

Photolabile Carboxylic Acid Protected Terpolymers for Surface Patterning. Part 2: Photocleavage and Film Patterning

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The surface properties of films made of *p*-methoxyphenacyl derivative terpolymers, associated with photocleavage by UV irradiation, and their optical patterning are investigated. The deprotection reaction has been monitored by UV and FTIR spectroscopy, contact angle measurements, and X-ray photoelectron spectroscopy, revealing the photoremoval of the protecting *p*-methoxyphenacyl group in high yields under mild conditions. Parallel and serial patterning of the films has been performed by selective irradiation through optical masks and by laser irradiation via fiber tips of a scanning near-field optical microscope, respectively. By irradiation of photolabile protected functional groups, free carboxylic groups become exposed to the surface with which fluorescent dyes and proteins can be associated specifically.

Introduction

As mentioned in part 1,¹ comprehensive work has been published in the past few years on functional monomers and polymers for the modification of surfaces.² In particular, the generation of functional patterned areas is of specific interest in a number of emerging technologies such as application in microelectronic devices, photonic materials, biosensors, displays, or light-directed chemical synthesis.^{3,4}

Recently, photolabile random terpolymers covalently tethered to silicon and gold substrates were demonstrated to be adequate for the preparation of patterned amino-functionalized surfaces by means of UV irradiation.^{5–8} Besides, there has been a relevant contribution in the literature concerning the patterning with light of organic materials. First, examples of patterning with self-assembled monolayers (SAMs), whether onto silicon/glass⁹ or gold substrates,¹⁰ using different protecting groups have been reported. Poly(methyl methacrylate) and polycarbonate films have been exposed to oxygen-rich environments to get free functionalities. Patterning here is a result of a combination of oxidative and thermal decomposition processes.¹¹ Also *o*-

nitrobenzyl-containing terpolymers were used for the photo-generation of polyelectrolyte bilayers.¹²

Here we present a simple method for the patterning of photochemically reactive terpolymers which avoids the harsh processing conditions employed in classical photoresist-based techniques and is capable of massive parallel processing. In contrast to the concept of chemically amplified photoresins, where also co- and terpolymers with protected acid or other polar functionalities are used,¹³ our aim is not the change in solubility and subsequent selective dissolution of imaged or nonimaged areas but the use of deprotected polymer films on the substrate for further modification reactions. For that the polymers exhibit a multifunctional composition which allows both the patterning with light and covalent attachment to the substrate. Random terpolymers have been built up from three functional units which allow the properties of the resulting materials to be tailored and optimized. The photolabile moieties (*p*-methoxyphenacyl derivative) provide free carboxylic acid units, allowing 2D patterning of the materials within short irradiation times (60 s) and with a lack of significant damage to the polymer surfaces. The carboxylic acid group is a versatile functionality with a wide reactivity spectrum and also a good candidate for the performance of noncovalent interactions through electrostatic charges or hydrogen bonding, providing many possibilities for patterning with functional organic (macro)molecules. A second component, methyl methacrylate, ensures the formation of films with good quality, and finally a third component allows the direct covalent anchoring of the materials to the surface without the presence of additional components or intermediate layers, enabling patterning and further anchoring of nanoelements to the deprotected areas in a fast two-step procedure. The capability of covalent attachment of the materials to the substrate also enables the manipulation of the materials in fluidic media.

Synthesis, characterization, and examination of the surface properties of phenacyl-protected terpolymers have been described in the first part of this study.¹ Here we devote special interest to the investigation of the deprotection reaction in films, covalently

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attached to a substrate, by using different characterization techniques (UV and FTIR spectroscopy, contact angle measurements, X-ray photoelectron spectroscopy, near-field optics, and surface potential measurements). Finally the formation of the patterned areas is proven by functional group specific labeling with a fluorescent marker and selective immobilization of proteins.

Experimental Section

Materials. The polymers PHE/MMA/SIL 1/1/1 and PHE/MMA/GLY 1/1/1 and the copolymers PHE/MMA 30/70 and PHE/MMA 70/30, used for the preparation of the films, have been described in part 1 of this work.¹ Dry dioxane and THF (over molecular sieves) were purchased from Fluka and directly used without further modification, e.g., for film preparation. 1,1'-[1,3-Propanediylbis-[(dimethylimino)-3,1-propanediyl]]bis[4-[(3-methyl-2(3*H*)-benzoxazolylidene)methyl]] tetraiodide (YOYO-1) (1 mM solution in DMSO) was purchased from Molecular Probes and further diluted to get a 0.04 mM solution in water (1/25). Trizma base (Sigma) and HCl (37%, Merck) were used without further purification. Polycationized ferritine (PCF) was purchased from Sigma and used after dilution (1/14) in water. Thermally oxidized silicon wafers (orientation, [100]; dopant, boron; SiO₂ layer, 30–50 nm) were purchased from Poliertech GmbH, Jena, Germany. Borosilicate glass wafers (22 cm × 22 cm, no. 0) were purchased from Wenzel & Glaser, and quartz substrates were purchased from Saint Gobain Quartz (75 mm × 25 mm). Thin films of the terpolymer PHE/MMA/SIL 1/1/1 were prepared as previously described.¹

Instruments. FTIR measurements were recorded in transmission mode using a Bruker IFS 66 v/s spectrometer (Bruker, Billerica, MA) with a resolution of 4 cm⁻¹ and 32 scans over silicon wafers.

