Biocompatible, sustainable coatings based on photo-crosslinkable cellulose derivatives

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Abstract

Materials derived from renewable resources have great potential to replace fossil-based plastics in biomedical applications. In this study, the synthesis of cellulose-based photoresists by esterification with methacrylic acid anhydride and sorbic acid was investigated. These resists polymerize under UV irradiation in the range $\lambda=254$ nm to 365 nm, with or even without the use of an additional photoinitiator concerning the sorbic acid derivative. Usability for biomedical applications was demonstrated by investigating the adhesion and viability of a fibrosarcoma cell line (HT-1080). Compared to polystyrene, the material widely used for cell culture dishes, cell adhesion to the biomaterials tested was even stronger, as assessed by a centrifugation assay. This is all the more remarkable since chemical surface modification of cellulose with methacylate and sorbic acid allows direct attachment of HT-1080 cells without the addition of protein modifiers or ligands. Furthermore, cells on both biomaterials show similar cell viability, not significantly different from polystyrene, indicating no significant impairment or enhancement. This will allow the future use of these cellulose derivatives as support structures for scaffolds or as self-supporting coatings also for cell culture, based solely on renewable and sustainable resources.
Keywords

photo-crosslinkable cellulose derivative; biocompatibility; HT1080

1 Introduction

The application of scaffolding materials combined with stem cell technology in regenerative medicine holds on enormous potential for tissue regeneration. 3D scaffolds are commonly fabricated using natural and synthetic polymers, such as cellulose, gelatin, chitosan, methacrylated hyaluronic acid or pullulan, collagen, polyglycolic acid (PGA), and polycaprolactone (PCL).[1, 2] Cellulose is a common and important biopolymer which is naturally available in almost unlimited quantities and meets the need for a green and biocompatible product.[3] In order to form the target tissue or even an organ, the scaffold should support the infiltration, growth, differentiation and proliferation of cells.[4, 5] Compared to other materials such as polystyrene (cell culture treated) [6], cells show a low affinity for adhesion to the hydrophilic material cellulose.[7, 8] Accordingly, at least the addition of matrix ligands such as FBS seems to be beneficial or even mandatory to facilitate cell adhesion on unmodified natural cellulose scaffolds.[7, 9]

Using above mentioned polymers, scaffolds are produced employing additive manufacturing methods, which are mostly based on “layer-by-layer” techniques, including the limitations associated with this method.[10] For example, stereolithography is one of the oldest laser-based rapid prototyping tool, which fabricates 3D architectures “layer- by- layer”,[11] but the minimum size resolution, the production speed and the accuracy of such structures are limited. Two photon polymerization (2PP) however offers a higher accuracy and enables a smaller minimum size resolution.[11] Furthermore, computer-assisted laser-based fabrication
techniques such as direct laser writing by two-photon polymerization (2PP-DLW) enables the fabrication of 3D scaffolds with highly ordered geometries to mimic natural 3D cell environments.[12]

Recently, the application of a cellulose-based photosensitive material was reported in direct laser writing with a resolution less than 1 µm.[13] Cellulose functionalized with methacrylic acid anhydride was polymerized using a photoinitiator and opens the door for a new class of bio-based photoresists based on cellulose and an application in medicinal scaffold creation.[13] The possibility of crosslinking these cellulose-based photoresists also via UV curing (1 photon absorption) makes these materials suitable for other additive manufacturing techniques and the application as bio-based coatings. The crosslinking reaction is usually initiated by a photoinitiator, which absorbs the incident light. Accordingly, the initiator molecule is cleaved and provides the reactive species. The need for a photoinitiator causes various undesirable issues such as tinting of transparent polymeric films or reduced long-term stability of the resist due to degradation.[14] As this can also lead to toxic residuals of the initiator in the crosslinked polymer, there is strong interest in developing photoresists which do not rely on a photoinitiator to start a polymerization reaction.[15, 16]

Here, we report for the first time a cellulose-based photosensitive material with a self-polymerization effect using sorbic acid (SORCA). Sorbic acid is introduced as a functional side group directly onto the cellulose diacetate (CDA) backbone in order to provide a conjugated carbon double bond system, which enables a self-initiated crosslinking upon UV irradiation. Sorbic acid is a food additive and can be obtained environmentally sustainable from the fruits of Sorbus species (e.g. Sorbus aucuparia L.).[17] Furthermore, the biocompatibility of the methacrylated cellulose diacetate
(MACA) and SORCA was investigated using adhesion and viability of the fibrosarcoma cell line (HT-1080).
2 Material and Methods

