

Hydrophobic and antibacterial textile fibres prepared by covalently attaching betulin to cellulose

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Abstract Betulin, a natural compound extractable from the outer bark of birch, can be used to improve the properties of cellulosic textile fibres. Herein, oxidation was performed to prepare carboxyl-functionalized cellulose, which was subsequently covalently attached by betulin through esterification. The surface-modified cellulosic textile fibres showed a substantially improved hydrophobicity, as indicated by a water contact angle of 136°. Moreover, the material showed excellent antibacterial properties, as indicated by over 99% bacterial removal and growth inhibition, in both Gram-positive and Gram-negative bacterial assays. The method of surface-modification of the cellulosic materials adapted in this study is simple and, to the best of our knowledge, has not been carried out before. The results of this study prove that betulin, a side-stream product produced by forest industry, could be used in value-added applications, such as for preparing functional materials. Additionally, this modification route can be envisaged to be applied to other cellulose sources (e.g., microfibrillated cellulose) to achieve the goal of functionalization.

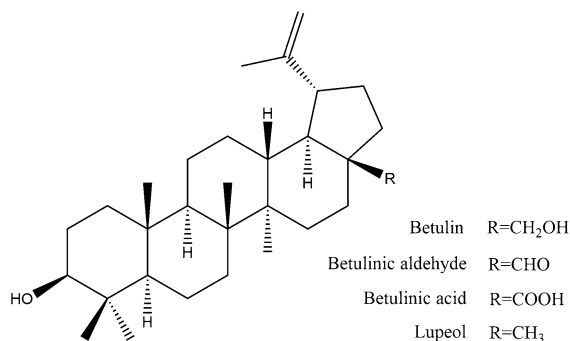
Keywords Antibacterial property · Betulin · Cellulose · Hydrophobicity · Surface modification

Introduction

Betulin (Scheme 1) is one of the main components of the extract from the outer bark of the birch tree. Up to 30% of the dry weight of birch bark extract is betulin, which gives the tree bark its white colour (Krasutsky 2006). Betulin can readily be obtained by solvent extraction with various organic solvents, such as ethanol and heptane (Cao et al. 2007). In addition to the conventional methods of obtaining betulin, it has also been reported that betulin could be extracted by supercritical carbon dioxide (SC-CO₂) (Yu-hong et al. 2003), classical reflux boiling and pressurized liquid extraction (Fridén et al. 2016). Recently, more sustainable approaches that utilize green solvents such as pine monoterpenes and limonene to extract purer betulin have been reported (Grazhdannikov et al. 2018).

Due to its high crystallinity, betulin can cause deposition problems in the papermaking process, as the kraft digestion process cannot fully dissolve betulin (Sharoyan 2011). Therefore, to minimize problems, the removal of betulin by efficient debarking is necessary. The debarking process generates a tremendous amount of birch bark, containing

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Scheme 1 Structure of major chemicals from birch bark extractive

abundant betulin. However, currently, the peeled bark is typically incinerated to provide thermal energy (Lu et al. 2006). According to the biomass value pyramid established elsewhere (Berbel and Posadillo 2018), using natural resources as solid fuels is ranked as the lowest valuable action, while production of chemicals or materials based on biomass can increase the value. This has motivated us to explore the possibility of using betulin in value-added applications, rather than only for energy production. Betulin has anti-inflammatory, anticancer and antimicrobial properties, and thus it has been used in the production of pharmaceuticals and medicines (Fălămaș et al. 2011; Fridén et al. 2016; Yu-hong et al. 2003). In our previous study, it was reported that betulin and its derivatives could be physically adhered onto cellulosic textile fibres to improve their hydrophobicity and water repellency (Huang et al. 2018). Considering these two aspects, we believe it is possible to fabricate a betulin-attached cellulosic textile with improved hydrophobicity as well as antibacterial property. However, the former product from our previous study is a kind of textile with physically adhered betulin particles. The betulin particles on that textile can be liberated when the textile is placed in an aqueous environment, such as by immersion, or by exposure to solvent extraction (Huang et al. 2018). Therefore, that textile cannot be used in antibacterial assays, which needs the textile sample to be immersed in solutions. Based on this, we hypothesize that by creating covalent bonds, which act as a binder, to attach betulin onto cellulosic textile may hinder betulin from being liberated in case of sample washing or solution-based assays.

