicon substrates (so called “Surfs”). A 10 mg/mL solution of Zeonor1060 R in o-xylene was spun at 2000 rpm for 60 seconds. The films were then annealed at 80°C for two hours.

2.2 Surface treatment

The UV/ozone treatment was performed with a PSD Pro Series Digital UV Ozone System from Novascan containing a mercury light source emitting at 185nm and 254 nm. The polymer samples were exposed to the UV radiation for 10 min in a sample chamber flushed with oxygen and set to a temperature of 50°C.

A FEMTO Plasma System from Diener Electronics was used for O₂-Plasma activation applying a 40 kHz plasma source with a power of 100 Watt. Unless otherwise stated, COP substrates were treated with O₂-plasma at a pressure of 0.4 mbar for 30 seconds.

2.3 Surface and thin film characterization

Static Contact Angles of water were determined by analysis of the profile of sessile drops using a Dataphysics System OCA15+ (Dataphysics, Germany). The drop volume was 3 µL and drop profile analysis by the Laplace method using the Software SCA 20.2.0 provided by Dataphysics.

Surface enhanced ellipsometric contrast (SEEC) microscopy, is a rapid optical technique for thickness and morphology determination of thin films in the nanometer range [13,14]. The technique is commercialized under the name SARFUS by Nanolane, and is based on the use of special, non-reflecting surfaces for crosspolarized reflected light microscopy. These surfaces (so called Surfs) generate a contrast enhancement of about twice the magnitude compared to a pure silicon wafer. The basic principle is based on the increase in colour and intensity of reflected light, which is proportional to the film thickness of the coating on the Surf. A LEICA DM4000 optical microscope with a 20x objective was used for the measurement. Image analysis and step height calibration were performed with the software Sarfusoft (Nanolane, France).

ATR-FTIR spectra were recorded on a Bruker ALPHA FT-IR spectrometer equipped with a single reflection diamond ATR crystal.

XPS spectra were recorded using a UHV-surface-analysis system by Omicron Nanotechnology. The x-ray source was a DAR400 aluminium-anode (excitation energy 1486.70 eV), and a quartz-crystal monochromator XM 500 was used. The kinetic energy was analysed with a hemispherical analyser, type EA 125, and the signal was detected with a pulse-counting channeltron.

3 Results and Discussion

In a first step, surface treatment was investigated by water contact angle measurement. Oxygen plasma treatment of COP resulted in a rapid decrease of the water contact angel from 91° to approx. 30° even for plasma treatment times as short as 30 seconds (data not shown). As shown in Figure 1, for UV/ozone treatment, the water contact angle typically dropped to values around 30° after surface treatment with UV/ozone within several minutes indicating the formation of a hydrophilic surface and polar functional groups (black circles). The influence of UV light in this process was investigated by shielding the surface from direct UV light using a metal plate placed approx. 10 mm above the polymer surface. This allowed unhindered access of ozone from the chamber atmosphere but prevented direct UV light reaching the COP surface. The water contact angle again decreased, but far slower compared to conventional UV/ozone treatment, and remained at a value around 45° even after 60 minutes of treatment (Figure 1, grey triangles). This shows that a certain degree of surface activation is also be achieved solely due to ozone present in the chamber. Nevertheless direct irradiation of the sample is by far more effective, either due to direct

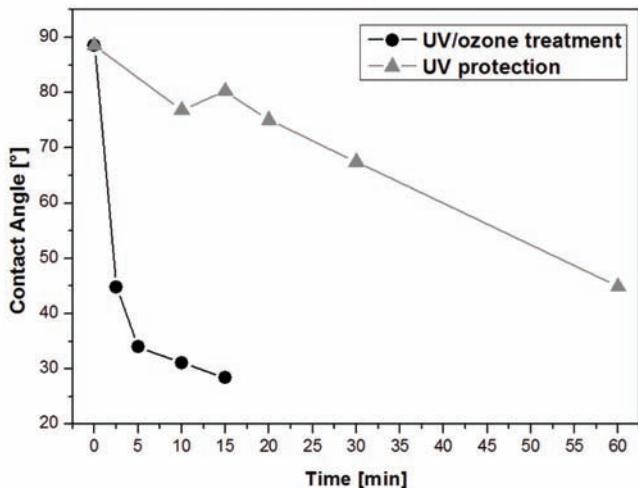


Fig. 1. Water contact angle after different duration of UV/ozone treatment, unprotected sample (black circles) and UV protected sample (grey triangles)