2.1 Synthesis of the photo-crosslinkable polysaccharides

Cellulose diacetate 1.2 g (pure, Carl Roth, Germany) was dissolved in 40 mL dichloromethane (DCM, 98%, Alfa Aesar, Germany) under permanent stirring. Subsequently, 1.65 g of sorbic acid (99%, Alfa Aesar, USA) or 2 mL of methacrylic acid anhydride (94%, Sigma-Aldrich, Germany), respectively, 0.017 g of 4-dimethylaminopyridine (DMAP, 99%, Acros Organics, USA) and 2.1 g of dicyclohexylmethanediimine (DCC, 99%, Alfa Aesar, Germany) were added to the solution. The reaction was allowed to proceed for 72 h at room temperature. MACA was precipitated into an excess of ethanol (99.5%, Chemsolute, Germany), while SORCA was filtrated through an 8-12 µm pore size filter paper (VWR, France) prior to precipitation. Subsequently, both derivatives were washed with ethanol using a centrifuge for five times. The product was obtained as a powder after evaporation of the ethanol. All chemicals were used as received without further purification.

2.2 Nuclear magnetic resonance spectroscopy

$^{13}$C NMR spectra were recorded on a Jeol ECS-400 NMR (Japan) with at least 2048 scans. The evaluation was performed by Delta v 5.0.4 software (Jeol). Samples were analyzed in d$_6$–acetone at 25 °C.

Fourier transform infrared spectroscopy:

Fourier transform infrared (FTIR) spectra were recorded on the powder of the cellulose derivatives using a Frontier MIR spectrometer (L1280018) performed with an attenuated total reflection (ATR) diamond (PerkinElmer, Germany). The spectra were recorded between 4000 and 400 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ and at least 8 scans.
2.3 Elemental analysis

The elemental composition of the biopolymers was measured using a Euro EA-Elemental Analyzer (Hekatech). Therefore, 1-3 mg powder in tin crucibles was analyzed and enables the calculation of the degree of substitution (DS).

2.4 Preparation of the photoresist/bio-based coating

The initiator-free photoresist is composed using 0.125 g of SORCA dissolved in 2.5 mL acetone and stirring under dark ambient conditions. To produce the MACA-based resist, 0.137 g MACA was dissolved in 3 mL of acetone. Subsequently, 3.3 mg of the photoinitiator Irgacure 2959 (2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, 98%, Sigma-Aldrich, Italy) was added and the reaction mixture was stirred for additional 30 minutes.

2.5 Measurement of absorption and fluorescence

130 µL of the photoresist were coated onto a glass substrate and cured for 1h under an UV lamp (Herolab UV lamp 8 watt, Germany). The crosslinked biopolymers were detached as films. For measurement, the foils were glued into a cuvette without side parts and then measured in the Clariostar (BMG, Germany) to determine the absorbance (λ=220-1000 nm, resolution 5 nm) and excitation spectra (Focal height 12.2 mm, Gain: 1000, Excitation range: 320-10-498-10, Emission 524-12).

2.6 2D-Scaffold preparation

Different cell culture plates (black, Brand, Germany, black clear bottom, Thermo, Germany) were first treated with 6 µL of acetone per well, followed by a short evaporation step. The biopolymer coatings were then applied by adding 10 µL of the photoresist to each well. After drying for 24 h at 20 °C, the resist was exposed to UV light (Herolab UV lamp 8 watt, Germany) for 1 h, followed by a second coating step to
obtain a smoother surface. Although the polymerization was activated by UV light, the plates were sterilized with ethanol before being transferred to the safety workbench. The plates were then washed with ethanol and PBS (phosphate buffer saline) and again sterilized by UV treatment for 1 h (original Hanau Quarzlampen GMBH, model: 306 A, Art. No. 53220, Germany). The coatings were hydrated by the addition of PBS immediately before the cell experiments were performed.