The wetting measurements were carried out with a Krüss DSA 10 drop shape analyzer (Germany). The instrument was located in a temperature-controlled laboratory maintained at 23 ± 1 °C. The measurements were carried out at a relative humidity of 50 ± 2% and using deionized reagent-grade water produced by an ELGA filtration system (Great Britain).

UV spectroscopy experiments were performed both in solution and in films using a Perkin-Elmer Lambda 800 spectrophotometer.

X-ray photoelectron spectroscopy (XPS) measurements were performed using an AXIS ULTRA photoelectron spectrometer (Kratos Analytical, Manchester, England). The spectrometer was equipped with a monochromatic Al K α ($h\nu$ = 1486.6 eV) X-ray source of 300 W at 15 kV. The kinetic energy of the photoelectrons was determined with a hemispheric analyzer set to a pass energy of 160 eV for wide-scan spectra and 20 eV for high-resolution spectra. During all measurements, electrostatic charging of the sample was avoided by means of a low-energy electron source working in combination with a magnetic immersion lens. All recorded spectra were aligned according to the C 1s peak, which was set to 285.00 eV.

Quantitative elemental compositions were determined from peak areas using experimentally determined sensitivity factors and the spectrometer transmission function. The backgrounds of the spectra were subtracted according to Shirley.¹⁴ The highly resolved spectra were dissected by means of spectrum deconvolution software. Free parameters of the component peaks were their binding energy (BE), height, full width at half-maximum, and Gaussian–Lorentzian ratio.

Fluorescent microscopy images were taken with a fluorescence microscope (Axiovert 200M, Zeiss, Jena, Germany) equipped with a RoberScientific Cascade 521B camera.

The AFM measurements were carried out using a NanoScope IIIa (Digital Instruments, Santa Barbara, CA) operated in tapping mode in air.

UV Decomposition Experiments. The irradiation was carried out with a 300 W high-pressure HgXe lamp (Oriel 6000) with a condenser (LOT-Oriel, Germany) at room temperature. The samples were not cooled during irradiation. Filters were not used. The distance from the irradiation source to the solution was 5 cm. The resulting

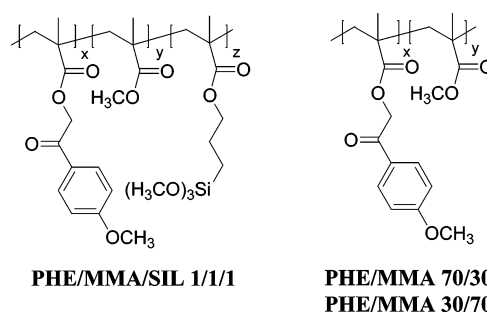


Figure 1. Chemical structures of the materials used for photocleavage studies and patterning by irradiation.

spectra were recorded with the spectrometer mentioned above. For the patterning of the films, a TEM grid (PLANO, 400 lines/in.) was placed as a mask between the irradiation source and the sample.

Labeling of Patterned Films with a Fluorescent Marker (YOYO-1). First, a buffer solution was prepared by dissolution of 1.21 g of Trizma base (Sigma) in 100 mL of water. The pH was adjusted down to 8.8 by slow addition of HCl (37%, Merck). Second, 300 μ L of this Trizma base pH 8.8 buffer solution and 10 μ L of YOYO-1 were stirred together, and 100 μ L of the mixture was added over the patterned area. After 1 min of exposure the sample was rinsed three times with distilled water and dried under a N₂ stream.

UV Writing and Imaging. For UV imaging, we used a He–Cd laser (Kimmon IK565IR-G, cw, ~20 mW at λ = 325 nm) that was focused onto the sample with a microscope objective (Zeiss Fluor 100*/1.30 oil, UV transparent). The sample was moved in the focal plane according to the desired pattern using a piezoelectric scanning stage to write structures of random shape. Created patterns were imaged with broad-band light from a Xe lamp (XBO 101) focused onto the sample. The transmitted light was collected by a multimode fiber coupled to the entrance slit of a grating monochromator (SPEX 1681B, 300 lines/mm), and the spectrum was monitored with a CCD detector (INSTASPEC4, Oriel).

Results and Discussion

Co- and terpolymers¹ containing an anchoring group (glycidyl or trimethoxysilane), a spacer (MMA), and a photolabile protected COOH group (*p*-methoxyphenacyl) have been employed in this study (see Figure 1). The polymers were studied in solution or as spin-coated films covalently attached to silicon surfaces.

Monitoring of the Photocleavage through Irradiation.

a. UV Spectroscopy. The photochemical reaction of these materials is of particular interest for the patterning of thin polymer films. The irradiation of these polymers with UV light produces a dramatic decrease in the absorption band ascribed to the *p*-methoxyphenacyl ester group both in solution and in films, as shown in Figure 2.