In this study, cellulosic textiles were first oxidized by 2,2,6,6-tetramethylpiperidine-1-oxy radical

(TEMPO)-mediated oxidation to selectively convert the primary alcohol groups located on the surface of cellulose fibrils or fibril aggregates to carboxyl groups, as described elsewhere (Marković et al. 2017; Saito et al. 2009). Betulin was then covalently attached onto the carboxyl-functionalized cellulose through tartaric acid-catalysed esterification (Hafrén and Córdova 2005; Hafrén et al. 2006; Zhao et al. 2010).

Oxidation and esterification were verified by Fourier transform infrared (FTIR) analysis. The mechanical properties of the prepared materials were tested by a tensile test. Scanning electron microscopy (SEM) was used to obtain surface morphology images. The hydrophobicity and antibacterial properties were the main characteristic properties of the prepared materials and were determined by static water contact angle measurements and antibacterial assays, respectively.

Experimental

Materials

Betulin was Soxhlet extracted from the outer bark of birch (*Betula verrucosa*) with n-heptane as solvent for 48 h and was obtained as a white powder. The purity of the betulin was 80%, as determined by quantitative ¹H NMR with TMS as internal standard. Commercial cellulosic textiles (100% cotton, Hemtex, Stockholm, Sweden) were used as the source of cellulose. The solvents and chemicals were used as received without further purification: tetrahydrofuran (THF, 99.99%), 1 M hydrochloric acid (HCl), toluene (≥ 99.5%), phenolphthalein, sodium hypochlorite solution (NaClO, 10%), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 98%), sodium chlorite (NaClO₂, 80%), acetic acid (CH₃COOH, ≥ 99.7%), sodium acetate anhydrous (CH₃COONa, ≥ 99%), sodium borohydride (NaBH₄, ≥ 98%), glutaraldehyde (50 wt%) (Sigma-Aldrich); sodium hydroxide (NaOH) (VWR International); L(+)-tartaric acid (Scharlau Chemicals).

Pretreatment of cellulosic textiles

The cellulosic textile (approximately 10 mg cm⁻²) was washed by an Electrolux EWC1350 laundering machine at 40 °C for 2 h with added non-bleaching washing powder to remove contaminants.

Subsequently, the cellulosic textile was washed with 2 g L^{-1} of NaOH solution at $98 \text{ }^\circ\text{C}$ for 90 min, followed by washing with distilled water. Afterwards, the cellulosic textile was dried at $105 \text{ }^\circ\text{C}$ overnight (Wang et al. 2006). Finally, three ($18 \text{ cm} \times 18 \text{ cm}$) pieces of cellulosic textile samples were cut out, and designated as Ref. cotton.

