Functional Patterning of Biopolymer Thin Films Using Enzymes and Lithographic Methods

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Two different lithographic techniques for the patterning of thin biopolymer films are developed. The first method is based on using a microstructured elastomeric mold for the structuring of thin films of regenerated cellulose. The thin films are manufactured by spin-coating of trimethylsilyl cellulose (TMSC) and subsequent regeneration. The microchannels formed by the mold and the cellulose film are filled with a cellulase solution by capillary action. In the areas exposed to the enzyme solution, the cellulose film is digested, whereas the area in contact with the mold is protected from the enzymatic activity. Optical thickness measurements, atomic force microscopy and fluorescent staining confirm a successful patterning of cellulose on several substrates by this method. The second method is based on the structured regeneration of thin TMSC films. TMSC surfaces are protected with metal masks and exposed to vapors of hydrochloric acid. These treatments result in hydrophilic cellulose structures surrounded by hydrophobic TMSC with differing physicochemical properties. Treatments of the obtained structures with cellulases allow the selective removal of pure cellulose, whereas a TMSC pattern remains on the surface. These TMSC can be regenerated back to pure cellulose by treatments with vapors of hydrochloric acid. The developed methods allow the effective fabrication of micropatterned biopolymer thin films suitable for further functionalization and application in, e.g., bioanalytical devices. This is shown by the immobilization and detection of single-stranded DNA on structured cellulose surfaces. Owing to the versatility of both patterning approaches the methods can be further extended to other combinations of substrates and enzymes.

1. Introduction

Cellulose is the most abundant biopolymer on earth and a major part of the cell walls of plants. It has remarkable mechanical properties and is stable under a wide range of environmental conditions. Therefore, it has found widespread applications ranging from fiber and membrane technology to chromatography and packaging.[1] Cellulose and many of its derivatives show excellent biocompatibility and have proven to be ideal materials for the interaction with other biomolecules in a large number of biotechnological and life science applications. These applications are for instance selective blotting or the use of cellulose as separation membranes, chromatography media, or for bioimaging and microarrays.[1–6] Pure cellulose surfaces are in general highly hydrophilic and usually strongly hydrated. Therefore, unspecific adsorption of proteins is often effectively suppressed and smooth cellulose surfaces show a low-fouling behavior.[7,8] These low levels of unspecific binding allowed for instance the application of cellulose thin films as support for the detection of single molecules.[9,10] Thin films of cellulose, deposited on solid and transparent supports made of glass or plastics are therefore promising for the development of DNA or protein microarrays and bioanalytical lab-on-a-chip devices. Such thin films are very stable because, despite its high hydrophilicity, cellulose is insoluble in water and most organic solvents. Common deposition techniques for such films are based on the regeneration of cellulose from organosoluble derivatives or solutions in ionic liquids.[11] A promising derivative for the preparation of cellulose thin films is trimethylsilyl cellulose (TMSC) which is soluble in toluene and can be regenerated to pure cellulose by a treatment with vapors or solutions of hydrochloric acid.[12,13] Besides the aforementioned advantages of cellulose and its thin films, many applications require a further patterning step to generate micro- or nano-structured surface features. In order to define reaction or detection zones in bioanalytical lab-on-a-chip devices, microarrays or cell growth scaffolds, microstructuring is regularly applied.[14] Within the large variety of different methods for

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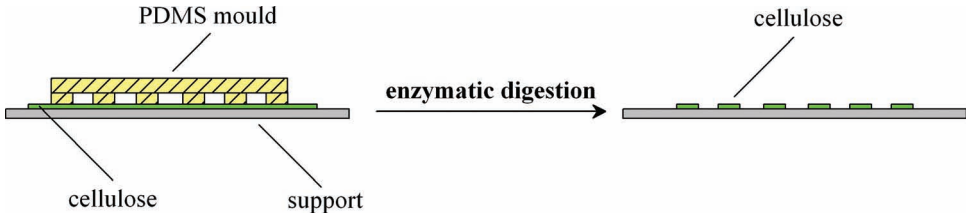


Scheme 1. Patterning scheme for a cellulose surface with enzymatic digestion.