In Figure 2a is shown an example corresponding to a solution of the copolymer PHE/MMA 30/70 (2×10^{-4} M), with a maximum at 269 nm that disappears within approximately 6 min. Figure 2b shows the UV spectra of a film prepared from a 2 wt % dioxane solution of the same material through irradiation. The thickness of the film can be estimated to be around 25–30 nm (conditions used for spin coating: 3000 rpm, 500 rpm/s for 60 s). The maximum of absorption is slightly shifted (275 nm) with respect to that of the solution spectrum (269 nm), and complete photodeprotection takes place only within longer irradiation times (50 min). The reaction is supposed to occur relatively homogeneously in the film. The group *p*-methoxyphenacyl was selected in this work versus other analogous phenacyl esters as the photoreactivity of other *para*-substituted/unsubstituted esters is supposed to be lower, decreasing in the

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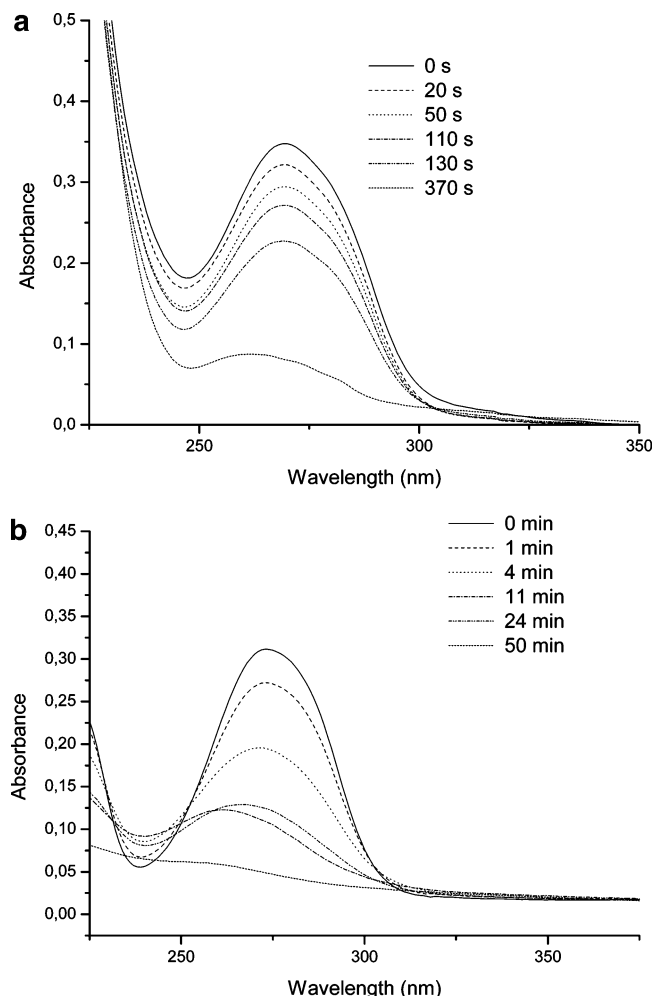


Figure 2. UV spectra during irradiation of the copolymer PHE/MMA 30/70 (a) in solution (THF, 2×10^{-4} M) and (b) in a film (spin coated, 2 wt % dioxane solution, 3000 rpm, 500 rpm/s for 60 s).

order $\text{MeO} < \text{Me} < \text{H} < \text{Br}$.¹⁵ The methoxy group causes a bathochromic shift of the UV absorption, and therefore, lower energy is needed for irradiation. Furthermore, the intermediate radical might be stabilized by the electron-donating group. Also groups with α -methyl substituents have been reported to exhibit reactivity comparable to that of *p*-methoxy derivatives.¹⁶

Together with the decreasing absorption band in both experiments, a slight shift to lower wavelengths of the maximum (10–15 nm) is observed, probably due to the products or intermediates formed during irradiation. In fact, the absorption spectrum of *p*-methoxyacetophenone, which is—according to the deprotection mechanism—the product of the decomposition reaction, was measured as a reference experiment in THF, and the maximum was found at 266 nm.

Very small differences in the height of the baseline of the spectra can also be observed. Tiny turbidity areas coming from the compounds originated in the photolysis could provide these differences; changes in film thickness or even nanoscopic dewetting processes could be suggested, although according to our studies the films seem to be not only anchored to the substrate but also self-cross-linked, so dewetting in principle could be excluded.

In Figure 3a the deprotection mechanism associated with *p*-methoxyphenacyl units as reported originally in the literature

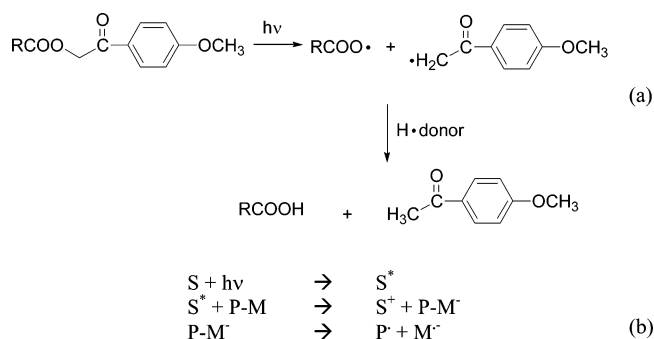


Figure 3. Mechanism of deprotection of *p*-methoxyphenacyl derivatives through photolysis¹⁷ (a) and strategy for photorelease using photoinduced electron transfer (b).²⁰

is shown.¹⁷ Accordingly, the irradiation with UV light of phenacyl ester compounds provides free carboxylic groups by homolytic C–O bond scission to give an acyloxy radical and a phenacyl derivative radical in good yields (average 70–90%, without the presence of filters).¹⁸ According to this mechanism, 4-methoxyacetophenone is generated as a photoproduct. The success of this approach relies on the rapid H atom transfer to the former to reveal the acid. It could be possible that, after the irradiation time required for the photolysis, *p*-methoxyacetophenone becomes chemically instable, hence being subjected to further modifications. This is supported by the fact that we have not observed any trace pointing to the presence of this photoproduct. To check this, an experiment was performed using a 10^{-4} M solution of *p*-methoxyacetophenone in THF. Within 90 s the absorbance decreased by 30% of the initial amount. Thus, after the necessary irradiation times for the photocleavage of the *p*-methoxyphenacyl units, at least a partial disappearance of the photoproduct *p*-methoxyacetophenone can be expected.

