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Enzymes as biodevelopers for nano- and micropatterned bicomponent biopolymer thin films

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ABSTRACT

The creation of nano- and micropatterned polymer films is a crucial step for innumerable applications in science and technology. However, there are several problems associated with environmental aspects concerning the polymer synthesis itself, crosslinkers to induce the patterns as well as toxic solvents used for the preparation and even more important development of the films (e.g. chlorobenzene). In this paper, we present a facile method to produce micro- and nanopatterned biopolymer thin films using enzymes as so called biodevelopers.

Instead of synthetic polymers, naturally derived ones are employed, namely poly-3-hydroxybutyrate and a cellulose derivative which are dissolved in a common solvent in different ratios and subjected to spin coating. Consequently, the two biopolymers undergo microphase separation and different domain sizes are formed depending on the ratio of the biopolymers. The development step proceeds via addition of the appropriate enzyme (either PHB-depolymerase or cellulase) whereas one of the two biopolymers is selectively degraded, while the other one remains on the surface. In order to highlight the enzymatic development of the films, video AFM studies have been performed in real time to image the development process in situ as well as surface plasmon resonance spectroscopy to determine the kinetics. These studies may pave the way for the use of enzymes in patterning processes, particularly for materials intended to be used in a physiological environment.

KEYWORDS enzymatic treatment, patterning, thin films, cellulose, poly-3-hydroxybutyrate
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INTRODUCTION

The patterning of thin films is a key step to realize advanced technologies in many emerging areas such as optics, electronics and biotechnology.\textsuperscript{1,2} The wide spectrum of thin film applications ranges from surface coatings\textsuperscript{3} and optoelectronic devices\textsuperscript{4} to microarrays\textsuperscript{5} and cell scaffolds\textsuperscript{6}, to name a few. When it comes to biopolymers, the use of classic patterning strategies (e.g. photolithography, soft lithography) is very often hampered by the degradative nature of the patterning procedure itself. Only a few examples exist in literature on how patterns of cellulose, the major biopolymer on earth, can be created in a non-destructive way by lithographic techniques.\textsuperscript{7,8} However, for very complex patterns, lithography is very often not straightforward, since suitable masks need to be manufactured for each pattern, which becomes costly and laborious at a certain point. An alternative approach to lithographic techniques is to exploit phase separation of polymers for the formation of micro- and nanopatterns. The idea is to mix two (or more) polymers (dissolved in a common solvent), which phase separate upon processing and to control the morphology and geometry of the domains by choosing the appropriate processing conditions.\textsuperscript{1,2} While the method of blend preparation is essential for surface morphology, the polymer molecular structures, molecular weights and the composition of the blends play an important role as well.\textsuperscript{9,10} In the case of spin coating, phase separation occurs upon solvent evaporation, leaving the film in a thermodynamically non-equilibrium state, giving rise to different surface morphologies depending on the parameters mentioned above.

Having these facts in mind, we became interested in whether we could exploit the concept of microphase separation of biopolymers to create micropatterns. There are only a few examples known where biopolymer blends have been used to realize such structures.\textsuperscript{11–13} In this paper, we
extend this approach and combine it with orthogonally active enzymes acting as bio-developers to obtain micro- and nanopatterned biopolymer structures.

MATERIALS AND METHODS

Materials. Poly-3-hydroxybutyrate (PHB, Biocycle L 91, $M_w = 260900 \text{ g mol}^{-1}$, $M_n = 111000 \text{ g mol}^{-1}$, PDI = 2.3 determined by SEC in chloroform) was purchased from PHB Industrial S.A. (Brazil) and purified by dissolution in chloroform followed by precipitation in cold ethanol. Afterwards it was dried under vacuum at 50 °C for two days. The process was repeated twice. Trimethylsilyl cellulose (TMSC, Avicel, $M_w = 185,000 \text{ g mol}^{-1}$, $M_n = 30,400 \text{ g mol}^{-1}$, PDI = 6.1 determined by SEC in chloroform) with a DS$_{Si}$ value of 2.8 was purchased from TITK (Rudolstadt, Germany). Chloroform (99.3%), potassium phosphate dibasic (KH$_2$PO$_4$, 99%), hydrochloric acid (37%), sodium acetate (99%), sodium hydroxide (99%) and cellulase from Trichoderma viride were purchased from Sigma Aldrich and used as received. PHB-depolymerase was isolated from Acidovorax sp. and expressed in E.coli bI21. Silicon wafers were cut into $1.5 \times 1.5 \text{ cm}^2$ squares. SPR gold sensor slides (CEN102AU) were purchased from Cenibra, Germany. Milli-Q water (resistivity = 18.2 $\Omega \text{ cm}^{-1}$) from a Millipore water purification system (Millipore, USA) was used for contact angle, SPR investigations and buffer preparation.