TEMPO-mediated oxidation and NaBH_4 reduction

The TEMPO-mediated oxidation of cellulose was performed as described elsewhere (Marković et al. 2017; Saito et al. 2009) with minor changes. A 3 g sample of Ref. cotton was added to 150 mL of 0.05 M acetate buffer (pH 4.5) and 60 g of TEMPO (0.38 mol) and 150 g of sodium chlorite (1.66 mol) were dissolved in the mixture. A total of 9.3 mL of 10% sodium hypochlorite solution (15 mmol) was added to the mixture, and the mixture was stirred at 500 rpm and $60 \text{ }^\circ\text{C}$ for a predetermined time of 15, 45 or 180 min to obtain three oxidized cellulosic textile samples. Next, the mixture was cooled to room temperature, followed by thoroughly washing with deionized water by filtration. A 2.5% NaBH_4 solution (pH adjusted to approximately 9 with 0.5% Na_2CO_3) was used to treat the washed textile samples for 2 h to reduce any trace ketone groups that had formed at the β -D-glucopyranose C2/C3 position to alcohol groups (Mishra et al. 2012; Tanaka et al. 2016). After the reaction, the reduced textile samples were thoroughly washed with deionized water by filtration. The sodium carboxylate groups in the reduced textile samples were protonated by immersing the textile sample in an HCl solution (pH 2) for 1 h and then thoroughly washing with deionized water by filtration. Finally, the oxidized cellulosic textiles, containing free carboxyl groups, were air-dried overnight and stored for further use. These samples were denoted as 15COOH, 45COOH and 180COOH, according to the oxidation times of 15, 45 and 180 min, respectively (Table 1).

Tartaric acid-catalysed esterification

Tartaric acid-catalysed esterification was performed as described elsewhere (Zhao et al. 2010). The oxidized cellulosic textile (approximately 100 mg) was immersed in 10 mL of betulin solution in toluene with a concentration of 0.16 mol L^{-1} , 25 mg of tartaric acid (0.16 mmol, 10 mol%) and activated

molecular sieves. The reaction was carried out at $110 \text{ }^\circ\text{C}$ for designated times of 6, 24 or 72 h for each type of oxidized textile to obtain 9 different betulin-grafted textile samples, as shown in Table 1. After cooling to ambient temperature, the cellulosic textiles were extracted (Soxhlet) using THF first then with water, followed by air dry overnight prior to further use.

Fourier transform infrared (FTIR) analysis

FTIR spectra of all samples were recorded on a Perkin-Elmer Spectrum 2000 FTIR equipped with a heat-controlled single-reflection attenuated total reflection (ATR) accessory (Golden Gate heat-controlled) under absorbance mode. Each spectrum was recorded between 4000 and 600 cm^{-1} with 16 scans and 4 cm^{-1} resolutions. All FTIR spectra were normalized to the band from 1320 to 1310 cm^{-1} .

Carboxyl content determination

Carboxyl content (CC) in the oxidized textile samples (15COOH, 45COOH and 180COOH) was determined by an acid–base titration procedure adapted from calcium-acetate methods described elsewhere (Kumar and Yang 2002; Marković et al. 2017; Praskalo et al. 2009; Yackel and Kenyon 1942). Oxidized textile sample (500 mg) was dipped into a solution containing 50 mL of deionized water and 30 mL of 0.5 M sodium acetate solution. After 2 h of ion interchange reaction under constant shaking, 30 mL of the liquid was transferred out and was titrated with 0.01 M sodium hydroxide with phenolphthalein as an indicator. Each measurement was repeated in triplicate, and the carboxyl content (mmol g^{-1}) was calculated using the following equation:

$$\text{COOH} = \frac{80}{30} \times 0.01 \text{ M} \times V(\text{NaOH}) / \left(m \times \left(1 - \frac{w}{100} \right) \right)$$

where 0.01 M is the concentration of NaOH, $V(\text{NaOH})$ is the volume (mL) of NaOH solution used for titration, and m and w are the weight (g) and moisture content (%) of the oxidized textiles added for titration, respectively.

Table 1 Experimental design for the preparation of each sample

	Denotation	Duration of oxidation (min)	Duration of esterification (h)
Original textile	Ref. cotton	–	–
Oxidized textile	15COOH	15	–
	45COOH	45	–
	180COOH	180	–
Betulin-grafted textile	15COOB6	15	6
	15COOB24	15	24
	15COOB72	15	72
	45COOB6	45	6
	45COOB24	45	24
	45COOB72	45	72
	180COOB6	180	6
	180COOB24	180	24
	180COOB72	180	72

Degree of oxidation (DO)

The DO was calculated using the following equation:

$$DO = \frac{162 \times CC}{1 - 14 \times CC}$$

where CC is the carboxyl content (mol g^{-1}), 162 g mol^{-1} is the molecular weight of a β -D-glucopyranose unit in cellulose, and 14 g mol^{-1} is the difference between the molecular weight of a COOH group and that of a CH_2OH group.