Laser flash photolysis experiments have shown that the initial step in the deprotection is a hydrogen atom transfer from the solvent (or additives) to the phenacyl carbonyl group.^{19,20} Dioxane or ethanol has been described to serve as a hydrogen donor in the solution experiments, and it is possible that the remaining traces of the solvent could serve as hydrogen donors in the film state. $-\text{CH}_2-$ groups in the polymer chain could have the same function.

It is worth mentioning that the possibility of competition between deprotection and decarboxylation has been reported. However, according to these authors, such a competition is mostly favorable in phenacyl esters whose acid components are themselves chemically labile to single-electron transfer. For example, 4-bromophenacyl acetate is mentioned to show elimination in competition with deprotection.¹⁹

The same authors described an alternative to avoid this problem, the use of photosensitizers. The presence of such molecules (i.e., dimethylaniline, ...) is described to promote the deprotection by a single-electron-transfer mechanism (see Figure 3b), providing the carboxylic groups in nearly quantitative yields. The photosensitizer seems to transfer an electron from the excited state to the caged molecule, creating an ion radical and finally rendering the functional group in its anionic form (i.e., conjugate base as M^-).²⁰ So far, our experiments on possible application of the

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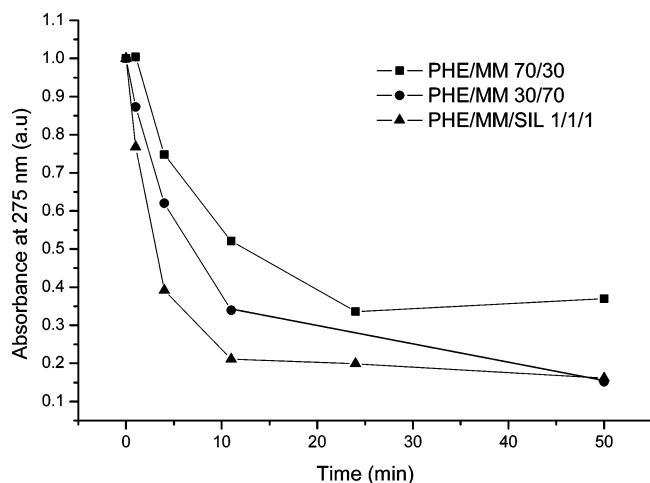


Figure 4. Normalized absorbance through irradiation of films of the copolymers PHE/MMA 70/30 (■) and PHE/MMA 30/70 (●) and the terpolymer PHE/MMA/SIL 1/1/1 (▲) prepared under similar experimental conditions.

polymers in nanotechnology have been performed without the presence of photosensitizer in the first stage.

After studying the differences in the absorption spectra in films and in solution of one of our copolymers taken as a reference, we looked into more details of the photolysis rate for different polymer compositions.

For this purpose the normalized absorbance of some films of our materials are compared in Figure 4 after different irradiation times. The copolymer PHE/MMA 70/30 with a higher content of photolabile units requires longer times for the conversion to the subsequent photoproducts than the copolymer PHE/MMA 30/70. A film prepared from a dioxane solution of PHE/MMA/SIL 1/1/1 containing roughly 30% glycidyl anchoring groups and photolabile units provided different deprotection rates compared with the copolymer PHE/MMA 70/30 and also with the copolymer PHE/MMA 30/70, containing a similar amount of photolabile units. Surprisingly, the decrease of the normalized absorption of the terpolymer film (▲) is higher compared to that of the copolymers.

There may be a number of different reasons for the differences in the observed photolysis rates. First, in very thin films (estimated thickness around 25–30 nm) the reactivity near the interfaces may be varied in comparison to that of the inner parts. The extent of this effect is dependent on the film thickness. Second, the variations in polymer structure may be accompanied by a varying permeability for oxygen, and oxygen quenching may have a strong influence on the photolysis rate if longer living transients are involved. Additionally, oxygen may take part in the radical reactions, changing the reaction mechanism. Third, structure formation of the polymers on a nanoscopic level may strongly depend on their composition. Therefore, self-quenching of excited states or the following radical reactions may not be influenced only by the nanoscopic structure. The situation is very complex and should be investigated in further work.

b. FTIR Spectroscopy. The photolytical cleavage by action of UV light was also investigated with FTIR spectroscopy. Films of co- and terpolymers were studied, and the changes observed in the spectra through irradiation were compared. In Figure 5a the FTIR spectrum of a copolymer PHE/MMA 30/70 film is shown. It was chosen as an example, because it exhibits all characteristic bands of the material. The film was prepared by spin coating of a 2 wt % dioxane solution at 3000 rpm and 500 rpm/s for 60 s. Figure 5b shows the spectrum of the film after

