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Dual Photosensitive Polymers with Wavelength-Selective Photoresponse

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Biomaterials that allow phototunable crosslinking are becoming of increasing interest for mimicking changes in the mechanical properties of the extracellular matrix during cell migration, ageing, fibrosis, or cancer progression.[1–3] Polymer matrices with photoreversible crosslinking are also interesting as reversible glues in surgery or dental restoration.[4] In microfabrication technologies, a "dual" photoresist material that can be used both as a negative and a positive would simplify processing and overcome mechanical stability limitations during fabrication of three-dimensional features with complex geometries.[5] For all these cases, the development of a photosensitive material able to polymerize and depolymerize under different exposure conditions would allow dynamic tuning of polymerization degree and related properties (mechanical stability, solubility etc.). Here we present a strategy to enable light-induced polymerization and depolymerization of polymeric backbones using wavelength-selective chromophores that activate chain growth or scission upon light exposure.

Photoremovable protecting groups (PRPGs or "cages") are chromophores that can be covalently attached to organic functional groups to inhibit reactivity. At a later time point the chromophore can be cleaved by light exposure and the functionality is restored.[6] PRPGs in polymer science have been intercalated in the polymer backbones to allow photolytic chain scission (i.e. photodegradation),[4,7,8] and have been linked to polar side-chain functional groups to make them hydrophobic and mediate solubility or swelling changes in polar solvents after light-triggered chromophore removal.[9–11] Interesting applications in the biomaterials field have been reported, e.g. photodegradable 3D scaffolds for tissue engineering[12] or phototriggerable particles for drug delivery.[13,14] Single and two-photon excitation has been applied to this purpose and demonstrated the potential of this approach for site-selective activation, including 3D micrometric resolution. Combining different families of PRPGs, sequential activation of individual protected reactive groups in a mixture has been realized using light of different wavelengths.[15,16] Wavelength-selective activation of PRPGs has been applied for sequential activation of protected anchored ligands anchored to a surface,[17–19] for liberating bioactive ligands from a polymer chain,[20] or activation of soluble biochemicals in vitro.[1,21–24]

We hypothesized that the chromic response of PRPGs could be applied to generate a dual photoresist material with phototunable polymerization degree containing two caged monomers with the ability to be activated or photocleaved with different wavelengths. The strategy is shown in **Figure 1**a. The monomer AA contains two PRPG attached to the reactive A groups AA (**PRPG**^{1}-AA-**PRPG**^{1}) and does not polymerize in the dark. Upon light exposure, the PRPG^1 can be removed and the free AA groups undergo light-triggered polymerization with a BB monomer. That BB monomer contains a second PRPG intercalated in its chemical structure (i.e. B-**PRPG**^{2}-B). Upon exposure of the polymerized mixture at a second irradiation wavelength, photodegradation of the polymer occurs as a consequence of the photolysis of the PRPG^2 groups intercalated in the polymer chain. In this way, a "positive" and "negative" tone are contained in a single resist formulation. Here, we demonstrate for the first time light-mediated polymerization and photodegradation of a polymer material by using light of different wavelengths under single and two-photon excitation. We have used caged diamines and caged photodegradable diols with two different PRPGs derived from the *o*-nitrobiphenylpropyl family[25,26] to achieve light-mediated polymerization and photodegradation of a polyurethane (PU) film. The same approach can be extended to other types of reactive monomers and polymeric backbones.

The photocleavable groups methoxynitrobiphenyl (PMNB)[27] and *p*-dialkylaminonitrobiphenyl (ANBP)[28] (Figure 1b) were selected for our studies. These are derivatives of the *o*-nitrophenylpropyl family [29,30] using the donor-acceptor biphenyl core and a –OR or $N(CH_3)_2$ substituent in the *p*- position. Both chromophores have shown highly efficient photophysical and photochemical properties under single and two-photon excitation (**Table 1**) for the release of carboxylate,[25,27,28] phenol[31] and phosphate [32] groups. PMNB can be photoremoved at 315 nm with 90% chemical yield and 9% quantum yield, or at 740 nm with a 3.2 GM two-photon uncaging cross section at 740 nm.[26] ANBP shows a 80 nm bathochromic shift (λ_{max} = 397 nm) with no significant efficiency loss in the photolytic reaction (95% release with 15% quantum yield).[28] The significant difference in λ_{max} between the two chromophores (80 nm) is a prerequisite for achieving wavelength selectivity