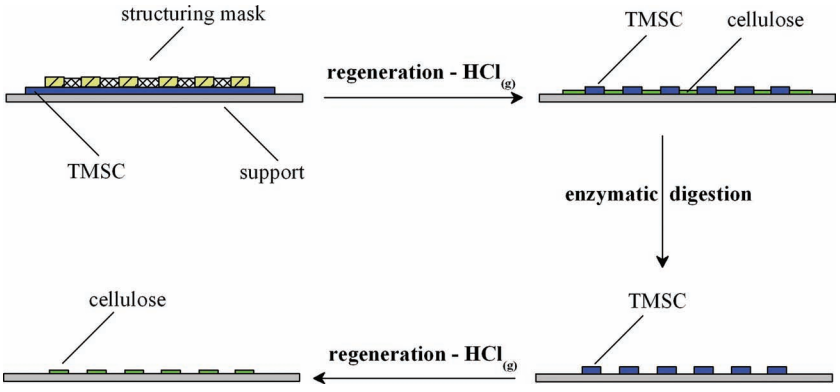
micropatterning of thin films, so called soft-lithography based techniques are the most versatile and frequently used ones. Originally introduced by the group of Whitesides, these techniques rely on the transfer of patterns by using soft elastomeric stamps. Several variants of this basic principle were developed and applied for patterning of a broad range of materials, from polymers to nanoparticles.[15] A frequently used soft-lithography method, called micromolding in capillaries (MIMIC) is based on placing an elastomeric stamp, with micro-channels onto a substrate. Subsequently, a solution which contains polymers, biomolecules or precursors is placed beside the stamp and the micro-channels are filled by capillary action.[16] MIMIC has also been used for the patterned deposition of protein thin films by selective adsorption or binding.[17,18] Alternatively biocatalytic reductive patterning of thin films and monolayers can be achieved by microcontact printing (μ-CP) or dip pen lithography.[19] In these approaches, enzymes are immobilized on stamps or AFM tips and then brought into contact with thin biopolymer films. A combination of the MIMIC technique with the selective catalytic properties of enzymes could therefore be used to microstructure thin biopolymer films.[20] Compared to biocatalytic microcontact printing or dip-pen lithography, this enzymatic "etching" method has the advantage that thicker films can be digested, that the digestion products can diffuse freely and that fresh enzyme solution can be provided. The selectivity of cellulase enzymes from *trichoderma viride* can further be used to allow selective digestion of pure cellulose thin films, whereas pure TMSC cannot be digested.[21,22] This can be advantageous if one aims at creating patterns of pure cellulose within a TMSC structure. This study aims at developing and investigating such micro-structuring methods for TMSC and cellulose thin films. In order to do so TMSC thin films will be prepared on various substrates and regenerated to pure cellulose. A PDMS-mold and cellulases are then used to selectively digest the exposed parts of the cellulose film (**Scheme 1**). In the second structuring approach a metal mask will be placed on a pure TMSC film and only parts which are exposed to vapors of hydrochloric acid are regenerated to pure cellulose with a high hydrophilicity. These parts can further be digested by cellulases to produce pure TMSC structures with a hydrophobic character. Subsequent regeneration to pure cellulose allows the manufacturing of cellulose grids on solid supports (**Scheme 2**). A detailed investigation on the manufacturing and surface properties of the obtained structures will be performed. Besides contact angle measurement and atomic force microscopy, fluorescent staining and the immobilization and hybridization of labeled and unlabeled single stranded DNA will be used to demonstrate the versatility and applicability of the elaborated methods.

2. Results and Discussion

2.1. Enzymatic Structuring Method

The successful patterning of cellulose thin films was demonstrated on several different substrates which are commonly used as supports for bioanalytical devices. Beside hydrophilic substrates such as glass and silicon dioxide, a hydrophobic cycloolefin polymer (COP) was chosen. Cycloolefin polymers are among others the most promising materials for plastic based microfluidics, lab-on-a-chip systems or microtiterplates.[23,24] The TMSC films were deposited on these substrates by spin coating. The silyl groups of TMSC were succesfully cleaved by exposure of the films to hydrochloric acid vapors. After complete evaporation of the silyl compounds, pure regenerated cellulose films were obtained. The completeness of the regeneration to pure cellulose throughout the whole film could be confirmed by complementary methods and was published elsewhere.[21,25]

This regeneration is also reflected in the increased hydrophilicity (contact angle of water: pure TMSC: 95.8 ± 2.8°, pure cellulose 25° ± 0.5) As confirmed by optical thickness measurements, the regenerated films are approximately 35 nm thick and cover the surface completely, and homogeneously.



Scheme 2. Patterning scheme for a TMSC surface with structured regeneration and enzymatic digestion.

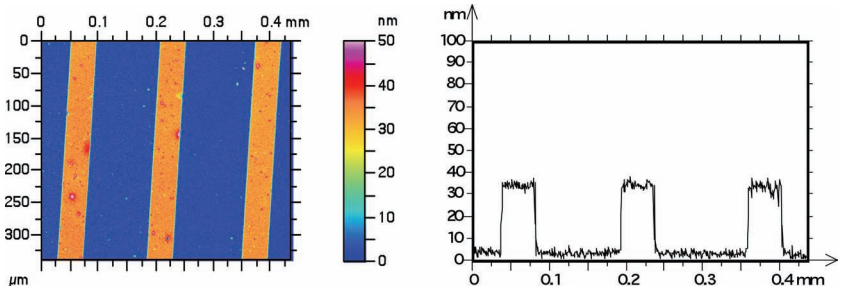


Figure 1. Sarfus image of enzymatically structured cellulose stripes.