photochemical reactions of the polymer or due to the higher concentration of ozone produced by photochemical processes in the vicinity of the surface. This result suggests that shadow mask techniques might be a suitable method for patterned surface modification by UV/ozone treatment.

Therefore, patterned surface modification was investigated by using either metal shadow masks or a pressure sensitive adhesive (PSA) film as protective tape. Results of contact angle measurements on exposed and protected areas of COP samples are shown in Figure 2.

It can be seen that UV/ozone treatment leads to a marked decrease of water contact angle on the unprotected areas. In contrast, no significant change of contact angles was observed on the areas that were protected by either metal shadow masks or protective tape directly in contact with the COP surface. This indicates that both masking techniques - metal shadow mask and protective tape - prevented or strongly restricted the access of UV light and ozone to the COP surface. The same patterning methods were investigated in combination with oxygen plasma treatment. Here the protective tape could also be successfully applied for surface patterning while the metal shadow mask did not show any effect in protecting the COP surface from plasma treatment.

These surface modification and patterning methods on COP surfaces were investigated in more detail using spectroscopic methods (ATR-IR and XPS). The comparison of ATR-IR spectra of protected and non-protected COP surfaces activated by UV/ozone treatment, showed that new IR bands could only be observed on the UV/ozone treated surface (formation of carbonyl groups, 1715 cm^{-1} and a broad band around 3400 cm^{-1} indicating hydroxyl groups) (see Figure 3a). The formation of carbonyl and hydroxyl groups on the surface is in good agreement with previous literature data on UV/ O_3 treatment of COP [6]. On the areas protected by either Al-masks or protective tapes the ATR-IR spectra were identical to the spectrum of pristine,

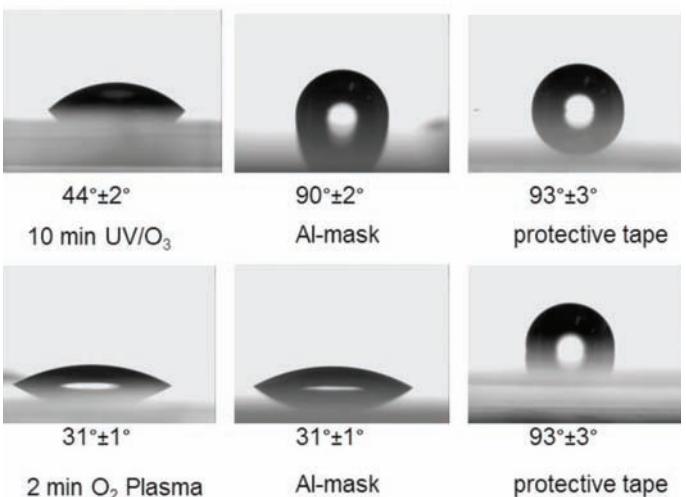


Fig. 2. Contact angles of water on COP surfaces after UV/ O_3 and O_2 -plasma treatment and different patterning principles (aluminium shadow masks and protective tape)