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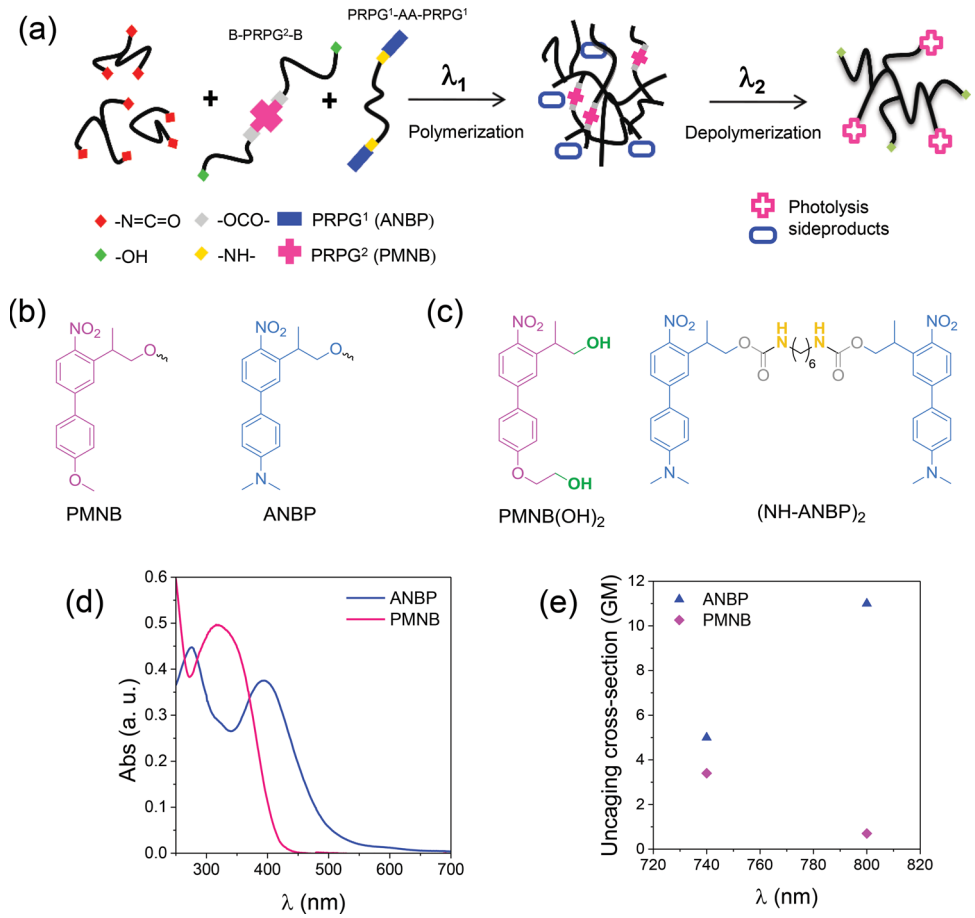

Figure 1. (a) Scheme of a dual photosensitive materials and structure of selected photoremovable groups (PMNB and ANBP) and monomers ($PMNB(OH)_2$ and $(NH\text{-}ANBP)_2$) (b,c) Chemical structure of the different PRPG (b) and the corresponding monomers (c). (d) UV-visible spectra of the ANBP and PMNB chromophores at 50 μM in PBS pH = 7.4, 50 mM, (e) Two-photon uncaging sensitivity for carboxylate release of ANBP and PMNB photoremovable groups.