In the first patterning method, a PDMS-mold has been used which was microstructured in a stripe like pattern with 40 to 100 µm wide channels separated by 50 µm wide partition walls. This mold was pressed against the cellulose surface and the microchannels were filled with cellulase solution by capillary action. The term cellulase relates to a group of enzymes that catalyze the hydrolysis of 1.4-beta-D-glucosidic bonds in cellulose and thus break down the macromolecule to cellobiose or glucose. The cellulase solution inside the microchannels selectively digested the cellulose film in the exposed areas (**Figure 1**). In contrast, the areas covered by the partition walls of the PDMS-mold were not affected by the enzymatic treatment. These areas were protected because the cellulose surface was in direct contact with the hydrophobic PDMS mold. After a reaction time of two hours at 35 °C the mold was removed and the surface was washed with water. The obtained miropatterned cellulose stripes were characterized by different microscopic techniques. Optical thickness imaging was carried out using a wide field microscopy technique (Sarfus) employing differential interference contrast with polarized light on special silicon substrates.[26] This technique enables rapid imaging of film thickness in the nm range and in a large field of view. After enzyme assisted soft lithography, a stripe like pattern of cellulose features is obtained (Figure 1). This features are of well defined shape with homogeneous width and spacings, and with sharp edges. The cellulose based features are separated by 40 to 100 µm wide areas from which cellulose was completely removed by enzymatic digestion. It can be concluded that the areas covered by the mold were effectively protected from enzymatic attack because the original film thickness has been preserved and the pattern width is well reproduced. The cellulose stripe pattern was insoluble in water and stable in a pH range from 4.8 to 9.0. As was shown elsewhere cellulose thin films are stable and are not hydrolysed in acetate buffer at pH 4.8.[21] The morphologies of the cellulose stripe patterns were further investigated using atomic force microscopy. As can be seen in **Figure 2**, the stripes showed well defined and sharp edges and a rather smooth surface. The height of each stripe is comparable with the data obtained from the Sarfus measurements.

Selective removal of cellulose by enzymatic lithography does not only lead to topographical patterning but also to chemical patterning of the surface. Therefore, beside pattern morphology, the chemical surface properties are relevant. Especially for bioanalytical applications, the differences in binding properties for labels and biomolecules are of special importance. Spatially selective functionalization of the cellulose covered areas was therefore investigated by staining with fluorescent dyes. 5-([4,6-Dichlorotriazin-2-yl]-amino)fluorescein (DTAF), a reactive fluorescein derivative that is capable of binding covalently to hydroxyl groups, was used for staining. Fluorescence microscopy of DTAF stained patterns clearly showed selective functionalization of the elevated, cellulose based stripes (**Figure 3**). Although, slight background fluorescence was observed on the areas from which cellulose has been removed, high contrast to the cellulose covered areas was achieved. This confirmed that the cellulose film was completely removed from the enzyme treated areas and mainly the bare, underlying substrate material is exposed. Therefore, such patterned cellulose structures are promising for bioanalytical applications like lab-on-a-chip systems and biosensors where surfaces with a selective reactivity are needed. In order to verify whether material from the mold has been transferred to the surface, a PDMS-mold was pressed on clean Surfs and incubated with cellulase under the same conditions as the cellulose model films. Neither residual PDMS nor deposited enzyme could be detected via an increase in thickness or the visibility of surface features. This also confirmed that only negligible amounts of enzyme adsorbed on the pure substrate after extensive rinsing with buffer solution and pure water.

With this method we were able to elaborate a versatile and new approach for the fabrication of micro-patterned cellulose thin films by an enzymatic MIMIC technique. This simple approach allows the patterning of cellulose films of several nm thickness with lateral a resolution in the micrometer range. The mild and selective hydrolysis by cellulases allows the removal of cellulose from the surface. In principle the removal could also be performed by a proper solvent or by strong acids. Nevertheless the selectivity of the hydrolysis and the use of water soluble harmless chemicals at moderate temperatures are advantageous.

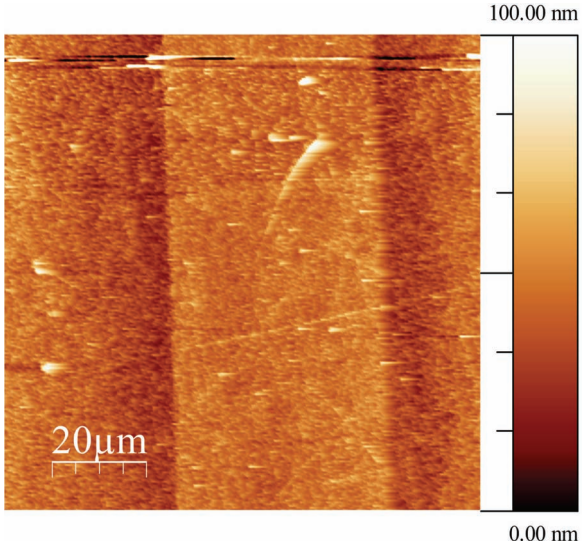


Figure 2. Atomic force microscopy image of a single cellulose stripe.