2.7 Cell culture
HT-1080 fibrosarcoma cells (human) were purchased from CLS (Germany). Cells were grown in Dulbecco’s modified Eagle medium (Thermo Fisher Scientific, Germany) containing 10% fetal bovine serum to a maximum confluence of 85%. Cells were passaged for experiments using Accutase (Gibco, REF: A11105-01) at a ratio of 1 to 3. Cell numbers were determined using the cell counter CASY TT (OLS-OMNI life science, Germany) with the following method settings. Capillary: 150 µm, Cycles: 3, Dilution: 1:100, evaluation cursor (CL: 12.10 µm, CR: 40.00 µm), normalization cursor (NL: 7.30 µm, NR: 40.00 µm), sample volume 400 µL.

2.8 Calcein staining, linearity of cell number and centrifugation assay
Polystyrene (PS) tissue culture plates (black, Brand, Germany) were coated as described above. For experiments, passages from 2 to 19 after thawing were used. Cells grown on 10 cm tissue culture dishes (Sarstedt, REF: 83.3902) were rinsed with PBS (Thermo, Gibco) and stained by incubation with 2.5 mL PBS containing 2 µM Calcein-AM (Sigma-Aldrich, Germany) and 2 mM dextrose (Sigma-Aldrich, Germany) for 20 minutes at 37° C. PBS was aspirated and the cells were detached using Accutase (Thermo Fisher Scientific, Germany). The cells were resuspended in PBS containing 2 mM dextrose. After centrifugation (Hettich, Germany Rotina 420R, 1000 rpm, 20 °C, 5 minutes). 100 µL of the cell suspension (1.6x10^5 cells/mL) were
added to the wells of the coated plate. The plate was incubated for 4 hours and then equilibrated for 30 minutes at room temperature. After replacing the buffer with PBS the initial fluorescence measurement was performed ($\lambda_{\text{ex}}$ 485 nm, $\lambda_{\text{em}}$ 535 nm; flashes 20) using the CLARIOstar (BMG, Germany). The wells were filled with PBS to a total volume of 380 µL, sealed with a transparent qPCR film (VWR, Germany) and centrifuged bottom up. After centrifugation (Hettich, Germany, Rotina 420R, 12 RCF, 24 °C, 5 min) the sealing was removed, the PBS was carefully replaced with 100 µL of PBS and a second fluorescence reading was performed. The adhesive fraction was obtained by normalizing the data of the second measurement to the values of the first (pre centrifugation) measurement.

\[
\% \text{ cell attachment} = \frac{\text{fluorescence intensity after centrifugation}}{\text{fluorescence intensity before centrifugation}} \times 100
\]

### 2.9 Viability assay

For the fluorescence cell-viability assay, a coated 96-well tissue culture plate (Brand, Germany, black) was treated with UV light for 1 h and rinsed twice with PBS. Cells were seeded at a density of $3.75 \times 10^4$ cells/well and incubated for 24 h at 37° C. Afterwards the medium was aspirated and the cells were stained, by incubation for 20 min with 100 µL PBS per well, containing 2 µM calcein-AM (Sigma-Aldrich, Germany) and 2 mM dextrose (Sigma-Aldrich, Germany). The staining solution was aspirated and replaced with PBS containing 2 mM dextrose and measured using the fluorescence reader CLARIOstar (BMG, Germany) (Gain: 1500, $\lambda_{\text{ex}}$ 483-14 nm, $\lambda_{\text{em}}$ 530-30 nm, $\lambda_{\text{dichroic}}$ 502.5 nm, focal height: 5.7 mm).

### 2.10 Morphology

A coated tissue culture plate (Eppendorf, Germany cell imaging plate) was used. HT1080-cells were seeded in a density of $3.0 \times 10^4$ cells/mL and grown for 24 h. Light
microscopy images were taken using a Zeiss Axio (Germany) with phase contrast and 10x objective and Axiocam 503 and ZenBlue 3.2

2.11 Statistical analysis

OriginPro 2021b (64-bit) SR2 9.8.5.212 was used to perform a one-way analysis of variance (ANOVA) on the data set to determine statistically significant differences between samples at confidence levels of p<0.001 (***) , p<0.01 (**) and p<0.05(*) .

3 Results and Discussion

3.1 Characterization of the biocompatible materials

Cellulose is a well-known and established material for biomedical applications due to biocompatibility. In this study, cellulose was used as starting polymer for materials with improved cell attachment compared to other conventional materials. To ensure that the cellulose diacetate can be photo-crosslinked, unsaturated side groups are introduced directly onto the cellulose backbone during synthesis. Methacrylic acid anhydride and sorbic acid were used to functionalize CDA, resulting in the derivatives methacrylated cellulose diacetate and sorbic cellulose diacetate (Figure 1).