Substrate Cleaning and Film Preparation. Prior to spin coating, SPR gold sensor slides/silicon wafers were immersed in a “piranha” solution containing H$_2$O$_2$ (30 wt.%)/H$_2$SO$_4$ (1:3 v/v) for 10 min, then extensively rinsed with Milli-Q water and blow dried with N$_2$ gas. PHB and TMSC were dissolved in chloroform by stirring over night at room temperature and filtered through 0.45 μm PVDF filters. PHB and TMSC solutions (0.75 wt.%) were mixed in different ratios (10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10). 100 µl of solution were deposited onto the
substrate and then rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 rpm·s<sup>-1</sup>. To convert TMSC into pure cellulose, the sensors/wafers were placed in a polystyrene petri-dish (5 cm in diameter) containing 3 ml of 10 wt.% hydrochloric acid (HCl). The dish was covered with its cap and the films were exposed to the vapors of HCl for 15 min. The regeneration of cellulose from TMSC was verified by water contact angle, XPS and ATR-IR measurements as reported elsewhere<sup>14–16</sup>.

**Enzymatic Treatment of Thin Films.** Buffer salts were dissolved in Milli-Q water. The pH value of the solutions was adjusted with 0.1 M acetic acid/hydrochloric acid or 0.1 M NaOH. PHB-depolymerase was dissolved in a phosphate buffer (c = 100 mM) at pH 7.4 at a concentration of 50 µg·ml<sup>-1</sup>. Cellulase was dissolved in an acetate buffer (c = 100 mM) at pH 4.8 at a concentration of 5 mg·ml<sup>-1</sup>. Enzymatic degradation was performed by depositing 100 µl of enzyme solution onto the bicomponent films at a temperature of 37 °C for a time period of 20 min (PHB-depolymerase) and 2 hours (cellulase), respectively. After enzyme treatment all samples were rinsed with Milli-Q water and dried in a stream of N<sub>2</sub> gas.

**Infrared Spectroscopy.** IR spectra were attained by an Alpha FT-IR spectrometer (Bruker; Billerica, MA, USA) using an attenuated total reflection (ATR) attachment. Spectra were obtained in a scan range between 4000 to 400 cm<sup>-1</sup> with 48 scans and a resolution of 4 cm<sup>-1</sup>. The data was analyzed with OPUS 4.0 software.

**Profilometry.** Film thicknesses were acquired with a DETAK 150 Stylus Profiler from Veeco. The scan length was set to 1000 µm over a duration of 3 seconds. Measurements were performed with a force of 3 mg, a resolution of 0.333 µm per sample and a measurement range of 6.5 µm. A diamond stylus with a radius of 12.5 µm was used. Samples were measured after scratching the
film (deposited on a silicon wafer). The resulting profile was used to calculate the thickness of different films. All measurements were performed three times.

**Contact Angle (CA) and Surface Free Energy (SFE) Determination.** Static contact angle measurements were performed with a Drop Shape Analysis System DSA100 (Krüss GmbH, Hamburg, Germany) with a T1E CCD video camera (25 fps) and the DSA1 v 1.90 software. Measurements were done with Milli-Q water and di-iodomethane using a droplet size of 3 µl and a dispense rate of 400 µl·min⁻¹. All measurements were performed at least 3 times. SCA were calculated with the Young-Laplace equation and the SFE was determined with the Owen-Wendt-Rabel-Kaelble (OWRK) method.¹⁷⁻¹⁹

**Atomic Force Microscopy – AFM.** Surface morphology and roughness of the films were obtained in tapping mode in ambient atmosphere at room temperature by a Veeco Multimode Quadrax MM scanning probe microscope (Bruker; Billerica, MA, USA) using Si-cantilevers (NCH-VS1-W from NanoWorld AG, Neuchatel, Switzerland) with a resonance frequency of 320 kHz and a force constant of 42 N·m⁻¹. Root mean square (RMS) roughness calculation and image processing was performed with the Nanoscope software (V7.30r1sr3, Veeco).