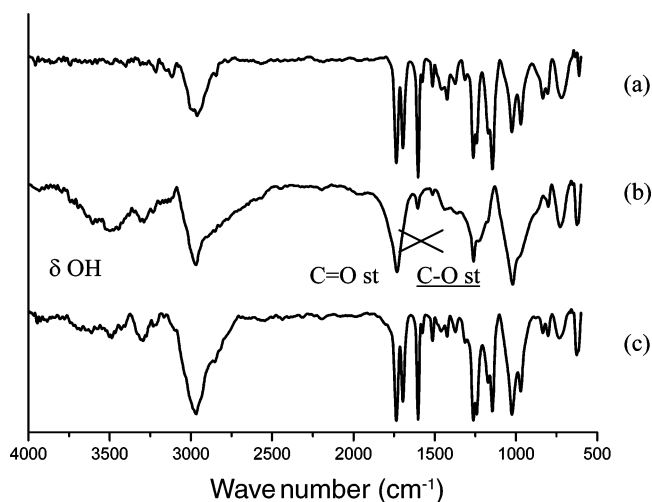


Figure 5. Monitoring of the deprotection of the *p*-methoxyphenacyl group through FTIR for thin films of the copolymer PHE/MMA 70/30 (a) as prepared, (b) after 180 min of irradiation, and (c) after 180 min of heating at 150 °C.

3 h of irradiation. We observe at first sight that the disappearance of the band at 1602 cm^{-1} associated with the aromatic signals and the appearance of the broad band at 3300 cm^{-1} associated with the OH group from carboxylic acids are remarkable. On the other hand, the two bands ascribed to both ester groups are reduced to one peak. It is possible that the photoproduct suffers from decomposition after the long irradiation time; however, the bands which can be associated with the main chain of the polymer remain invariable. The resulting structure of the polymer would correspond to that of a random copolymer of methacrylic acid and methyl methacrylate, and the resulting spectra resemble the tabulated FTIR spectra of poly(methyl methacrylate). To ensure that the deprotection is promoted only by action of the light and not by local heating of the sample due to irradiation, a film of the copolymer after deposition was heated at 150 °C for 180 min. The spectrum resulting from this experiment is designated as (c) in Figure 5. No significant change was observed from the spectrum of the original material shown in Figure 5a, which confirms that the deprotection is promoted by the action of the light and in no case by heating of the sample. Also, TGA analysis of these polymers reported in part 1 of this work revealed no significant weight loss at temperatures below 230 °C.

c. Contact Angle Measurements. Possible changes in the water contact angles on the film surfaces were considered as an indicator for the exposure of the deprotection of carboxylic groups to the surface of the films after irradiation. A decrease in the obtained contact angles should be a consequence of the irradiation-induced increase in the polarity of the surfaces. Spin-coated films prepared from 2 wt % dioxane solutions were irradiated up to full deprotection using the appropriate exposure time according to UV spectroscopy. The contact angles were measured before and after the irradiation. The increase of free functional groups in the film by irradiation should provide an increase in the polarity of the films of co- and terpolymers. In measurements of contact angles of the protected polymers shown in part 1 of this work,¹ both advancing and receding contact angles were shown to be somehow a rough indicator for the composition of the polymers.

Thus, we took from the literature the advancing and receding contact angles of poly(methyl methacrylate), $\theta_a = 76^\circ$ and $\theta_r = 52^\circ$ ($\Delta\theta = 24^\circ$).²¹ The polymer PHE/MMA 70/30 exhibits values highly similar to these values as stated in the first part of this work.¹

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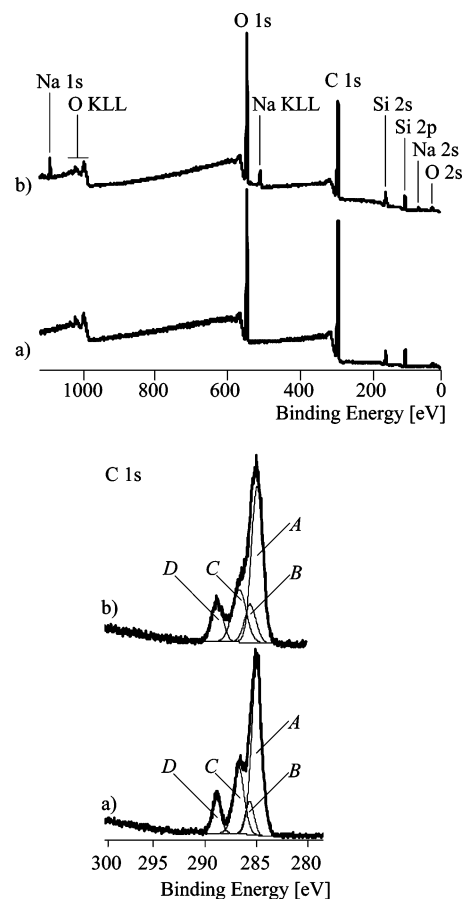
Table 1. Advancing and Receding Contact Angles (deg) of the Polymer Films before and after UV Irradiation

polymer	before irradiation			after irradiation		
	θ_a	θ_r	$\Delta\theta$	θ_a	θ_r	$\Delta\theta$
PHE/MMA 70/30	79	69	10	49	36	13
PHE/MMA 30/70	75	69	6	67	56	11
PHE/MMA/GLY 1/1/1	78	63	15	71	57	14