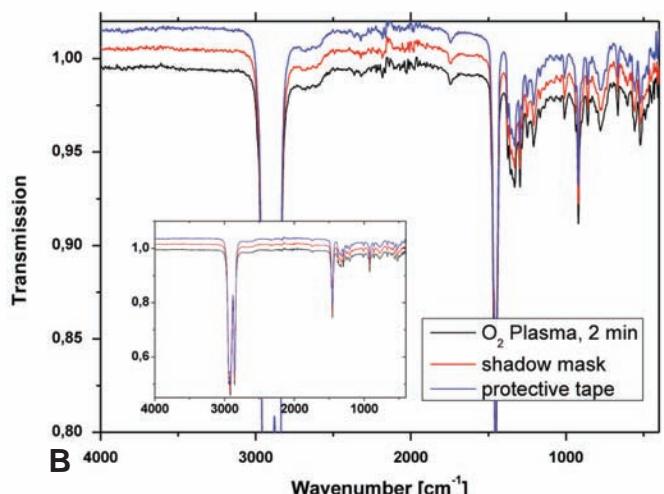
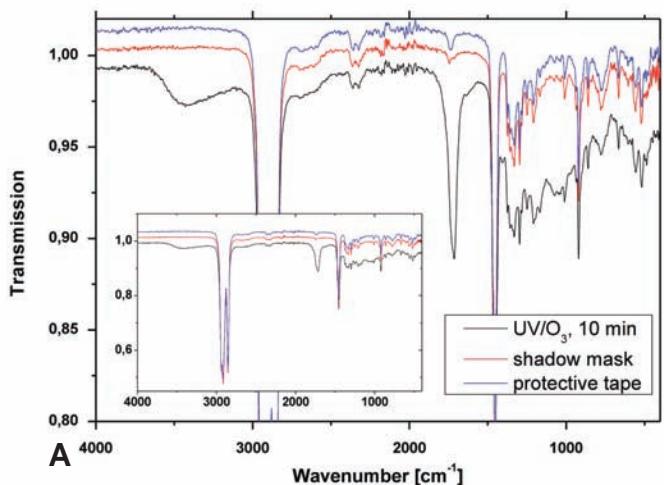


Fig. 3. ATR-IR spectra of UV/ozone (a) and oxygen plasma (b) treated surfaces, no treatment protection (top), shadow mask (middle) and protective tape (bottom)