Betulin fraction

Betulin fraction (mg/g) stands for the mass of covalently attached betulin per gram of betulin-grafted textile. Betulin-grafted textile was first weighed and then saponified (using 0.5 M NaOH for alkaline hydrolysis at 60 °C for 48 h) to give betulin and textile containing sodium carboxylate groups. The textile was taken out accompanying by rinsing with water. The remaining insoluble betulin was then filtered followed by air drying and weighing. Finally, betulin fraction of each betulin-grafted textile was calculated: Betulin fraction = m_b/m_t , where m_b is weight (mg) of filtered betulin and m_t is weight (g) of betulin-grafted textile prior to saponification.

Tensile test

Tensile properties of betulin-grafted cellulosic textiles in the warp direction were determined using an Instron universal testing machine 5944 (UK) equipped with a 500 N load cell. All samples were conditioned at 23 °C and 50% RH for 24 h prior to tests. The breaking tensile strength and elongation at break of at least three specimens (5 mm × 30 mm) from each sample were tested at 23 °C, with an initial distance between the grips of 10 mm and a separation rate of $1\% \text{ s}^{-1}$. The test results are presented as the mean \pm standard deviation.

Hydrophobicity test

The static water contact angle (WCA) was measured on a KSV Instrument CAM 200-m connected to a Basler A602f camera. A 4 μL sessile water droplet was placed on 5 different positions of the surface, and pictures were taken every 4.8 s for a total period of 120 s. The average contact angle and corresponding standard deviation for each sample were calculated over the 5 positions.

Antibacterial assays

The antibacterial tests were slightly modified from a standard method for antibacterial textiles (ISO 20743). The Gram-negative bacteria *Escherichia coli* (*E. coli*)

K-12 and Gram-positive *Staphylococcus aureus* (*S. aureus*) (BioRad, Solna, Sweden) were incubated in a nutrient broth (Difco, Stockholm, Sweden) with continuous shaking at 37 °C for 18 h, and the cells were harvested by centrifugation at 5000 rpm for 5 min with a bench-top centrifuge (VWR, Stockholm, Sweden). The nutrient broth was exchanged with ¼ Ringer's solution, and the cells were washed twice before final redispersion. The antibacterial effects of the betulin-grafted textile were examined from the aspect of both bacterial removal and bacterial growth inhibition. In the bacterial removal test, 8 mg each of the Ref. cotton and modified textiles (15COOB24, 45COOB72 and 180COOB24 betulin-grafted textile cut approximately 5 mm × 15 mm) were added, as well as the “+” control to Eppendorf mini tubes (maximum 2 mL) along with a bacterial concentration of 2×10^6 CFU mL⁻¹. The samples were then placed in an incubator with continuous shaking at 37 °C for 18 h. After incubation, 0.2 mL of solution from each sample was taken and diluted in 1.8 mL of ¼ Ringer's solution. The solutions were further diluted 3- and 4-fold, and 1 mL of each diluted solution was dropped onto a piece of Petrifilm™ (3 M, Sollentuna, Sweden). The bacterial colony counts were carried out by a particle analyser using ImageJ.

To test the bacterial growth inhibition, 0.2 mL of nutrient broth was added to each textile-bacteria suspension after the bacterial removal test. All samples were incubated for 18 h at 37 °C with continuous shaking. The optical density (OD) of each suspension was determined at $\lambda = 620$ nm on a MultiSkan FC microplate spectrophotometer (Thermo Scientific, Stockholm, Sweden) to estimate the number of bacteria. The true viability was also examined by counting the number of bacterial colonies on the Petrifilm.