In the present case we observe a significant decrease of the contact angles after irradiation correlating with the composition of the protecting group and the release of a free carboxylic acid. In Table 1 both the advancing and the receding contact angles of the investigated polymer films are reported. The contact angles of the irradiated films of the copolymer PHE/MMA 70/30 experienced a decrease from 79° to 49° for θ_a and from 69° to 36° for θ_r , indicating an increase in the polarity of the surface, ascribable to the release of the carboxylic groups. For the copolymer PHE/MMA 30/70, with lower content of phenacyl methacrylate, a lower decrease was experienced (from 75° to 67° for θ_a and from 69° to 56° for θ_r). Also in the case of the terpolymers a decrease in contact angle was noticeable, although less significant (see Table 1). Thus, in general one can conclude that contact angle measurements provide evidence for the exposure of carboxylic groups to the film surface as a consequence of the deprotection reaction in thin films.

d. X-ray Photoelectron Spectroscopy. In the previous study¹ a detailed discussion about the chemical constitution of the nonirradiated PHE/MMA/SIL 1/1/1 layer on a silicon wafer substrate was given as an example. The XPS studies showed that the surface composition of the polymer film largely correlates with the stoichiometry of the applied polymer. Here, the surface chemical composition is now studied before and after irradiation. The high-resolution C 1s spectrum of the nonirradiated sample PHE/MMA/SIL 1/1/1 was deconvoluted into four component peaks (A, B, C, and D) showing bonds of the carbon atoms (Figure 6a). The main component peak A represents hydrocarbons (C_xH_y). Component peak D relates to carbon atoms of the ester groups ($O=C-O-C$). The corresponding component peak B, showing the carbon atoms which are in the β -position to the ester groups ($C-C(O)-O-C$), is found to be a little smaller, because the carbon atom which is a constituent of the phenyl ring did not show a pronounced shift and contributes to component peak A. Component peak C summarizes all $C-O$ bonds which are on the alcoholic side of the ester bonds ($O=C-O-C$), the methoxy group in the *para*-position of the phenyl ring ($C-O-CH_3$), and the $O-CH_2-O$ groups.

The XPS results shown in Figure 6 give further evidence that the deprotection is successful and that the laser irradiation of the PHE/MMA/SIL 1/1/1 sample eliminates the 4-methoxyphenyl methyl ketone unit from the polymer backbone. Compared with that of the untreated sample, the wide-scan spectrum of the laser-treated sample (Figure 6b) shows a smaller C 1s peak, indicating a relative increase of the oxygen content (Table 2). The increase of oxygen cannot be explained only by differences in the silicon content. The elimination of the 4-methoxyphenyl methyl ketone unit removes a larger number of carbon atoms than oxygen atoms. Hence, the relative oxygen amount in the remaining polymer must be increased. The photoreaction significantly lowers the number of $C-O$ bonds which contribute to component peak C. The high-resolution C 1s spectrum clearly shows a smaller area of component peak C (for the untreated sample $[C] = 23.8\%$ of the whole C 1s peak area, and for the laser-treated sample $[C] = 21.7\%$ of the whole C 1s peak area). Furthermore, during deprotection the phenyl ring is removed where the carbon atom is situated in the β -position to the ester group. As mentioned

**Figure 6.** Wide-scan core-level photoemission and high-resolution C 1s spectra of a nonirradiated PHE/MMA/SIL 1/1/1 sample (a) and a laser-irradiated sample (b), spin-coated on a silicon wafer. The assignment of the component peaks (A, B, C, and D) shown in the C 1s spectra is explained in the text.**Table 2. Chemical Composition of the Nonirradiated and Laser-Treated PHE/MMA/SIL 1/1/1 Sample Surface**

	nonirradiated sample	laser-treated sample
[O]/[C]	0.423	0.594
[Si]/[C]	0.077	0.122

above this carbon atom does not contribute to component peak B. Its removal equals the number of strong electronegative functional groups (ester and carbonic acid groups) and the number of carbon atoms standing in the β -position, which explains the chemical shift of the electronegative centers. The C 1s spectrum of the laser-treated sample shows equal areas for component peak D appearing from the strong electronegative groups (ester and carbonic acid groups) and component peak B ($[D] = 12.94\%$ of the whole C 1s peak area, and $[B] = 12.97\%$ of the whole C 1s peak area).

e. Nonconventional Patterning of the Films Using Near-Field Optics and Surface Potential Measurements. Besides the macroscopic proof of photodeprotection of the phenacyl derivative films as shown above by FTIR spectroscopy, contact angle measurements, and X-ray photoelectron spectroscopy, we also carried out experiments for surface patterning using near-field optical microscopy and following the area-selective changes in the film surface by surface potential measurements using Kelvin probe force microscopy (KPFM) as already shown previously for diazosulfonate and amine-protected polymeric thin films.^{5,22}