(Figure 1d). The substitution of the methoxy group by the $-NR_2$ better electron-donating group lead to an unprecedented increase in two-photon sensitivity with a cross-section of 11 GM at 800 nm, >20 times higher than that of PMNB at the same wavelength (Figure 1e). This is a very promising scenario in terms of two-photon wavelength selective activation of the two chromophores coexisting in a mixture. It should be noted that ANBP and PMNB are the most efficient PRPGs candidates for two-photon excitation at 800 and 740 nm available to date.

Based on the PMNB and ANBP chromophores, the monomers $(NH\text{-}ANBP)_2$ and $PMNB(OH)_2$ (Figure 1c) were conceived. $(NH\text{-}ANBP)_2$ is a diamine end-capped by two ANBP units through a carbamate link. Light exposure photocleaves the ANBP units and releases free amine groups able to react with the isocyanates to form a PU polymer (Figure SI1). On the other hand, $PMNB(OH)_2$ contains the PMNB photocleavable group flanked by two hydroxyl groups. The free hydroxyl groups can react with isocyanates to form PU chains and can be photodegraded upon exposure via the photolysis of the intercalated PMNB groups. The synthesis of the chromophores and monomers is described in the SI (Figure SI2).

Table 1. Photochemical properties of donor-acceptor biphenyl PMNB and ANBP cages for carboxylate release.[26]

Caging group	λ_{max} [nm]	ε [M^{-1} cm^{-1}]	photo-release [%]	Φ_u	$\delta\Phi_u$ (λ) GP [nm]
PMNB	317	9900	90	0.09	3.2 (740)
ANBP	397	7500	95	0.15	11 (800)

We prepared a positive, a negative, and a dual photoresist formulation. For this purpose monomer mixtures containing different ratios of $PMNB(OH)_2$ and $(NH\text{-}ANBP)_2$ and commercially available di-/tri-isocyanates, di/tri-alcohols and/or diamines were prepared. The mixtures were spin-coated onto glass substrates and tested for obtaining PU films with good film-forming properties, crosslinking and/or depolymerization kinetics within minutes, and convenient crosslinking degree for avoiding swelling of the crosslinked parts in solvents used for pattern development (THF and ethanol mixtures). The optimized composition of the positive resist contained 40 mol% IPDI, 38 mol% PPG and 12 mol% $PMNB(OH)_2$ in THF with 10 mol% triisocyanate DRE as crosslinker and 0.5 mol% of DBTDL initiator. This mixture formed a stable crosslinked PU film at 90 °C. The optimized composition of the photopolymerizable monomer mixture (negative photoresist) contained

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35 mol% IPDI, 15 mol% DRE and 50 mol% of the photoactivatable monomer $(NH\text{-}ANBP)_2$ in THF. The optimized monomer ratio in the dual photoresist formulation was 25 mol% of each of the photocleavable chromophores $(NH\text{-}ANBP)_2$ and $PMNB(OH)_2$ and 35% IPDI and 15% DRE multifunctional isocyanates in THF. With these compositions, homogeneous films of ca. 2 µm thickness were obtained in all cases after spin-coating.