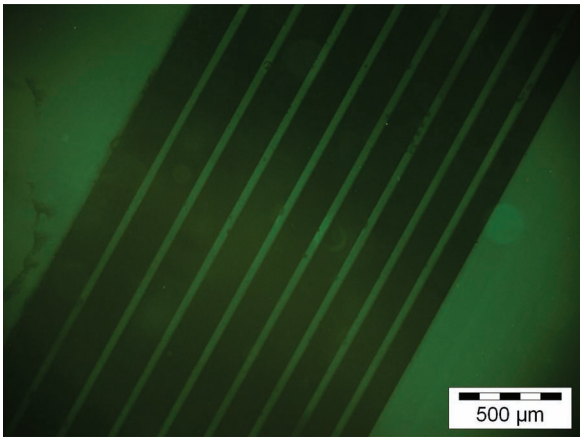


Figure 3. Fluorescence micrograph of a structured cellulose thin film on glass, stained with DTAF.

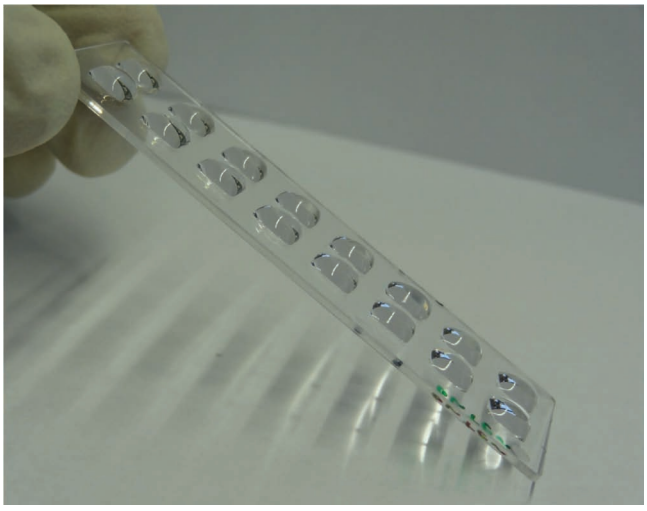


Figure 4. Water droplets on COP slides coated with structured cellulose/TMSC.

The technique is somehow similar to other soft-lithographic techniques such as biocatalytic microcontact printing.[27,28] Nevertheless, the MIMIC approach presented here has several important advantages for the reductive patterning of biopolymer films. For biocatalytic μ-CP, the enzyme has to be immobilized on the elastomeric stamp surface by adsorption, covalent binding, or affinity interactions. This immobilization step always bears the risk of compromising enzyme activity and active site accessibility. Especially active site orientation is critical when the substrate is present in the form of a thin solid film and thus cannot diffuse towards the enzyme. In contrast, in the MIMIC approach presented in this work, the enzyme is present in its natural configuration in buffer solution and thus is free to diffuse towards the substrate. Furthermore, in (bio)catalytic as well as other reactive variants of μ-CP, chemical reactions occur in the nanoscale confinement between the substrate and the stamp which is in contact with the substrate.[29] While this confinement is advantageous for the transfer of self assembled monolayers and often accelerates chemical modification of monolayers, it is detrimental for enzymatic degradation reactions where a large number of low-molecular degradation products such as glucose are formed. In the MIMIC approach described here the reaction products are easily dissipated into the comparatively large volume of the buffer solution. Therefore, it is possible to successfully fabricate several nm high features from biopolymer thin films. With the method presented here one could achieve a lateral feature size of 50 µm. This size is limited by the manufacturing of a PDMS mold that provides stable channels for the exchange of the enzyme solution and the digestion products. From our practical experience the lower limit of this method will therefore be at around 20 µm for lateral cellulose features.

2.2. Structured Regeneration of TMSC Surfaces

2.2.1. Structured Wettability of the Substrates

The structured regeneration of pure TMSC films on a solid support is an alternative method to produce cellulose patterns with versatile functionalities. In this method the structuring is obtained by placing a mask onto a TMSC coating and by subsequent exposure of the protected surfaces to vapors of hydrochloric acid. Only the parts of the TMSC coating which are not covered by the mask are regenerated to pure cellulose. Already known structuring methods for cellulose thin films allowed the manufacturing of hydrophilic/hydrophobic domains from TMSC and other hydrophobic cellulose derivatives by a phase separation process.[30] Nevertheless the method described in the present work allows controlling the geometry of the obtained features very selectively by applying a defined mask. The coated functionalized slides are transparent, homogeneous and stable against rinsing with water. The successful patterning leads to an increased wettability of the cellulose part (contact angle of water: 24.4 ± 1.2°), whereas the pure TMSC is not wetted by water (contact angle of water: 101.4 ± 1.3°). **Figure 4** shows water droplets on a coated COP slide that was structured in this way. It can be clearly seen that water droplets are retained in the form of squares and do not roll off even when the slide is tilted to 45°. This is especially interesting if one aims to use the slides for protocols which require sufficient separation of different test solutions on the slide. This gives the opportunity to conduct experiments in parallel on one slide. The cellulose patches are insoluble in water and most of the organic solvents but provide a hydrophilic surface which is advantageous in the immobilization of biological relevant compounds such as proteins or cells. The obtained cellulose feature size is defined and limited by the applied mask which allows the diffusion and exchange of vapors of HCl, water and cleaved siloxanes. The smallest lateral cellulose features that could be obtained by applying a mechanically drilled mask in our experiments were 100 µm (data not shown).