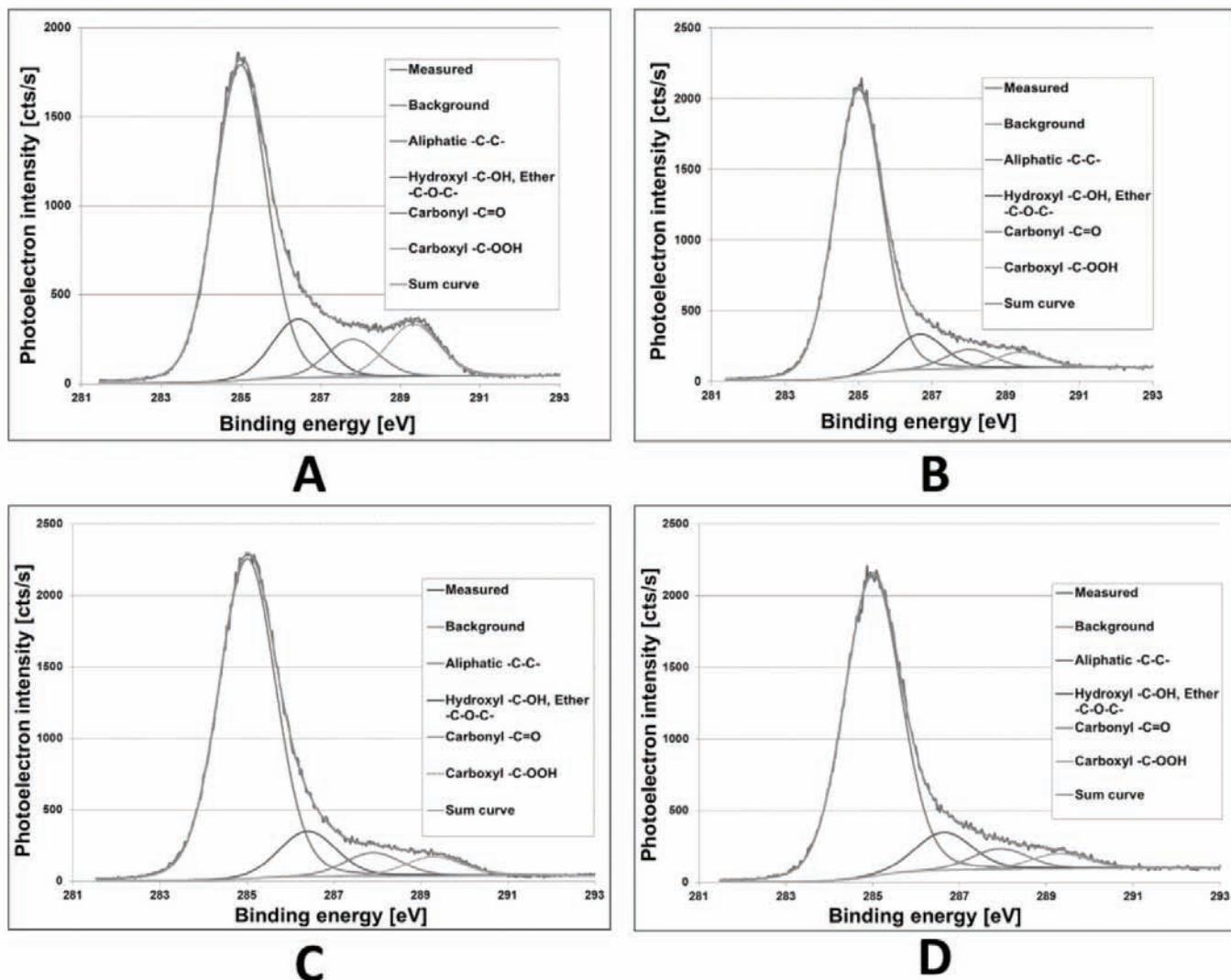


Fig. 4. XP-spectra of the C1s band on COP substrates after UV/ozone treatment (A), after oxygen plasma treatment (B), after UV/Ozone treatment followed by washing with 2-propanol (C), and after oxygen plasma treatment followed by washing with 2-propanol (D)

untreated COP. Despite the clearly observed modification of COP surfaces by oxygen plasma treatment the ATR-IR spectra of pristine COP, and exposed and protected areas of oxygen plasma activated COP were virtually identical (see Figure 3b). The lack of signal change in oxygen plasma activated surfaces can be ascribed to the fact that plasma surface activation only modifies the uppermost few nanometers of the surface, and these changes cannot be detected with the used ATR-IR instrumentation and settings.

Therefore, the expected formation of functional groups was further investigated using XPS as a more surface sensitive technique. High resolution XP-spectra of the C1s band proved the formation of carbonyl, hydroxyl, and carboxylic acid groups on the surface of UV/ozone as well as oxygen plasma treated COP (see Figure 4 A and B). Similar distributions of C-O groups have been reported in previous literature on UV/ozone and plasma modification of COP/COC polymers [6,8].

These functional chemical groups on the surface allow on the one hand a direct biofunctionalization of the polymer and

on the other hand the attachment of subsequent coatings for a well-controlled covalent immobilization of biomolecules.

In order to determine the stability of the activated surfaces against organic solvents, activated surfaces were rinsed with 2-propanol for 5 min. While on the oxygen plasma treated surfaces this rinsing step did not show any apparent effect, for UV/ozone treated COP surfaces a marked increase of the water contact angle to about 65° was observed. Again XPS was applied to investigate this phenomenon. A comparison of the XP-spectra in Figure 4 reveals that for the oxygen plasma treated COP surfaces, there was hardly any change in functional groups after washing with 2-propanol, while for UV/ozone treatment a significant loss in functional groups took place after rinsing. Especially the band at 289 eV, indicative for carboxylic acid groups, decreased substantially.