AFM investigations of the enzymatic degradation were carried out using a FastScan Bio Atomic Force Microscope (Bruker AXS, Santa Barbara, CA/USA) operated by a Nanoscope V controller. All experiments were conducted in a small-volume (60 µl) flow cell (Bruker AXS, Santa Barbara, CA/USA) and FastScan D cantilevers with a nominal spring constant of 0.3 N·m⁻¹ in tapping mode. A vacuum chuck was used to immobilize the silicon wafer specimen with the corresponding PHB/Cellulose thin films. Degassed buffer solution was then injected into the small-volume cell with attached probe and carefully lowered onto the film. Preliminary to image scanning the experimental setup was allowed to equilibrate to the air conditioned room
temperature of 22 °C. Prior to injection of enzymes multiple reference images were recorded. Continuous image scanning was started by injecting 180 µl of a buffer enzyme mixture with enzyme concentrations of 50 µg·ml\(^{-1}\) for either the PHB-depolymerase or 5 mg·ml\(^{-1}\) for the cellulase. Please note, 180 µl correspond to 3 times the volume of the cell and ensures a complete exchange of buffer to buffer/enzyme solution. Image recording was performed until either all PHB or cellulose was degraded. Setpoints, scan rates and controlling parameters were chosen carefully to ensure lowest possible energy dissipation to the sample and to exclude tip driven artifacts. Data analysis of images was performed using Nanoscope Analysis 1.50 (Build R2.103555, Bruker AXS, Santa Barbara, California/USA) and Gwyddion 2.38 (Released 2014-09-18, http://gwyddion.net/). Movie presentations were compiled using Windows Movie Maker (Version 2012, Build 16.4.3508.0205, Microsoft Corporation, Redmond, WA/USA). All images were plane fitted at 1st order unless otherwise stated.

**Multi Parameter Surface Plasmon Resonance Spectroscopy – MP-SPR.** MP-SPR spectroscopy was accomplished with a SPR Navi 200 from Bionavis Ltd., Tampere, Finland, equipped with two different lasers (670 and 785 nm, respectively) in both measurement channels, using gold coated glass slides as substrate (gold layer 50 nm, chromium adhesion layer 10 nm). All measurements were performed using a full angular scan (39–78°, scan speed: 8°·s\(^{-1}\)). Enzymatic degradation was performed by exposing the films to enzyme solution at a flow rate of 50 µl·min\(^{-1}\) and a temperature of 25°C for a time period of 5 min, followed by rinsing with buffer.

**RESULTS AND DISCUSSION**

For the proof of concept, we chose blend thin films composed of poly-3-hydroxybutyrate, a biopolymer produced by bacteria, and cellulose. Both biopolymers are readily degradable by
enzymes developing either selectively PHB (by *PHB-depolymerase*) or cellulose (by *cellulases*) micro/nanopatterns. In terms of the experimental approach, a soluble precursor for cellulose, which is solvent compatible to PHB, has to be used, since cellulose is insoluble in common organic solvents. A suitable precursor for this purpose is trimethylsilyl cellulose (TMSC), as its solubility can be easily adjusted by the degree of substitution with TMS groups. The concept is to convert organosoluble TMSC (DS$_{Si}$ ca. 2.8) back to cellulose after spin coating using HCl vapors; a procedure which has been extensively employed during the past years.$^{20,21}$

In order to create different micro/nanopatterns, PHB and TMSC are dissolved in chloroform at different ratios and subjected to spin coating. Afterwards, the morphology of the resulting films is investigated before and after exposure to HCl vapors and the enzymatic development of PHB and cellulose is studied. As revealed in the AFM images (*Figure 1*) the microphase separation seemingly proceeds according to the transient bilayer theory, describing the vertical stratification of the two phases followed by interfacial instabilities, caused by a solvent-concentration gradient throughout the film, leading to lateral phase separation.$^{22}$