Further surface chemistry with cellulose allows several immobilization techniques on the solid support. Even adsorptive immobilization of polymers which exploits the selective interaction of polysaccharides with cellulose can be performed on these surfaces.[31–34] The different chemical composition and reactivity of the structured coatings can further be elucidated by fluorescent labeling as will be shown in the next section.

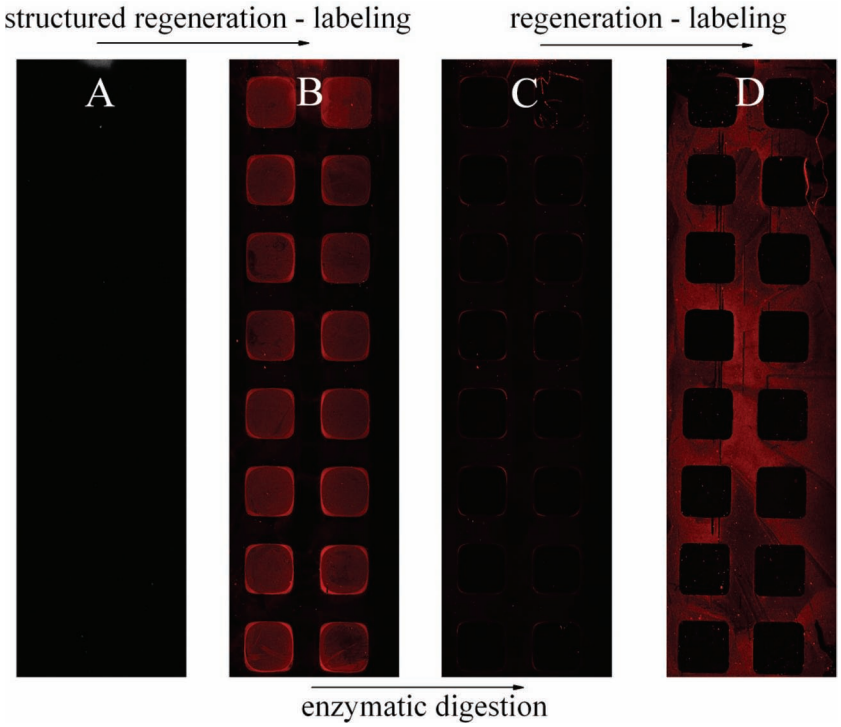


Figure 5. Slides of structured and regenerated TMSC/cellulose on COP, fluorescent-dyed with TRITC. A) pure TMSC, B) cellulose patches surrounded by TMSC, C) enzymatically digested cellulose patches surrounded by TMSC, and D) cellulose grid obtained by regeneration of slide (C). The width of one slide is 25 mm.

2.2.2. Chemical Composition and Enzymatic Digestion of the Structures

As can be seen in **Figure 5**, a structured regeneration of TMSC to cellulose is able to produce squared patterns which can be fluorescent labeled by TRITC (tetramethylrhodamine isothiocyanate) (B). In contrast, pure TMSC coated COP slides cannot be labeled (A) since the reactivity and unspecific binding of TRITC on these surfaces is low. Therefore this structuring method provides the possibility to create surfaces that can selectively be labeled with reactive compounds. This allows the functionalization of the cellulose surfaces in a desired way, in order to create surfaces with specific interaction sites. It is further possible to digest the cellulose part by treatments with cellulases. These enzymes and pure buffer solutions at pH 4.8 are not able to hydrolyze TMSC.[21] However pure cellulose is removed completely and a TMSC mask remains on the surface which is indicated by the vanished fluorescence intensity of the former cellulose patches (C). It is worth noting that the hydrolysis of pure cellulose is only caused by the action of cellulases and not by the buffer solution.[21] After digestion the COP surface is exposed and the static contact angle of water on this surface is 90° which is a proof of a complete removal of the cellulose coating. Further regeneration of the obtained TMSC structure produces a cellulose coating that can again be dyed with TRITC (D). These experiments show that TMSC in combination with structured regeneration and enzymatic digestion can successfully be applied to produce functional coatings on polymeric microscope glass slides.

Both structuring methods are innovative and reliable for the micro-patterning of cellulose and TMSC thin films on different supports. These supports are commonly used for the fabrication of microfluidic analysis systems. It can be assumed that both approaches are rather generic methods and can be applied to a broad range of other biopolymer substrates. By selecting suitable enzymes most other biopolymer films could be patterned following the same methodology. Since enzymatic degradation is also known for a large number of synthetic polymers, the method could be further extended to synthetic polymer thin films.[35,36]

3. Application of Structured Cellulose Surfaces

As it was discussed above the structured cellulose thin films can be used as solid support for the immobilization of biomolecules. Functional (bio) molecules can be attached to cellulose surfaces by a recently developed technique.[37] This technique exploits the relatively specific adsorption of water soluble carboxymethyl cellulose (CMC) to solid cellulose surfaces. Coupling of functional molecules to CMC with the well established carbodiimide chemistry in solution and subsequent adsorption of these CMC-conjugates to solid cellulose allows the irreversible attachment onto the cellulose surface. Such a procedure was successfully applied to structured cellulose surfaces. As an example for this application primary amino-modified single-stranded DNA was immobilized on cellulose patches on a COP microscope slide as shown in Figure 4.