Scanning electron microscopy (SEM)

SEM images were captured by a Hitachi S-4800 field-emission scanning electron microscope (JEOL Ltd., Tokyo, Japan). Textile samples of Ref. cotton, 180COOH and 180COOB72 were adhered onto a round metal stud and were coated with a 5 nm platinum layer by a Cressington 208HR high-resolution sputter coater (Cressington, England, UK) that was connected to a Cressington thickness monitor controller. The textile samples incubated with bacteria

were immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C for proper fixation. Thereafter, the specimens were rinsed in water and then solvent exchanged in 50%, 70%, 90%, 96% and absolute ethanol for 10 min, respectively, and finally dried using a critical point dryer. The dried specimens were coated with a 10 nm platinum layer and analysed under the SEM at a voltage of 5 kV.

Results and discussion

Oxidation of cellulose

The FTIR spectra of Ref. cotton, 15COOH, 45COOH and 180COOH are shown in Fig. 1a. The noteworthy absorption band at 1727 cm⁻¹ corresponds to the C=O stretching vibration of carboxyl groups (Habibi et al. 2006) and was clearly identified in each oxidized sample, except in Ref. cotton. This indicates that the primary hydroxyl groups at the C6 positions in cellulose were oxidized to carboxyl groups. The carboxyl content (CC) and degree of oxidation (DO) of 15COOH, 45COOH and 180COOH were determined (Table 2). Both the CC and DO of cellulose slightly increased when the reaction was prolonged from 15 to 45 min. When the oxidation reaction continued to 180 min, the CC and DO increased to a maximum value of approximately 500 $\mu\text{mol g}^{-1}$ and 8.5%, respectively. Results from the FTIR analyses and carboxyl content determinations successfully verified the desired oxidation.

Esterification process

Prior to ion exchange treatment, sample 180COOH was designated as 180COONa, which was also analysed by FTIR, along with sample 180COOH and Ref. cotton (Fig. 1b). The band at 1604 cm⁻¹ due to C=O stretching vibration confirmed the existence of carboxylate groups (Fujisawa et al. 2011), and this band overlapped with the band at 1640 cm⁻¹ arising from a small amount of adsorbed water (da Silva Perez et al. 2003). After the ion exchange treatment, the carboxylate groups were converted to carboxyl groups, and thus the C=O absorption band at 1604 cm⁻¹ was shifted to 1727 cm⁻¹. This fact is useful for identifying the ester bonds that exist among abundant carboxyl groups. Since the C=O absorption