A similar behaviour has also been reported for UV/ozone modification of other polymer surfaces like poly(styrene) and poly(methylmethacrylate) [15,4].

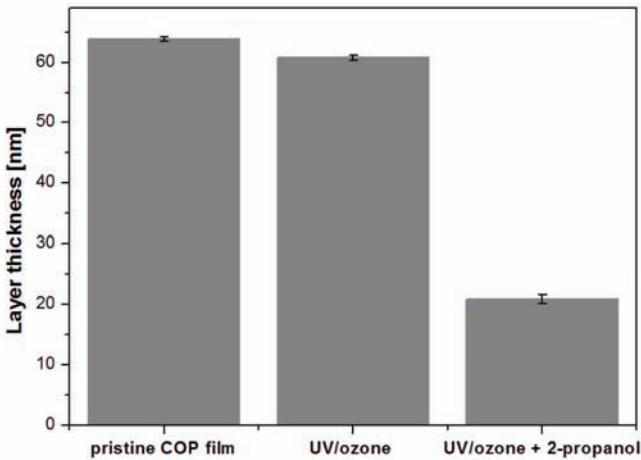


Fig. 5. Layer thickness of COP model film on Surfs, pristine COP film, UV/ozone treated and UV/ozone treated and washed with 2-propanol

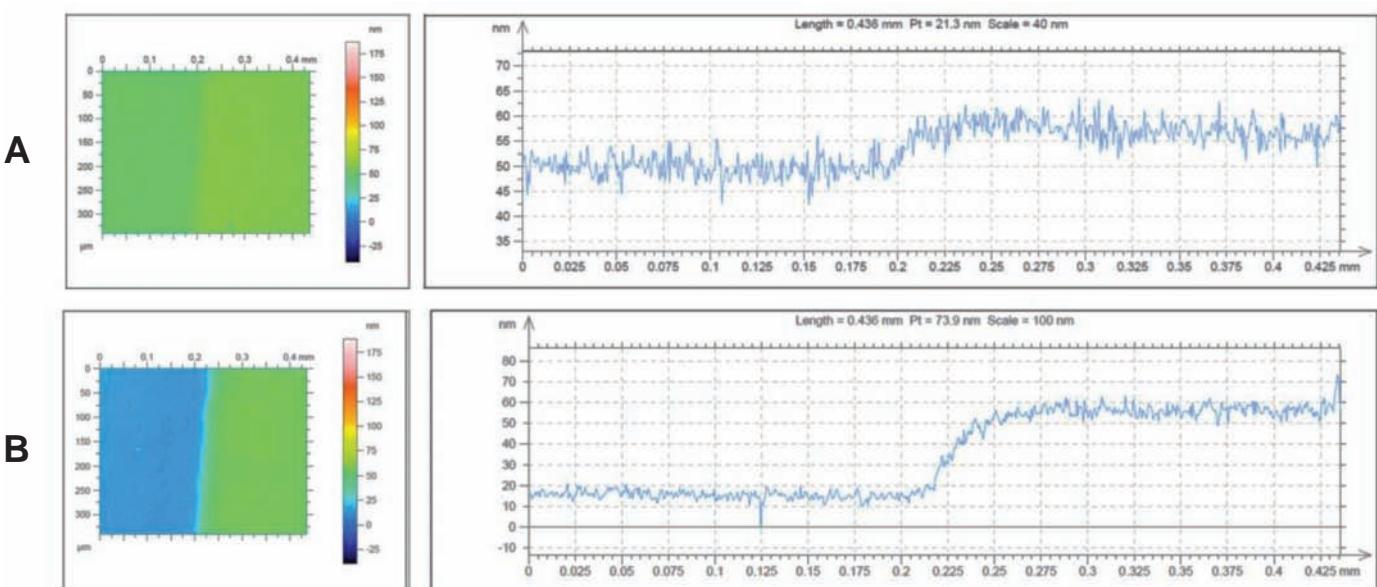


Fig. 6. SEEC microscopy thickness images and extracted profiles of COP model films after UV/O₃ treatment (A) and after subsequent rinsing with 2-propanol (B). The surface on the right part of the images was covered with an Al-shadow mask for patterning during UV/O₃ treatment while the left part was exposed to UV radiation. The respective film thickness profiles represent a horizontal line in the central part of the images.