*Figure 1.* AFM images $10 \times 10 \ \mu m^2$ of PHB/TMSC thin films (different ratios) before (a) and after exposure to HCl vapor (b).
The final structure of the resulting domains is defined by a variety of factors, such as film thickness, relative humidity, temperature, the type of substrate, solubility of the blend components in the solvent used and surface segregation, which is the preferential migration of one component to the interface, depending on surface free energy. This complex interplay of parameters complicates the explanation of the emerging phase structures without evidence from time-resolved small-angle x-ray scattering or light reflectivity during the spin coating process. In the herein investigated system, PHB probably forms the bottom layer and TMSC the top layer during the initial phase of spin coating, due to the relatively high surface free energy and higher polarity of PHB, compared to the highly substituted TMSC (DSi ca. 2.8). Therefore, interaction of PHB with the hydrophilized gold/silicon support is more pronounced. Additionally, the lower surface free energy compound TMSC tends to migrate to the air-polymer interface. Apparently, a rather thin TMSC layer is present for blend films with PHB/TMSC ratios of 10:1, 5:1, 3:1 and 1:1 during vertical stratification, which contracts to droplets, that will be surrounded by the second phase at the end of the spin coating process, seen in the droplet shaped cellulose domains that are distributed in a continous PHB phase. A thicker layer of TMSC during the early phase of spin coating favors PHB distribution in a continous cellulose phase, due to a dewetting process, leading to hole formation and subsequent filling with the polymer from the lower layer. This is observed for PHB/TMSC ratios of 1:5 and 1:10. Films with a PHB/TMSC ratio of 1:3 exhibit two continuous laterally separated phases, which usually occur at ratios close to one, however this deviation can be attributed to the difference in molecular weight of the polymers. The resulting morphologies are different compared to other TMSC-based blend films known in literature. PHB/TMSC films with ratios of 10:1, 5:1, 3:1 and 1:1 show circular TMSC domains embedded in a PHB matrix, similar to those reported by Kontturi et al. for
PMMA/TMSC films. For these blend films, the TMSC domains form cavities with a depth of up to 10 nm (before and after regeneration) compared to the PMMA phase. Further, polystyrene/TMSC blend films show low roughness and protruding polystyrene domains are only obtained after HCl treatment. A special case is the lignin palmitate/TMSC system, where the 3:1 ratio yielded rather flat films, while the 1:3 ratio led to the formation of lignin palmitate cavities. After regeneration, these cavities inverted into protruding pillar like domains due to shrinkage of the TMSC after conversion. In contrast to that, all the herein investigated PHB/TMSC films showed protruding TMSC features (see Table 1) which were converted into cavities just after the regeneration step. For the PHB/TMSC blend films with ratios of 1:3, 1:5, 1:10, the situation was different since the continuous phase was inverted. The protruding domains shrank but instead of cavities rather flat films (in terms of height difference) were obtained after regeneration. The shrinkage amounts to approximately 60-70%, which agrees well with values reported in literature.

**Table 1.** Average TMSC/cellulose domain height (in nm) in respect to PHB for the different blend ratios before and after the regeneration step.

<table>
<thead>
<tr>
<th>PHB/TMSC</th>
<th>10:1</th>
<th>5:1</th>
<th>3:1</th>
<th>1:1</th>
<th>1:3</th>
<th>1:5</th>
<th>1:10</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMSC domain height</td>
<td>36 ± 9</td>
<td>83 ± 24</td>
<td>128 ± 45</td>
<td>100 ± 18</td>
<td>88 ± 8</td>
<td>116 ± 14</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>cellulose domain height</td>
<td>-17 ± 4</td>
<td>-15 ± 7</td>
<td>-8 ± 6</td>
<td>-40 ± 14</td>
<td>30 ± 12</td>
<td>24 ± 4</td>
<td>18 ± 10</td>
</tr>
</tbody>
</table>