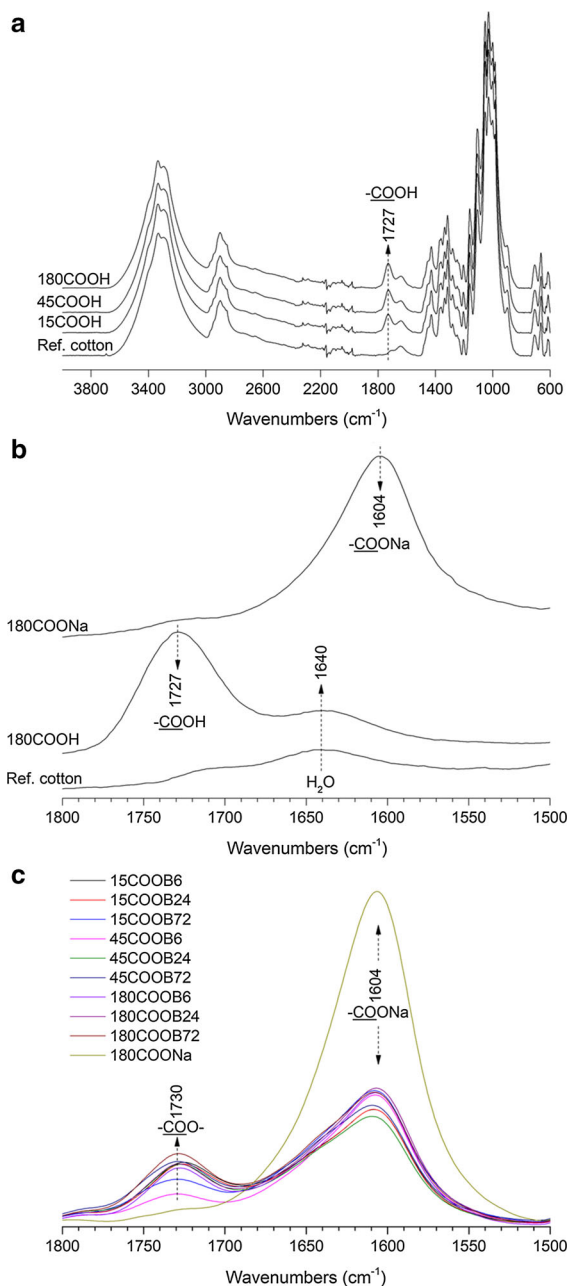


Fig. 1 FTIR absorbance spectra of **a** Ref. cotton and its oxidized products after 15 min (15COOH), 45 min (45COOH), and 180 min (180COOH); **b** Ref. cotton and its 180-min oxidized products before (180COONa) and after (180COOH) ion exchange through an acidic treatment; **c** all betulin-grafted textile samples after alkali treatment ion exchange, as well as 180COONa

band of ester bonds majorly overlaps with that of carboxyl groups at approximately 1730 cm^{-1} , those carboxyl groups should be converted to carboxylate

groups by alkali treatment in order to differentiate the carboxyl bands from the ester bands. The alkali treatment should be carried out with an alkaline solution at low concentration for a short period of time at room temperature to cause ion exchange, but with little hydrolysis. Herein, all 9 betulin-grafted samples (Table 1) were immersed in NaOH solution at pH 10 for 1 h at room temperature, followed by washing thoroughly with water to convert the unconsumed $-\text{COOH}$ groups to $-\text{COONa}$, as described elsewhere (Li et al. 2017). The alkali-treated samples were also analysed by FTIR, along with $-\text{180COONa}$ (Fig. 1c). As seen, after shifting the $-\text{COOH}$ band at 1727 cm^{-1} to a $-\text{COONa}$ band at 1604 cm^{-1} by alkali treatment, the band assigned to the ester bonds at 1730 cm^{-1} is clearly exposed in all betulin-grafted samples, suggesting that the esterification was successful. However, the peak heights of the bands at 1730 cm^{-1} (Fig. 1c) associated with ester bonds is different among those samples. A stronger 1730 cm^{-1} peak indicates a greater number of ester bonds present. Moreover, it is worth noting that the so-called betulin used in esterification is actually the extractive directly obtained from birch bark, and it contains 80% betulin as determined. This purity corresponds well to the average betulin content of birch bark extractive, according reported literatures (Krasutsky 2006). Along with betulin as the major component, other impurities consist the rest 20% of the extractive, such as betulinic acid (4.3%), betulinic aldehyde (1.2%) and lupeol (7.9%) (Fălămaș et al. 2011; O'Connell et al. 1988). It should be emphasized that those impurities are 4–5 times less reactive than betulin in the esterification since they only own secondary hydroxyl groups (Scheme 1) (Jeromenok 2012). The combination of low content and low reactivity of the impurities will result in the dominated involvement of betulin in the esterification. The fraction of covalently attached betulin on each betulin-grafted textile is shown in Table 3.

Surface morphology

Changes in the morphology of textile samples from each step were observed by SEM. Ref. cotton (Fig. 2a) contains longitudinal smooth fibers and rough fibres distributed by tiny grooves at the micrometre level, which is a classic characteristic of pristine cellulosic textile fibre surfaces (Tissera et al. 2015; Xue et al.