The degree of substitution (DS) of the cellulose derivatives was investigated by elemental analysis and revealed a DS of 2.0 for the cellulose diacetate. The DS was determined to be 0.4 and 0.3 for the methacrylic and sorbic acid moieties. The reaction yields obtained were about 95% for MACA and 70% for SORCA.
Figure 1: Schematic depiction of the synthesis routes of MACA (above) and SORCA (below). R = H, acetate, methacrylate or sorbic acid moiety depending on the DS.

Comparative Fourier Transform Infrared Spectroscopy analyses of the educt CDA and the products MACA and SORCA confirmed the successful esterification of CDA with the unsaturated anhydride and acid (Figure 2). The absorption band at 1738 cm\(^{-1}\) is assigned to the carbonyl C=O ester stretching vibration. [13, 18] Other significant vibrations of CDA are located at 1367 cm\(^{-1}\) and 1216 cm\(^{-1}\) referring to the methyl in-plane bending vibration and the C-O stretching vibration of the acetyl moiety. [13, 19]

Methacrylation results in further absorption bands at 1638 cm\(^{-1}\), 950 cm\(^{-1}\) and 813 cm\(^{-1}\), which are assigned to the alkene C=C stretching vibration and the C=CH\(_2\) out-of-plane deformation vibration of the methacrylate group. [13, 20] Accordingly, functionalization of the CDA with sorbic acid, led to additional absorption bands at 1644 cm\(^{-1}\), 1617 cm\(^{-1}\), 957 cm\(^{-1}\) and 801 cm\(^{-1}\). These bands correspond to the alkene C=C stretching vibration [21-23] and the C=CH out-of-plane deformation [23, 24] of the conjugated carbon-carbon double bonds. Comparing the spectra of CDA with those of MACA and SORCA at 3485 cm\(^{-1}\) (stretching vibration of the hydroxyl groups), a decrease is noticeable,
which is due to the substitution of the hydroxyl groups by unsaturated moieties during the syntheses. [13, 18, 19] Since the absorption band of the acetyl ester around 1740 cm$^{-1}$ overlaps with the ester bonds of the unsaturated group, a direct monitoring is not possible. [13] MACA can be crosslinked via one-photon and two-photon absorption, as previously reported [13] leading to a decrease or disappearance of the signals related to the C=C bonds at 1638 cm$^{-1}$, 950 cm$^{-1}$ and 813 cm$^{-1}$.

![FTIR spectra of CDA, MACA and SORCA.](image)

**Figure 2:** FTIR spectra of CDA, MACA and SORCA. For better readability, the spectra are vertically shifted.

SORCA, synthesized from two natural products - cellulose and sorbic acid - can also be crosslinked using a photoinitiator, similar to MACA. More remarkable however is the self-crosslinking property of SORCA, confirmed by several FTIR spectra recorded during the crosslinking reaction induced by UV light. Figure 3 illustrates the decreasing or even vanishing of the alkene absorption bands during UV induced photocrosslinking. Using sorbic acid, conjugated double bonds were introduced into the cellulose-based material.
Figure 3: FTIR spectra of SORCA films, which were crosslinked for 0h, 2h, 72h and 21d via UV irradiation without photoinitiator. The spectra are vertically shifted for better readability.

These conjugated systems cause the absorption of UV light triggering the crosslinking via an activated radical species.[17, 25] This radical species probably reacts with a double bond of a surrounding sorbic moiety leading to an inter-chain crosslinking. [26, 27]

Table 1: Signal assignment $\delta$ for the $^{13}$C NMR spectra of the cellulose derivatives recorded in $d^6$-acetone.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Assignment $\delta$ (ppm)</th>
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<tbody>
<tr>
<td>C=O</td>
<td>C=O</td>
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<tr>
<td>(CDA)</td>
<td>(MACA/ SORCA)</td>
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<tr>
<td>C=O</td>
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<tr>
<td>(CDA)</td>
<td>(MACA/ SORCA)</td>
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<tr>
<td>C=C</td>
<td>C1–C6</td>
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<tr>
<td>(CDA)</td>
<td>(MACA/ SORCA)</td>
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<tr>
<td>$\text{CH}_3$</td>
<td>$\text{CH}_3$</td>
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<tr>
<td>170.1</td>
<td>100.4 – 62.3</td>
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<tr>
<td>–</td>
<td>20.0</td>
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A further characterization of the material was achieved using NMR spectroscopy. Characteristic signals obtained from the $^{13}$C NMR experiment for CDA show chemical shifts of 170.1 ppm and 169.3 ppm assigned to the acetate ester (Table 1). The signals at δ 20.0 ppm and 19.8 ppm represent the methyl group of the acetyl side group. The doublets of the ester signal as well as the methyl group indicate the presence of two hydroxyl groups substituted by acetate groups, confirming cellulose diacetate as educt.[28, 29]