Amino modified single-stranded DNA (21 mer) was coupled to CMC with water soluble 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl). This conjugation product was immobilized on the cellulose patches by simple incubation for 60 min and subsequent washing. Hybridization with a fluorescent labelled complementary DNA_{comp} strand was proven at different concentrations of labelled DNA_{comp}. As can be seen in the **Figure 6** DNA concentrations from 10 µM to 80 nM can be detected easily on one structured cellulose/TMSC slide. The fluorescence intensity of a patch incubated with 80 nM labeled DNA_{comp} can easily be distinguished from the background signal. The inset in Figure 6 shows a structured cellulose slide (16 cellulose patches) with hybridized labeled DNA_{comp} of different concentrations (10 µM to 80 nM). Before hybridization the upper row was modified with unlabeled DNA coupled to CMC in the presence of EDC·HCl, whereas the lower row only contained a mixture of uncoupled CMC and DNA (in the absence of EDC·HCl). After hybridization complementary labeled DNA_{comp} was only bound to cellulose patches which contained immobilized CMC-DNA conjugates on the surface. The low unspecific binding of both, labeled and unlabeled DNA to cellulose and the possibility to conduct different

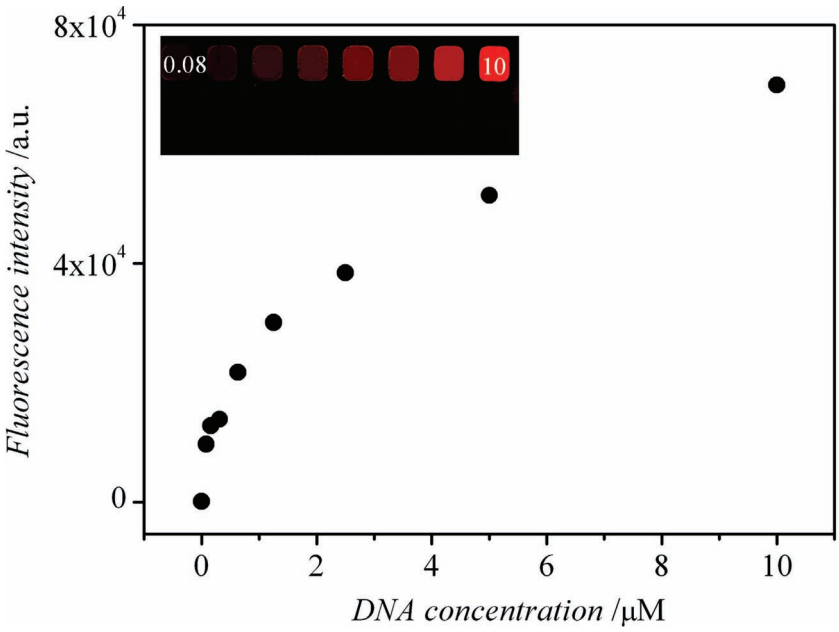


Figure 6. Concentration-dependent fluorescence intensity of labeled DNA, detected on structured cellulose patches.

experiments simultaneously on one sensor slide shows that structured cellulose surfaces have a high potential to be used in the field of biosensors.

4. Conclusion

We were able to manufacture microstructured patterns of pure cellulose and the derivative trimethylsilyl cellulose with a thickness in the nanometer range and a width of micrometers on various substrates. Two approaches were used to produce these structures. In the first approach pure regenerated cellulose thin films were digested by cellulases in combination with a soft-lithographic PDMS-mold. This successfully produced cellulose stripes with a thickness of 35 nm and a width of 50 μm as confirmed by optical thickness and AFM measurements. For a further immobilization or derivatisation of the functionalized and patterned surfaces, successful staining experiments with fluorescein were conducted. In the second method, pure thin films of trimethylsilyl cellulose were successfully patterned by covering the surfaces with metal masks followed by a spatially separated regeneration to pure cellulose. This resulted in cellulose structures that are strongly wetted by water and are reactive towards an isothiocyanate fluorescent dye. For further structuring the cellulose parts could be removed by digestion with cellulases. This treatment produced purely hydrophobic trimethylsilyl cellulose structures, which are not digestible by cellulases and do not react with isothiocyanates. A subsequent regeneration of the trimethylsilyl cellulose produces again cellulose. Both structuring methods can in principle be applied to any silylated polysaccharide, or polymer in general. A combination of diverse enzymes and polymers would allow the production of more complex structures with selective surface properties.

A potential application of structured cellulose surfaces in the field of biosensors arrays was demonstrated by the immobilization of DNA and the subsequent detection of complementary fluorescent labeled DNA. Owing to the low unspecific binding of the structured cellulose surfaces, complementary DNA with a concentration of 80 nM could easily be detected. Therefore the methods develop in this work can be seen as versatile and with a potential application in the field of biosensors and microarrays.