Table 2 Carboxyl content and degree of oxidation of oxidized textile samples

Sample	Duration of oxidation (min)	Carboxyl group content ^a ($\mu\text{mol g}^{-1}$)	Degree of oxidation (%)
15COOH	15	343 \pm 2	5.6
45COOH	45	361 \pm 12	5.9
180COOH	180	522 \pm 13	8.5

^aData are presented as the mean \pm standard deviation of three replicates

Table 3 Betulin fraction of betulin-grafted textiles^a

Sample	Fraction (mg/g sample)
15COOB6	N/A
15COOB24	46 \pm 16
15COOB72	91 \pm 21
45COOB6	N/A
45COOB24	70 \pm 10
45COOB72	106 \pm 24
180COOB6	48 \pm 17
180COOB24	95 \pm 14
180COOB72	141 \pm 25

Data are presented as the mean \pm standard deviation of three replicates; N/A: data are not available

^aBetulin fraction stands for weight (mg) of grafted betulin on per gram of betulin-grafted textile

2016). The grooves will lead to surface roughness (Xue et al. 2016) in favour of hydrophobization treatment. Generally, the oxidation was mild, since compared with Ref. cotton (Fig. 2a), no obvious damage was seen on the fibre surface of the oxidized one (Fig. 2b). On the other hand, sample 180COOB72 (Fig. 2c), obtained after a long esterification process, showed some burls or nubs, with dimensions ranging from 0.5 to 1 nm, as seen in the zoomed-in area of Fig. 2c. The burls or nubs were unidentified and require further investigation.

An additional control sample was prepared by dipping a piece of Ref. cotton into a betulin solution that was prepared identically to that prior to the esterification process (0.16 mol L^{-1} , toluene) for 5 min, followed by air drying. This control sample was also analysed by SEM to investigate the original morphology of free betulin particles (Fig. 2d). The fibre surface was densely covered by cylinder-like betulin particles, with an average length of approximately 5 μm . This special cylinder shape of free

betulin particles corresponds well to the findings in our previous study (Huang et al. 2018). Compared with the textile fibres in Fig. 2d that were densely covered by free betulin particles, it can be concluded that in sample 180COOB72, the non-attached betulins were removed to a large extent by Soxhlet extraction, since no remaining betulin particles could be seen.

Wettability

The wettability of each sample after esterification was determined by water contact angle measurements. Only samples 45COOB72, 180COOB24, 180COOB72 and 15COOB72 exhibited measurable water contact angles. Figure 3 shows the water contact angles of the above four samples as a function of time as well as the slide views of the water droplets captured during the test. The water contact angle of 15COOB72 reduced from 128° to 77° , with a steady trend over 2 min. Particularly, the value was lower than 90° after 100 s, indicating that the sample was hydrophilic after that time point. In the case of the five easily wetted samples, perhaps due to an insufficient reaction time of either oxidation or esterification, the amount of covalently attached betulin might be too low to contribute to improving the hydrophobicity of the samples. This could result in the capillary force of textile fibres being large enough to wick water droplets into the samples despite the small amount of hydrophobicity provided by trace levels of grafted betulin (Bae et al. 2009).

By contrast, the static water contact angles of 45COOB72, 180COOB24 and 180COOB72 were generally constant over time (Fig. 3) with similar mean values, suggesting that stable hydrophobicity was achieved. Constructively, higher degree of esterification with betulin leads to higher hydrophobicity on the product surface.