In order to find the spectral window for the wavelength-selective photoactivation of $(NH\text{-}ANBP)_2$ and $PMNB(OH)_2$ within the monomer mixture, UV spectra of the films of the negative and positive resists were taken after irradiation at different wavelengths and increasing times. The critical factor for the wavelength-selectivity is the photostability of the PMNB chromophore at a wavelength at which ANBP can be effectively cleaved. The absorption maxima of the ANBP and PMNB chromophores are located at $\lambda_{max} = 397$ ($\varepsilon = 7500\ M^{-1}\ cm^{-1}$) and $\lambda_{max} = 317$ ($\varepsilon = 9900\ M^{-1}\ cm^{-1}$) respectively (Figure 1d and Table 1). In principle, this is a promising difference for achieving selective photoresponse. Moreover, the UV absorption band of $PMNB(OH)_2$ is comparatively narrow and its absorbance coefficient at 397 is weak ($\varepsilon_{397} = 2590\ M^{-1}\ cm^{-1}$). We first tested the photoresponse of both chromophores at wavelengths close to λ_{max} of ANBP. **Figure 2**a shows the recorded UV spectra of $(NH\text{-}ANBP)_2$ and $PMNB(OH)_2$ containing films after exposure at 405 nm. The intensity of the UV absorption band of ANBP at $\lambda_{max} = 397$ decayed and while the absorbance at longer wavelengths increased, indicating the development of a new absorption band in the spectrum (Figure 2a). These changes were associated with the formation of the vinylnitrobiphenyl photolytic byproduct[28] and indicate that the photoreaction took place. Complete photocleavage of ANBP in the 2 µm thick positive resist film was achieved after 60 minutes exposure at 405 nm with our LED lamp. The time for full exposure depends on the chromophore concentration in the mixture, the thickness of the film and the intensity of the lamp. Under these exposure conditions, a film with comparable concentration of $PMNB(OH)_2$ chromophore also showed photoreactivity. The UV spectrum showed a decay in the absorbance at $\lambda_{max} = 317$ and the appearance of a new absorption maximum at 385 nm (Figure 2a). This change corresponds to the formation of vinylnitrobiphenyl photolytic byproduct[27] and indicates that no selectivity between