5. Experimental Section

Materials: Trimethylsilyl cellulose (TMSC, DS TMS: 2.55, M_w:175 kDa, M_n:36 kDa) was prepared according to a published literature procedure and dissolved in toluene at a concentration of 10 mg mL^{-1} for the coatings on glass and SiO_2 substrates.[38] For the coating of COP-slides (cyclo olefin polymer) TMSC (20 mg) was dissolved in a mixture of toluene (100 μL) and 2-butanone (900 μL). To this solution 2-butanon (1 mL) was added (final TMSC concentration: 10 mg mL^{-1} solvent). A HCl solution (10 wt.%) was prepared by dilution of concentrated HCl (37 wt.%, Roth, Germany) with Milli-Q water. Cellulase (5 mg mL^{-1}) from *trichoderma viride* (Sigma-Aldrich, Austria) was dissolved in 100 mM sodium acetate buffer at pH 4.8. The solutions for fluorescent labeling were prepared by dissolving 5-([4,6-Dichlorotriazin-2-yl]amino)fluorescein hydrochloride (0.1 mg mL^{-1}, DTAF, Sigma-Aldrich, Austria) in bicarbonate buffer (100 mM, pH 9) and by dissolving tetramethylrhodamine isothiocyanate (0.1 mg mL^{-1}, TRITC, mixed isomers, Sigma-Aldrich, Austria) in pure water. The washing buffer for the COP-slides was composed of 15 mL SSC (saline-sodium citrate, 20 × concentrate, Sigma-Aldrich, product no. S6630), surfactant Tween 20 (600 μL Merck, Germany) and double distilled water (584.4 mL). Sylgard 184 (DowCorning Corp.) silicone elastomer (10:1 mixture of base and curing agent) was used for the fabrication of the PDMS mold. PDMS was casted on a micromilled stainless steel master with lines ranging from 40 to 100 μm spaced by 50 μm (depth 50 μm) and cured for two hours at 80 °C. The aluminum mask for the structured regeneration of TMSC was prepared by drilling 18 equidistant squares (50 × 50 mm^2) into an aluminum slide (75 × 25 × 1 mm^3).

Coating of Substrates: Standard microcope glass slides (Roth, Germany), antireflective Surfs with a SiO_2 surface (Nanolane, France) and COP (Zeonor1060R) slides (SonyDADC, Austria) were used as substrates for the preparation of thin TMSC. Glass and COP slides were cut into pieces of 1 × 1 cm^2, washed with ethanol and double distilled water and dried in a stream of nitrogen. The antireflective SiO_2 Surfs were used as received. The TMSC in toluene (10 mg mL^{-1}) was spin-coated by placing 50 μL of the solution on the static substrates and spinning at 4000 rpm for 60 s at an acceleration of 2500 rpm s^{-1}. For the coating of COP slides (25 × 75 mm^2) 2 mL TMSC solution (10 mg mL^{-1}) in toluene/butanone (1:9, v:v) were dropped on the static substrate and spin coated at 4000 rpm for 60 s and an acceleration of 2500 rpm s^{-1}.

Enzymatic Structuring Method: TMSC on glass, SiO_2-Surfs and COP were completely regenerated by exposure to HCl vapors for 10 min in a 20 mL petri-dish. The regeneration of TMSC to pure cellulose was followed by contact angle and thickness measurements applying the Sarfus technique. A detailed description of the regeneration can be found elsewhere.[39] For the structured hydrolysis of cellulose by cellulases, the PDMS mold was pressed on the cellulose coated substrate with a pressure of 98 kPa. A volume of 2 mL cellulase solution was placed next to the substrate. The enzyme solution immediately

filled the PDMS-channels due to capillary action and wetting of the cellulose substrate. The cellulose film was digested for 120 min at 35 °C. Afterwards the substrates were washed with double distilled water and dried in a stream of nitrogen. For fluorescence staining, the patterned substrates were incubated with 200 µL of 5-([4,6-Dichlorotriazin-2-yl]amino)fluorescein hydrochloride (DTAF) solution (0.1 mg mL^{-1} in 100 mM bicarbonate pH 9) for two hours, and afterwards rinsed thoroughly with double distilled water and dried in a stream of nitrogen.