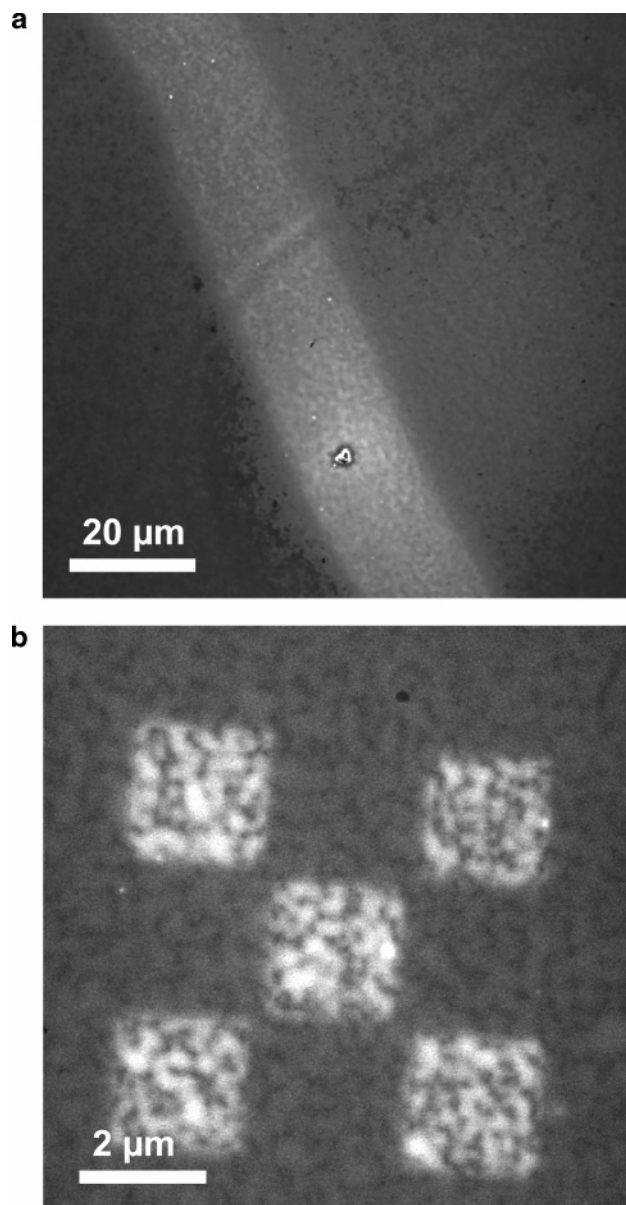


Figure 7. Kelvin probe microscopy images of (a) a line used as a guiding structure and (b) $2 \times 2 \mu\text{m}^2$ squared areas written into the ~ 20 nm thick polymer film via the local SNOM technique. At 325 nm wavelength, the polymer locally deprotects (white areas) and enables both a variable optical transmission and a changed surface potential value, as shown above. The potential change measures $\sim +157$ mV, indicating an increase in surface hydrophilicity.

Near-field patterning was performed using a tapered multimode optical fiber focusing 325 nm light from a He–Cd laser to a micrometer spot. Areas were patterned down to $\sim 2 \times 2 \mu\text{m}^2$ spot size and then inspected with both white light illumination and KPFM to record the local optical transmission spectra and surface potential differences before and after laser exposure, respectively. The combined near-field/KPFM approach was chosen to record the surface potential changes on the same surface spot.

Figure 7 shows two KPFM images displaying the surface potential changes recorded on PHE/MMA/SIL samples locally modified by our SNOM technique. The polymer film of approximately 20 nm thickness was spin coated from a 2 wt % dioxane solution at 3000 rpm onto glass substrates, cured as described previously,¹ and finally ultrasonically cleaned in a dioxane bath. The rms surface roughness taken over a $50 \times 50 \mu\text{m}^2$ surface area measures ~ 5.4 nm. To allocate certain surface

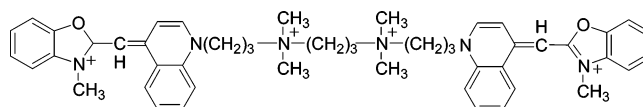


Figure 8. Chemical structure of the fluorescent marker YOYO-1.

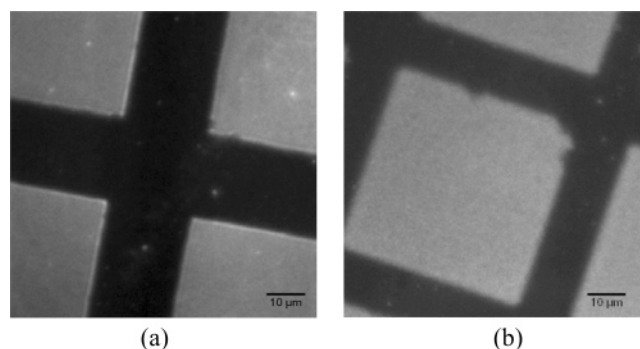


Figure 9. Fluorescence microscope images of films of the terpolymer PHE/MMA/SIL 1/1/1 after exposure to a Hg–Xe lamp and treatment with the fluorescent marker solution (a) after 1 min of exposure and (b) after 15 min of exposure. Contrast values calculated for (a) and (b) are 0.19 ± 0.5 and 0.32 ± 0.7 , respectively (see the calculation and 3D intensity images in the Supporting Information).

areas to the micrometer scale, a broad line was optically written into the polymer film as shown in Figure 7a; the change in surface potential is reflected as a white stripe crossing the picture from top left to bottom right. In fact, the surface potential on this brighter area was changed through the photodeprotection from initially -970 ± 80 to -813 ± 80 mV after exposure. Similarly, squared patterns $2 \times 2 \mu\text{m}^2$ in size, arranged in a checker-board-like fashion, were written (see Figure 7b). Further inspection with the KPFM method again revealed a potential difference on irradiated and nonirradiated areas of ~ 150 mV.