The methacrylation is verified by additional signals at δ 166.3 ppm, 136.3 ppm, 125.9 ppm and 17.6 ppm compared to the $^{13}$C NMR spectrum of CDA.[13] The ester signal at δ 166.3 ppm confirms the successful reaction of the methacrylic acid anhydride with CDA. Furthermore, the signals at δ 136.3 ppm and 125.9 ppm represent the carbon double bond and the signal at 17.6 ppm can be assigned to the methyl group.

Similarly, the ester signal at δ 166.1 ppm verifies the functionalization of CDA with sorbic acid. Sorbic acid contains two conjugated carbon-carbon double bonds, accordingly four corresponding signals at δ 118.4 ppm, 129.7 ppm, 140.3 ppm and 145.7 ppm were detected. The signal at δ 18.1 ppm can be assigned to the methyl group of the unsaturated acid, according to data already published. [30-33] Therefore,
the evaluation of the $^{13}$C NMR spectra validates a successful modification of CDA with methacrylic as well as sorbic side groups.

The solubility of both materials in different solvents has been investigated to give an indication of the different applications. MACA and SORCA are soluble in various organic solvents such as acetone, ethyl acetate, dimethylsulfoxide, tetrahydrofuran, dimethylformamide, dichloromethane, acetonitrile and dimethylacetamide. However, the crosslinked polysaccharide-based coatings are insoluble in commonly used organic solvents such as those mentioned above.

Furthermore, absorption and emission spectra of the materials investigated were recorded, also concerning interference in following biocompatibility measurements. Therefore, the side walls of a cuvette were removed and a foil of the coating was stuck as one sidewall of the cuvette. Due to the experimental settings, no thickness and pathway length could be specified and consequently the results can only be considered qualitative. The absorbance maxima of all materials were shown at $\lambda=230$-$320$ nm. Scanning of the emission spectra with $\lambda_{ex}=330$-$28$ nm, the lowest value measurable with the instrument used, showed no fluorescence in the range of $\lambda=400$-$600$ nm (Figure 4). The biomaterial studied shows only low autofluorescence and even less in the range up to $\lambda=600$ nm, which is probably due to the absence of aromatic chromophores. Low autofluorescence of photoresists is preferable to reduce the signal-noise ratio concerning imaging experiments [34].
Figure 4: A) absorption spectra of different coatings B) emission spectra of different coatings $\lambda_{ex} = 330$ nm ($\delta = 28$ nm).

3.2 Biocompatibility of materials

Eukaryotic cells show a moderate affinity to unmodified cellulose [7], but good cell adhesion is required using the coatings investigated in tissue regeneration. Furthermore, the direct attachment of cells performing the cell-specific phenotypic function is a key challenge in tissue engineering [35]. The adhesion of cells to the biomaterials was determined using a modified centrifugation assay [36] based on the method of Reyes et al. [37]. HT1080 fibrosarcoma cells seeded on the different coatings were exposed to shear stress via centrifugation and normalization of the post centrifugation cell count to the original seeding counts yields the percentage of cells remaining. Quantification of cell number pre and post centrifugation was possible using Calcein-AM as fluorescent staining agent. Living cells convert the nonfluorescent Calcein-AM to a green fluorescent Calcein by intracellular esterases [38]. Accordingly,
this cell-permeant dye is suitable to determine cell viability [39] of eukaryotic cells as well as relative cell number in centrifugation assay used. To exclude interference of the biomaterial with the readout, the fluorescence of coated wells without cells and medium was measured at an excitation wavelength of $\lambda_{\text{ex}}=496$ nm and an emission wavelength of $\lambda_{\text{em}}=516$ nm, which are the characteristic wavelengths for Calcein measurement. No significant change in emission intensity was observed but nevertheless, the values without cells were subtracted from the values measured. To quantify the cells pre and post centrifugation, the response of cell number used should be described by a linear fit. [37] The response at which the fluorescence values show a linear dependence both pre and post centrifugation was determined to be between $1.07 \times 10^5$-$2.03 \times 10^5$ cells/mL (Figure 5).