Structured Regeneration and Enzymatic Digestion: TMSC coated COP slides were covered with an aluminum mask (75 × 25 mm^2, thickness: 1 mm) bearing 16 squared holes (50 × 50 mm^2). The slides were placed into a 1000 mL desiccator containing 200 mL of 10 wt.% hydrochloric acid. The TMSC was regenerated for 10 min and the slides were taken out of the desiccator. 40 µL of a 0.1 mg mL^{-1} TRITC solution was placed on the regenerated cellulose squares and incubated for 1 hour. The slides were thoroughly washed with pure water and dried with nitrogen gas. After that the slides were scanned in the microarray fluorescence scanner. Following this, the cellulose squares were digested with cellulase solutions (5 mg mL^{-1} in 100 mM sodium acetate buffer pH 4.8). For that purpose, 40 µL cellulase solution was placed on the cellulose squares and incubated for one hour at 35 °C. The cellulase solution was exchanged once, and a fresh enzyme solution (40 µL) was dropped on the squares and incubated again for one hour at 35 °C. Following this, the enzyme solution was replaced with 40 µL of pure acetate buffer and incubated for 15 min at 35 °C. The acetate buffer incubation was repeated once. The acetate buffer was twice exchanged against SSC buffer (40 µL, 15 min each) at room temperature. Afterwards the SSC buffer was twice exchanged against pure water (40 µL, 15 min each) at room temperature. Afterwards, the whole slides were rinsed with pure water, dried with nitrogen gas and scanned in the microarray scanner. The slides treated in this way were again exposed to vapors of hydrochloric acid in the desiccator as described above. Staining with a TRITC solution (40 µl, (0.1 mg mL^{-1}) rinsing with pure water and drying with nitrogen was followed by a measurement in the microarray scanner.

Atomic Force Microscopy: Atomic force microscopy (AFM) was performed in tapping mode using an Agilent 5500 AFM multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA, USA). The images were scanned using silicon cantilevers (ATEC-NC-20, Nanosensors, Germany) with a resonance frequency of 210–490 kHz and a force constant of 12–110 N m^{-1}. The scanned image was 100 × 100 μm^2 and structured cellulose samples on COP were measured.

Fluorescence Microscopy and Sarfus Measurements: Fluroescence microcopy was performed with an Olympus BX51 microscope with blue excitation and green emission (Olympus MWIB filter cube). Optical thickness measurements on standard SiO_2 Surfs (Nanolane, France) were performed with a Leica DM4000 polarization microscope with differential interference contrast (DIC). The Sarfus technique was used for that purpose. Sarfusoft (Nanolane, France) software packages were used for recording and processing of the images.

Contact Angle Measurements: Contact angle measurements were conducted on a Dataphysics OCA20+ contact angle measurements system (Dataphysics, Germany). The static contact angles of water were measured using a drop volume of 3 µL.

Microarray Scanning: Microarray fluorescence scanning was performed on a microarray scanner DNAscope LM+ from GeneFocus, USA with green excitation (523 nm) and red emission. The images were colored red by the software.

DNA-Immobilization, Hybridization, and Detection: A specific technique was used for the immobilization of unlabeled single-stranded DNA on structured cellulose coated COP microscope slides. A detailed description of this technique can be found elsewhere.[37] Carboxymethyl cellulose (CMC), DS = 0.7, M_w: 90 kDa, Sigma-Aldrich was dissolved in water (4 mg mL^{-1}) stirred overnight and filtered through a 0.2 µm PFTE syringe filter. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), Sigma-Aldrich was dissolved in water (19.4 mg mL^{-1}). 100 µM single stranded 5′-amino modified DNA (5′ to 3′ sequence: NH_2-AGCAAAAAGCGACGCAATGAG, Microsynth, Balgach Switzerland) was used as received. The CMC, EDC and DNA solutions were mixed in a ratio of 2:1:1 (v:v:v) giving a final molar concentration of CMC carboxylic groups: 6.4 mM, EDC: 25.3 mM and DNA: 25.0 µM. The mixture was reacted for 3 h. As a comparison, mixtures with the same concentrations but without EDC were prepared in the same way.

20 µL of CMC/EDC/DNA mixtures were dropped on one cellulose patch of a structured TMSC coated microscope slide and incubated for 60 min. 8 patches in one column were modified in this way. 20 µL of mixtures without EDC were applied on the cellulose patch in the second column of one slide and incubated for 60 min. The solutions on each slides were exchanged against 40 µL washing buffer (15 mL SSC (sodium saline citrate—0.3 M sodium citrate, pH 7.0, 3 M NaCl), 600 µL surfactant Tween 20 and 584.4 mL pure water) and incubated for 15 min. This washing procedure was repeated twice. Afterwards each patch was incubated with 40 µL pure water for 15 min. This procedure was repeated twice. Finally the slides were blow dried with nitrogen. All experiments were done at room temperature.

Complementary Cy3-labeled DNA (5′-3′sequence CTCATTGCGTCGCTTTTTGCT, Microsynth, Balgach Switzerland) with a concentration of 10.0, 5.0, 2.5, 1.25, 0.63, 0.31, 0.16, and 0.08 µM in a hybridization buffer (20 × SSC: 46.8 mL, blocking reagent (bovine serum albumin): 0.93 g, Tween 20 undiluted: 187.5 µL, 1 M NaH_2PO_4: 75 mL, water: 28.02 mL) was used for hybridization. 20 µL of each DNA concentration was placed on the cellulose patches. The slides were hybridized for 1 h at 50 °C. The DNA was exchanged against 40 µL washing buffer for 15 min at 50 °C. The washing was repeated twice. The patches were further incubated with 40 µL pure water (15 min) at room temperature. This procedure was repeated twice. Finally the slides were blow dried with nitrogen. The slides were scanned with a microarray scanner before DNA immobilization and hybridization as described above.

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