The changes observed and quantified in Figure 7 by the locally measured surface potential values correlate well with an increase in surface hydrophilicity by the release of functional groups at the film surface upon laser irradiation. Potential reading was initiated shortly after exposure and followed for more than 6 h. No changes in the reported potential values were observed within this time frame, indicating that no measurable relaxation of the exposed polymer film took place and the photoreaction was carried out to completion. Furthermore, as shown in Figure 7b, the film shows some local heterogeneity probably due to the coating process and the surface attachment reaction. However, the roughness in the imaged areas did not increase compared to that in the nonimaged areas as checked by AFM.

f. Patterning of the Films Using UV Light. Proof for the creation of free acid functions was provided by the selective attachment of fluorescence markers and proteins after UV light patterning of the films. Pursuing this goal, thin films of the terpolymer PHE/MMA/SIL 1/1/1 were prepared as described in section e and irradiated with a Hg–Xe lamp through masks, which were slightly pressed to the films, at different irradiation times. Afterwards, the samples were treated with 100 mL of a YOYO-1 solution prepared as described in the Experimental Section. YOYO-1 is a positively charged dye suitable for the interaction of deprotected, carboxylic group-containing areas. Its chemical structure of YOYO-1 is shown in Figure 8.

Figure 9 depicts fluorescence images of the terpolymer PHE/MMA/SIL 1/1/1 after selective irradiation and postexposure to the YOYO-1 solution. Figure 9a shows an image taken after 1 min of irradiation. A clear pattern is observed. The irradiated areas of the film appear light due to the staining with the dye. The nonirradiated area appears dark. Interestingly, despite the

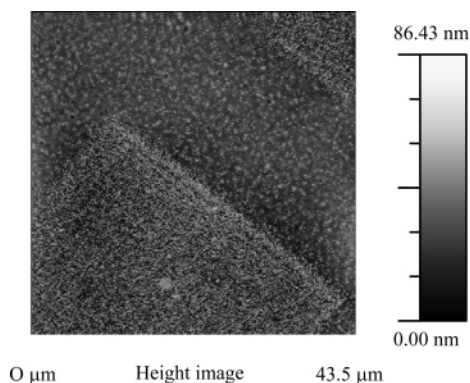


Figure 10. AFM image of a PHE/MMA/SIL 1/1/1 terpolymer film irradiated for 1 min through a 400-mesh TEM grid and treated with PCF.

relatively long exposure times required for the complete photocleavage in films, already after 1 min of irradiation, enough carboxylic groups are available for reaction with the fluorescent marker molecule and a good patterning of the film following the outline of the mask grid is achieved.

Figure 9b shows an image taken after 15 min of irradiation. In this case the contrast of the staining is higher due to a higher fluorescence intensity of the irradiated film area. Thus, a higher degree of deprotection allows a higher amount of the fluorescent marker to be attached to the imaged areas.

Besides labeling with YOYO-1, the exposure of carboxylic groups to the surface of the deprotected areas was also pursued with cationically charged proteins such as PCF. Ferritine is a cage-shaped protein responsible for the transport of iron in the blood in the form of FeOOH complexes which are placed in the core. PCF has been previously used as a marker of negative surfaces of proteins²³ and charged centers^{24,25} and also for the bionanofabrication of ordered nanoparticle arrays.²⁶

Figure 10 shows an AFM image of a terpolymer film irradiated for 1 min and afterward treated with a PCF solution. Although some of the ferritine molecules are nonspecifically adsorbed onto the nonirradiated areas, a much higher density of protein is observed onto the irradiated areas where negatively charged functional groups are exposed at the film surface. We suppose that the less dense nonspecific PCF adsorption at the nonirradiated film area is due to the preparation of the sample. In particular,

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when the sample is dried after the protein deposition, unbound PCF molecules might remain on the areas covered by the grid without specific interaction. This hypothesis is supported by roughness measurements. The roughness analysis of both irradiated and nonirradiated areas reveals an increase of the roughness in the irradiated areas and thus the deposition of larger particles. A roughness value of 3.48 nm was found for the PCF-covered, irradiated area and a value of 1.35 nm for the nonirradiated areas. This latter value is similar to the roughness value of a polymer film which was treated with PCF but not irradiated before, which is 1.65 nm.

The section analysis of the sample (not shown) reveals a difference in height of approximately 10 nm between the areas treated with PCF and the nontreated areas. This is in accordance with the size of the spherical protein, which has a diameter of 13 nm, since the height of dried proteins on surfaces is usually decreased due to capillary forces during the drying process.

Conclusions

The photocleavage of a novel family of photolabile protected COOH-functional terpolymers in thin films covalently attached to a substrate has been investigated by using different techniques such as UV and FTIR spectroscopy, contact angle measurements, and XPS. These techniques reveal the release of the carboxylic groups in adequate yields. Thus, the *p*-methoxyphenacyl unit is demonstrated to be an efficient photoremovable protecting group for carboxylic acid groups. Furthermore, patterning of the films by using serial SNOM laser writing and parallel UV irradiation through a mask was carried out, leading to the selective attachment of positively charged entities such as YOYO-1 dyes and polycationic ferritine on the irradiated areas of the surface and thus demonstrating the availability of these deprotected COOH groups for the establishment of further interactions or modification reactions. The area-selective exposure of functional groups on the surface and the high stability of the covalently attached thin films even in water or buffer solution make these materials attractive for application in microsystems and nanotechnology.

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Supporting Information Available: Calculation of contrast values for fluorescence images. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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