Before enzymatic development is performed, it is of crucial importance to confirm the conversion of TMSC to cellulose and to exclude any influence of HCl vapors on the PHB domains of the films. Comparing the ATR-IR spectra (Figure S1) of TMSC and cellulose, it
can be clearly seen that the characteristic bands for methyl groups of TMSC at 2960 cm$^{-1}$ ($\nu_{\text{asym}}$) and 2872 cm$^{-1}$ ($\nu_{\text{sym}}$) and the Si-C contributions at 1251 cm$^{-1}$ and 842 cm$^{-1}$ diminish in the regenerated TMSC film and TMSC containing blends. Additionally, the OH stretching band between 3600 and 3000 cm$^{-1}$ appears due to the formation of cellulose. ATR-IR spectra (Figure S2) of PHB bulk and PHB films before and after the regeneration step confirm that there is no influence of HCl vapor on PHB. This is further corroborated by AFM studies (Figure S3), profilometry (constant thickness) and wettability (constant contact angles) determinations as well. However, a change in thickness of films containing TMSC is observed due to shrinkage of TMSC domains by cleaving off TMS groups during conversion. As expected, the shrinkage is higher for films containing a larger amount of TMSC (Figure S4). During the regeneration step, surfaces change from rather hydrophobic to more hydrophilic. Figure 2 depicts this shift of surface free energy from circa 30 mJ·m$^{-2}$ with hardly any polar contribution for non-regenerated blends to higher values with increased polar contributions for regenerated ones. Interestingly, the highest surface free energies are determined for blend films with PHB/cellulose ratios of 1:1 to 1:10, which are even higher than for pure cellulose ($63 \pm 1$ mJ·m$^{-2}$). Maybe, this behavior originates from the roughness of the films, which is a major factor besides the presence of chemical groups for variations in SCA and SFE.
Figure 2. Surface free energy of bicomponent thin films before (a) and after (b) exposure to HCl vapor. Please note that the error bars are very small and hardly visible in the diagrams.

The phase separated blend thin films were subjected to enzymatic development (37°C) aiming at bioorthogonal degradation of one component without influencing the other. For this purpose, the films have been exposed to enzyme solutions for different periods of time. It was observed that PHB-depolymerase degrades PHB very fast (20 minutes) under the chosen conditions. In contrast, the cellulose films were degraded after 2 hours, which is in line with specific investigations on the degradation of cellulose films. However, since in these studies different cellulase cocktails and operating conditions had been employed, further quantitative comparisons are not possible.

After drying the films, AFM investigations (Figure 3) confirm the selective removal of PHB by PHB-depolymerase and of cellulose by cellulases from blend films of all tested ratios, while the other phase was left intact. Remaining pillars and pores/cavities after enzymatic treatment are in the nano to micro size range depending on the PHB/cellulose ratio. An overview on the average and median feature sizes of the blend films after enzymatic degradation is presented in Table 2. Further, the wettability of the remaining patterned biopolymer phases was nearly
identical for all PHB and cellulose phases and in the same range as pure PHB or cellulose films (Figure 4).

Figure 3. AFM images (size $10 \times 10 \mu m^2$) of enzymatic degradation of PHB/cellulose thin films with PHB-depolymerase (a) or Cellulase (b).

Table 2. Average and median feature sizes of PHB/cellulose blends after enzymatic treatment.

<table>
<thead>
<tr>
<th>PHB/cellulose</th>
<th>10:1</th>
<th>5:1</th>
<th>3:1</th>
<th>1:1</th>
<th>1:3</th>
<th>1:5</th>
<th>1:10</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td>50 ± 15</td>
<td>71 ± 28</td>
<td>126 ± 50</td>
<td>534 ± 209</td>
<td>1337 ± 204</td>
<td>1405 ± 380</td>
<td>126 ± 65</td>
</tr>
<tr>
<td>median</td>
<td>49 ± 12</td>
<td>57 ± 24</td>
<td>106 ± 40</td>
<td>498 ± 174</td>
<td>1260 ± 182</td>
<td>1430 ± 290</td>
<td>100 ± 48</td>
</tr>
</tbody>
</table>
Figure 4. Static water contact angle of bicomponent thin films after treatment with PHB-depolymerase (a) or cellulase (b).

The high efficiency of PHB-depolymerase from Acidovorax sp. led us to examine its adsorption behavior onto the herein investigated blends by means of surface plasmon resonance spectroscopy (SPR). The adsorption of PHB-depolymerases is a fundamental step for the degradation function of the enzyme. Usually, these kinds of enzymes dock to the surface with a substrate binding domain and linker regions, followed by a conformational change activating the catalytic domain. However, we were not able to monitor the complete adsorption process of PHB-depolymerase, because almost immediate degradation of PHB set in at the chosen concentration (50 µg·ml⁻¹). This is even more astonishing since all SPR experiments were performed at 25 °C, keeping in mind that the optimum working temperature of the used PHB-depolymerase is 37 °C. The SPR-angle increases slightly upon enzyme injection (5 min, 250 µl, 50 µg ml⁻¹), followed by an immediate decrease for neat PHB and PHB/cellulose blend ratios of 3:1 and 1:1. This indicates that both processes, adsorption and degradation, proceed at the same time, whereas the blend film with a PHB/cellulose ratio of 1:3 is not degraded at all at 25°C. (Figure 5 and Figure S5). A closer look reveals that we monitor faster adsorption of PHB-depolymerase (steeper slope of increasing SPR-angle) and earlier start of degradation (decrease in SPR-angle) with increasing PHB contents in the films (Figure S6). However, for blend films with a PHB/cellulose ratio of 1:3, we did not observe any activity of the enzyme at this concentration. A potential explanation for this is the lower temperature (25°C) in the SPR experiments compared to the patterning trials (37°C) concomitant with lower activity of the enzyme in general. Furthermore, the surface free energy of the 1:3 PHB/cellulose blend film is the highest among all investigated films, which may impede efficient interactions of the enzyme.
with the remaining PHB in the blends, since the main binding interactions of \textit{PHB-depolymerase} are of hydrophobic nature.\textsuperscript{40,41}