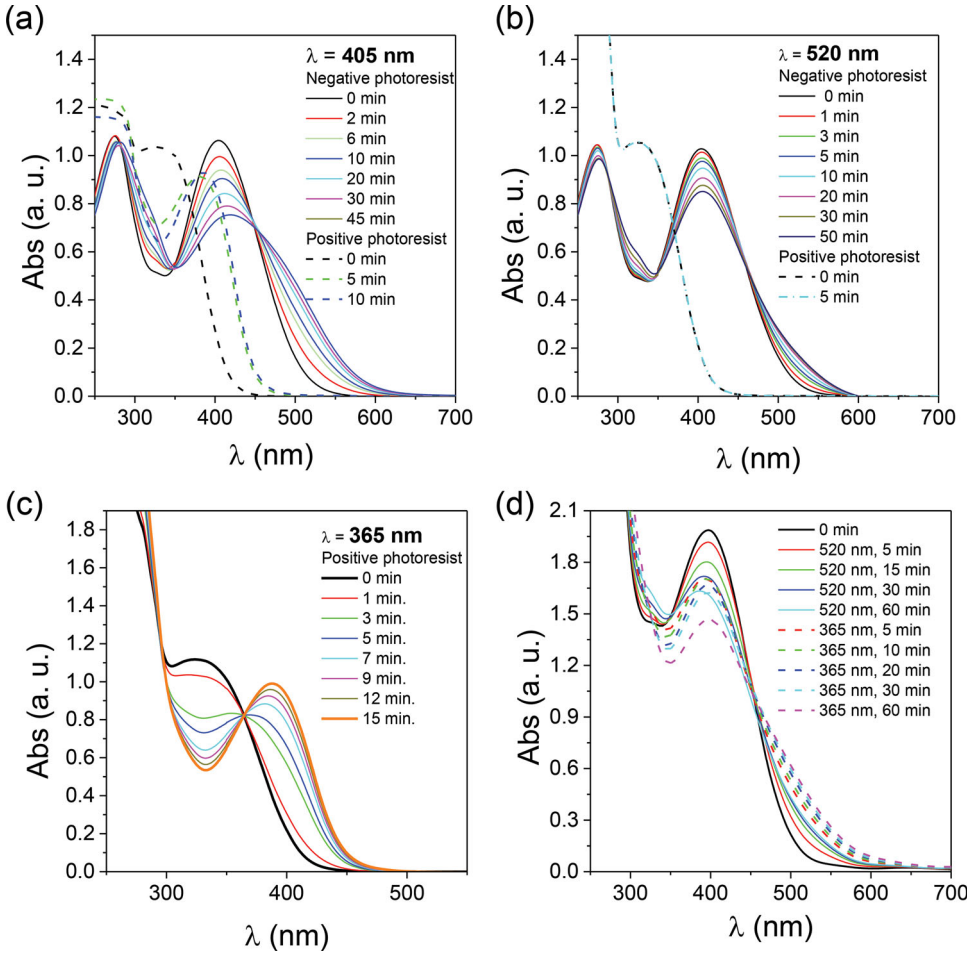

Figure 2. (a,b) UV/Vis spectra of $IPDI/DRE/(NH\text{-}ANBP)_2$ (negative photoresist) and $IPDI/PPG/GP/PMNB(OH)_2$ (positive photoresist) films after light exposure at (a) 405 nm and (b) 520 nm for increasing times. (c) UV/Vis spectrum of $IPDI/DRE/PPG/PMNB(OH)_2$ (positive photoresist) film after exposure at 365 nm (d) UV/Vis spectra of 35% IPDI, 15% DRE, 25% PMNB(OH)2 and 25% (NH-ANBP)2 film (dual photoresist) after exposure at 520 and 365 nm.

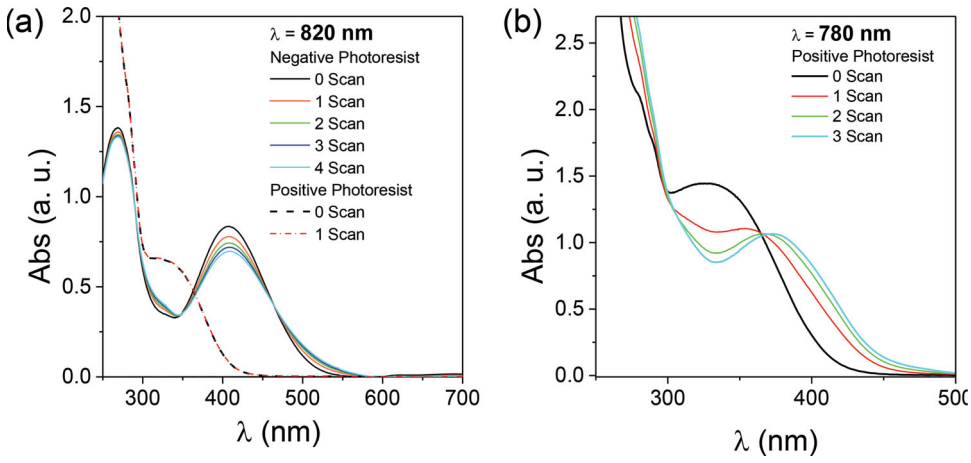

Figure 3. (a) UV/Vis spectra of IPDI/DRE/$(NH\text{-}ANBP)_2$ and IPDI/DRE/PPG/ $PMNB(OH)_2$ films after TPE exposure at 820 nm (b) UV/Vis spectrum of IPDI/DRE/PPG/ $PMNB(OH)_2$ film after TPE exposure at 780 nm.

both chromophores can be achieved at 405 nm. Similar experiments were performed at longer wavelengths, where ANBP still shows appreciable absorbance, whereas $PMNB(OH)_2$ does not. Experiments performed at 520 nm allowed cleavage of the ANBP chromophore while $PMNB(OH)_2$ remained stable (Figure 2b) and, therefore, realization of the wavelength-selective response. Therefore, for the following experiments with the dual resist, the first exposure (activation of $(NH\text{-}ANBP)_2$ and polymerization – negative tone) step was performed at 520 nm, while depolymerization (photodegradation $PMNB(OH)_2$ – positive tone) was performed at 365 nm (Figure 2c). Note that exposure of $(NH\text{-}ANBP)_2$ at 365 nm after full exposure at 520 nm did not change the spectrum of the ANBP chromophore any further (Figure SI3). Figure 2d shows the changes in the UV/Vis spectrum of the dual resist after sequential exposure at the selected wavelengths.