There might be different reasons for the observed changes of COP surface properties upon rinsing with 2-propanol. Most probably the UV radiation induces photochemical damage leading to degradation of the polymer. This might lead to excessive oxidation of the polymer and selective leaching of highly oxidized surface groups. Similarly it may cause the formation of a soluble fraction containing a large number of functional groups (e.g. carboxylic acid groups) and/or reduction of molecular weight by e.g. chain scission. Similar phenomena were also observed for other photochemical surface modifications of polymers. Even for fluoropolymers significant loss of functional groups occurred upon extraction of a photochemically modified surface in aqueous solution [16].

In order to investigate whether the rinsing in 2-propanol causes only selective leaching of a certain number of functional groups or it leads to the dissolution of a whole thin surface layer, optical thickness measurements in the nanometer range were performed using SEEC microscopy. For these experiments, spin coated COP model films of about 60-65 nm thickness on special silicon substrates (Surfs) were applied because SEEC microscopy cannot be performed with bulk polymer samples.

UV/O₃ treatment of these COP films reduced the layer thickness by about 5 nm compared to the initial film thickness probably due to etching of the surface and vaporization of degradation products (see Figure 5). This etching rate is rather low compared to oxygen plasma treatment of COP films, where

etching rates of approximately 60 nm/min were determined (data not shown). These differences might be due to the more efficient vaporization of polymer degradation products at the low pressure (ca. 0.4 mbar) during plasma treatment. After rinsing the UV/ozone treated COP films with 2-propanol, another reduction in layer thickness by about 40 nm was observed (Figure 5). These findings support the assumption that after UV/ozone treatment, which is a dry and ambient pressure process, a large part of low molecular weight degradation products remain on the activated surface and are subsequently washed away during rinsing with solvents.

SEEC microscopy was further used to image the film thickness of COP films partly covered with metal shadow masks for patterned UV/O₃ surface modification. Examples of these etch depth analysis are shown in Figure 6, illustrating the film degradation after UV/ozone treatment and washing with 2-propanol, respectively. In agreement with the data presented in Figure 5, a height difference around 5 nm was found between the masked and the exposed surface area upon UV/ozone treatment. This height difference increased by another 40 nm after rinsing in 2-propanol. Besides the decrease of the surface thickness, the sharp edge between the protected and non-protected surface area could be clearly shown. This underlines the applicability of the chosen surface treatment and masking methods for patterned surface activation of COP substrates for e.g. subsequent biofunctionalization or coating.

4 Conclusions

UV/ozone and oxygen plasma were applied for patterned surface activation of COP surfaces using metal shadow masks or protective tapes to define the areas to be activated. Both kinds of surface treatments have been found to be suitable for COP surface activation, yielding carboxyl, and hydroxyl groups on the exposed surface areas. These surfaces will furthermore be applicable for the subsequent attachment of biomolecules or polymeric coatings for e.g. biofunctionalization. Nevertheless, it has been found that especially after UV/ozone treatment a thin film of degradation products remain on the COP surface and is at least partly removed in the following washing or rinsing steps. Although the number of remaining surface groups will probably be sufficient for biofunctionalization, this might still limit the applicability of this surface modification technique in the field of biosensors and biochips. For these applications a slow dissolution or decomposition of the polymer surface in aqueous buffer systems would be undesired. Therefore the oxygen plasma procedure is most probably better suited for these applications.

Further investigations would be required in order to clarify the exact mechanism of this surface film dissolution on COP. This should involve a more systematic study on the effect of different aqueous and organic solvent systems, leaching times, etc. on these surfaces.

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