\textbf{Figure 5.} SPR sensograms of enzymatic treatment with PHB-depolymerase (50 µg·ml\textsuperscript{-1}) of PHB/cellulose bicomponent thin films acquired at 25°C.

In order to gain better insights into the degradation process, the enzymatic treatment of PHB/cellulose blend ratio 1:1 was monitored in real time by fast scan AFM (ESI). The resulting videos further substantiate our findings concerning \textit{PHB-depolymerase}, revealing initial swelling of the PHB domains, followed by fast degradation compared to other enzymes of this kind.\textsuperscript{41,42}

The swelling step could be an indication for a change in PHB surface morphology upon enzyme adsorption\textsuperscript{43-45} or a very thin cellulose layer on top of the PHB phase, which is swollen by the buffer, thereby breaks up and is then penetrated by the enzyme, which is then able to digest the underlying polymer; however there is no evidence for both assumptions. In-situ AFM investigations regarding the reverse patterning of the blends by enzymatic degradation of cellulose domains by \textit{cellulase} reveals a slower process compared to PHB depolymerase. This behavior originates from the different types of enzymes that need to work cooperatively in the
case of cellulases (glucosidases, exo- and endoglucanases, etc.) whereas PHB-depolymerase consists just of a single enzyme.

Further, it is known that similarly to PHB-depolymerase the optimum working temperature of cellulase from Trichoderma viride to digest cellulose thin films is 37 °C. However, the outcome of the enzymatic digestion experiments at lower temperature is in good agreement with results from degradation at optimum temperature. In contrast to PHB, cellulose pillar-like domains are degraded uniformly from top to bottom, generating holes with a size of approximately 500 nm. PHB-depolymerase initially degrades only some parts of the continuous PHB domains which in turn are nearly completely digested. As soon as the first domains have been degraded, it seems that the film gets more accessible to the enzymes and further breakdown of PHB is observed.

CONCLUSION

In summary, an efficient approach for bioorthogonal templating of biopolymer blend films is presented at the example of PHB and cellulose. Depending on the ratio of the components in the blend films, different surface morphologies and feature sizes were obtained. This pre-structuring by phase separation is then further utilized to create micro and nano sized domains by selective enzymatic development of just one of the two biopolymers. While patterning at 37°C is working very well for all blend film systems, a decrease of temperature to 25°C showed that the activity of the PHB-depolymerase decreased significantly for those films that feature rather high surface free energies. This behavior is particularly pronounced for the film with the highest surface free energy among all films (PHB/cellulose 1:3) where hardly any degradation can be observed at 25°C. Currently, we are investigating how to exploit this very interesting phenomenon, namely
to induce switchable temperature triggered development of biopolymer films which can be further tuned by variation of enzyme concentration to increase the activity.

The enzymatic developing step of this ‘bioresist’ was further visualized by video AFM studies which revealed different degradation mechanisms for the corresponding biopolymer types relating to the different enzymes (one component vs multi-component in the case of cellulases). Furthermore, the herein demonstrated approach presents a general method which is applicable for templating purposes with various other renewable or bio-based polymer systems, aiming at the replacement of polymers derived from fossil resources. The resulting patterned surfaces may be subject of further investigations concerning their utilization in various fields such as biosensors or antifouling surfaces.

ASSOCIATED CONTENT

Supporting Information. Materials, detailed experimental procedures, AFM videos of enzymatic degradation, supplementary IR spectra and contact angle data (Fig. S1-S6).

AUTHOR INFORMATION

Notes

The authors declare no competing financial interests.

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