The possibility of wavelength-selective photocleavage of the chromophores using two-photon excitation was also tested. Two-photon excitation occurs by simultaneous absorption of two photons with half of the energy (i.e., double wavelength) required for the single photon excitation. The photocleavage of ANBP was tested by exposure at 820 nm. A 1 mm^2 area of the film was scanned and analyzed by UV/Vis spectroscopy after increasing the number of scans (**Figure 3**a). The observed changes in the absorption bands in the UV spectrum were comparable to those observed in the single photon exposure (Figure 2a), indicating that equivalent photochemical processes took place under single or two-photon excitation. Note that the overall change in the spectrum in Figure 3a is less pronounced than in Figure 2a due to the fact that the depth of the scanned region is smaller than the film thickness. Films containing the PMNB chromophore did not show any change in the UV spectrum at these wavelengths, indicating that the chromophore remained stable under these exposure conditions (Figure 3a). We then tested the possibility of uncaging PMNB at a shorter wavelength. The shortest wavelength available in our experimental equipment for two-photon excitation (Ti:Saphire laser) was 780 nm. The PMNB containing film showed comparable changes in the UV spectra after two-photon exposure (Figure 3b) as those observed after irradiation at 365 nm (Figure 2d). These results demonstrate that ANBP and PMNB chromophores are suitable for wavelength-selective activation using single and two-photon excitation.

In order to demonstrate the possibility of selectively triggered with our caged monomers upon exposure, we used the PU films as resists in an optical lithographic process. The positive and negative resist films were exposed through a mask (single photon excitation) or scanned with the laser (two-photon excitation) and washed with a good solvent (THF/EtOH, 1/1) to develop a surface pattern. The differential solubility in irradiated and not irradiated areas in the presence of a solvent is expected to generate a surface relief that can be visualized under the microscope. **Figure 4**a shows a pattern of holes with 200 µm diameter that were photodegraded from a 2 µm thick positive resist, demonstrating the ability of $PMNB(OH)_2$ monomer to mediate effective single-photon induced degradation of the PU network. Figure 4b shows light-induced polymerized circular pillars obtained on the negative resist using the same mask. These results clearly proof the ability of $(NH\text{-}ANBP)_2$ to mediate photopolymerization of the PU network. To our knowledge, this is the first example of photo-initiated polymerization of polyurethanes. Drying of the film during polymerization stopped the crosslinking of the films and, therefore, the films partially dissolved during the development step. This factor leads to microfeatures shorter than the film thickness (i.e., 1 µm pillars from 2 µm film thickness) after development. This issue might be solved if exposure and polymerization occurs under controlled solvent atmosphere or if illumination times are reduced using more intense light sources.

The possibility of wavelength-selective activation of polymerization and depolymerization processes on the same film was tested with the dual photoresist. Spin-coated films of the dual photoresist were irradiated at 520 nm with a line mask and heated to 90 °C to allow polymerization of the free amine groups of the photocleaved $(NH\text{-}ANBP)_2$ with the isocyanates and formation of the PU network at the exposed areas. In a second illumination step, the film was irradiated at 365 nm through a mask of circles to photocleave the $PMNB(OH)_2$