\[ \text{Figure 5: A) Cell number vs fluorescence intensity measured via Calcein-AM staining before centrifugation. B) Cell number vs fluorescence intensity measured via Calcein-AM staining after centrifugation (n=3).} \]

Accordingly, $1.6 \times 10^5$ cells/mL were seeded in coated 96 well plates for the following experiments. After 4 hours incubation at 5% CO$_2$, 95% humidity and a temperature of 37 °C the initial fluorescence values were determined. The fluorescence values prior to centrifugation did not differ significantly between the different materials, but a wider
spread of the values was observed (Figure 6). Due to the handmade coating, the surface of the biomaterials is apparently not as smooth as the standardized surface of industrially manufactured polystyrene.

**Figure 6:** Fluorescence intensity measured via Calcein-AM staining of different coatings (MACA, SORCA) and polystyrene (PS) as reference polymer before centrifugation **p<0.01** (one-way ANOVA) (n=3).
Figure 7: Fraction of adherent cells on different coatings (MACA, SORCA) and as reference polymer polystyrene (PS) 4 hours after seeding in relation to cell number measured before centrifugation, measured via Calcein-AM staining. *** p<0.001 (one-way ANOVA) (n=3).

The fluorescence intensity post centrifugation was normalized to the fluorescence intensity pre centrifugation. Normalization seems to be all the more important since various experiments have shown that surface roughness and topology are some of the many factors influencing the cellular response concerning attachment. [40, 41] Additionally, the signal was normalized to the fluorescence intensity of polystyrene (Figure 7). Cells show a moderate affinity for unmodified cellulose [7], however the modification introduced, led to a two fold improvement in cell attachment as observed using polystyrene (cell culture treated). Noteworthy, the addition of fetal bovine serum
(FBS) and other matrix ligands is not required to facilitate cell attachment, as described using native cellulose. [7, 9]

Adhesion was assessed after 4 hours, and the next step was to determine whether the material had an effect on cell viability or morphology with longer contact times. Cell viability was measured 24 hours after seeding in media containing FBS to assess whether cells could survive on the coatings for a longer period.

**Figure 8:** Viability of cells grown 24 hours on different coatings relative to polystyrene (PS) using Calcein-AM. ***p<0.001 (one-way ANOVA) (n=3).

The fluorescence values of HT-1080 cells seeded on the coatings were normalized to those on polystyrene (100%). Cells on both biomaterials show similar cell viability, which does not differ significantly to polystyrene indicating neither a significant impairment nor an improvement in cell viability after 24 h. Similar was shown growing
HT-1080 cells on Ormocer® an organic-inorganic hybrid material also suitable for 2PP-induced polymerization. [42]

To additionally verify the results, cells grown on the different coatings were visualized using a microscope (Zeiss Axio, camera: Axiocam 503 color) (Figure 9). The HT-1080 cells adhere to the surface and retain the epithelial morphology. [43] The cells display a typical flat irregular cell shape, which is characteristic for neoplastic cells. [44] Accordingly, cells grown on the coatings seem to have the same morphology compared to cells grown on polystyrene.

![Figure 9: Pictures taken with microscope (Zeiss Axio; objective 10x, phase contrast)](image)

A) PS, B) MACA, C) SORCA.

4 Conclusion

Working towards the development of cellulose-based photoresists for manufacturing tissue engineering scaffold materials, we have demonstrated for the first time that chemical modification of cellulose with sorbic acid can be used for photopolymerization and more remarkable for self-photopolymerization. These non-hydrogel formulations do not swell in water and are processible using additive manufacturing techniques such as direct laser writing. Ligand free attachment of cells without significant influence on viability measured via Calcein-AM Assay and morphology was determined, indicating
biocompatibility of the biomaterial tested. This enables a future implementation of these cellulose derivatives as support structures for scaffolds or as self-supporting coatings for cell culture based on renewable and sustainable resources.

5 Acknowledgement

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6 References