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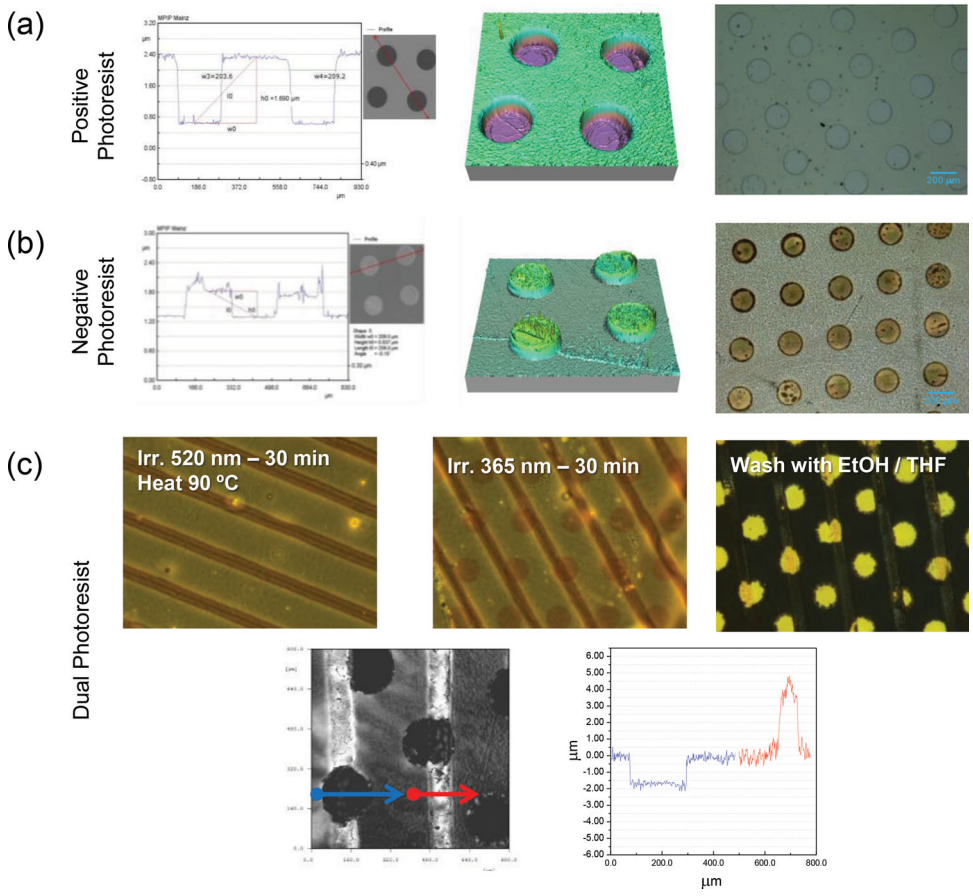

Figure 4. (a,b) Confocal and microscopic pictures of micropatterns obtained with (a) the IPDI/PPG/GP/PMNB(OH)$_2$ mixture (positive photoresist) after exposure at 365 nm, and (b) with the IPDI/DRE/(NH-ANBP)$_2$ mixture (negative photoresist) after exposure at 405 nm. (c) Microscope pictures of the different steps in the λ-selective polymerization (lines) and depolymerization (circles) of the IPDI/DRE/PMNB(OH)$_2$/(NH-ANBP)$_2$ mixture (dual photoresist) and profile measurements of the polymerized (red lines) and depolymerized (blue line) areas in the PU film.

units on the exposed areas. Washing with THF/EtOH (1/1) removed unexposed areas containing not polymerized (NH-ANBP)$_2$ as well as exposed areas containing photocleaved PMNB(OH)$_2$. The two superposed patterns became clearly visible, polymerized lines remain after washing the film (red line in Figure 4c) and the depolymerized circles were dissolved (blue line in Figure 4c). Overall, these results clearly demonstrate the possibility of phototriggering crosslinking and depolymerization steps by using the selective response of ANBP and PMNB to single-photon excitation at different wavelengths.

We also attempted to generate 3D patterns using two-photon excitation. For this purpose a square of 1 mm^2 was scanned with the focused laser in the spin-coated film. In the positive resist, scanning was performed at 780 nm. Development with EtOH revealed a 0.2 µm deep square in the 2 µm film, demonstrating successful site-selective two-photon depolymerization of the sample (Figure SI4). Two-photon induced polymerization could not be achieved in the negative PU photoresist because the film dried during the long exposure times and crosslinking reaction stopped due to the low monomer diffusion. This might be avoided if the exposure process is performed in a close chamber, which we did not have.

In summary, the photocleavable monomers (NH-ANBP)$_2$ and PMNB(OH)$_2$ allow sequential photoactivation of independent crosslinking and depolymerization steps in a dual PU mixture using single photon exposure at 520 and 365 nm, or two-photon exposure at 820 and 780 nm. Using masked irradiation or scanning lasers, 2D and 3D control of the reaction steps can be achieved. This approach is not restricted to urethane prepolymer mixtures and can be applied to other types of reactive monomers and polymeric backbones. Taking into account that these chromophores are typically applied in experiments with living cells,[27,33] we envision that this approach will not only be attractive for microfabrication, but also be applicable to biomaterials research.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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