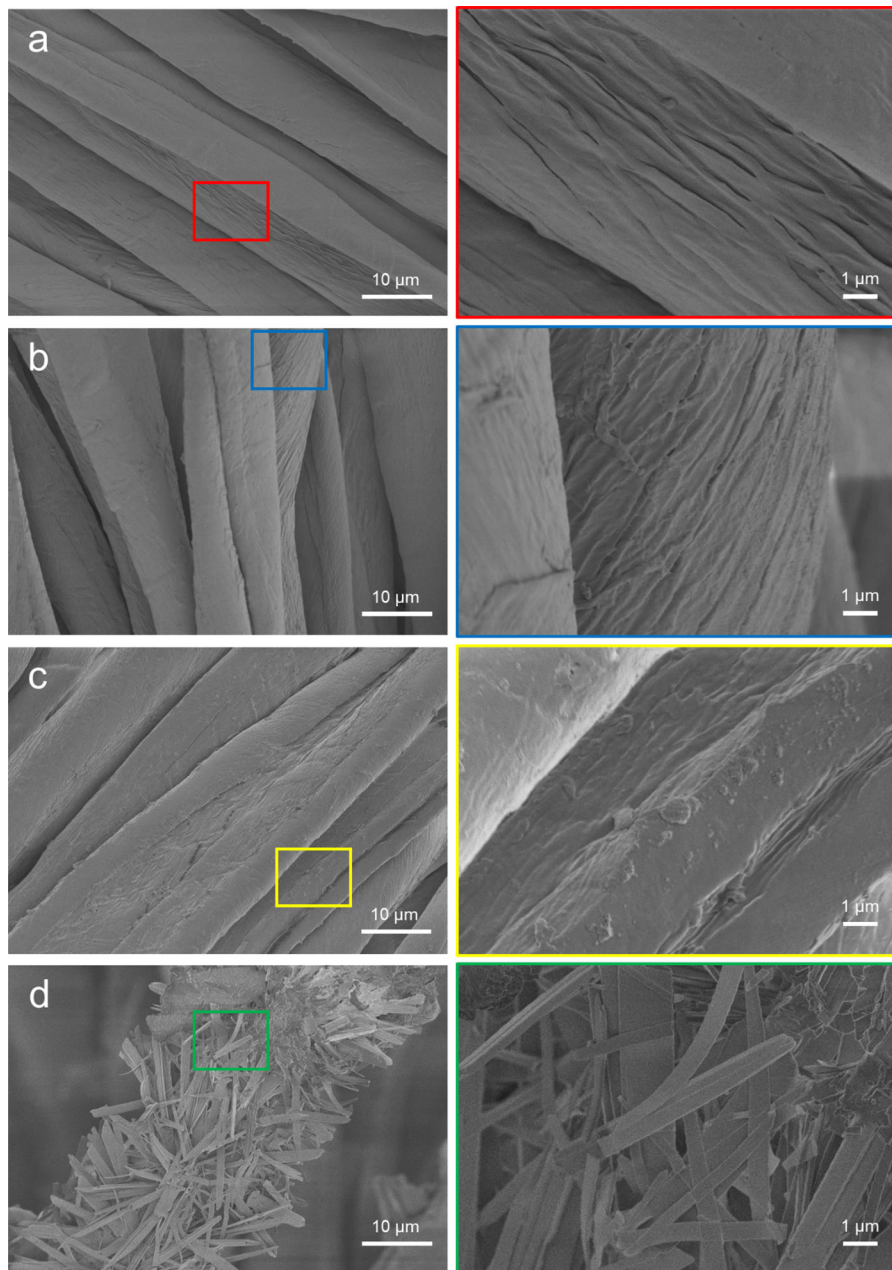


Fig. 2 SEM images of **a** Ref. cotton: untreated cellulosic textile, **b** 180COOH: cellulosic textile treated with 180 min of oxidation, **c** 180COOB72: cellulosic textile treated with

180 min of oxidation, followed by 72 h of esterification, and **d** Ref. cotton impregnated with 0.16 mol L^{-1} betulin solution in toluene

Betulin shows desirable hydrophobicity, as its CH groups make it a low surface energy chemical, as discussed in our previous study (Huang et al. 2018). Since the samples were sufficiently Soxhlet extracted, the non-attached betulin molecules were removed, which was also confirmed by SEM. Therefore, the

exhibited hydrophobicity of samples 45COOB72, 180COOB24 and 180COOB72 can mainly be attributed to the covalently attached betulin moieties on the sample surface. Additionally, the roughness caused by hairy protruding textile fibres on a macro scale, and dense grooves on textile fibre surfaces on a micro

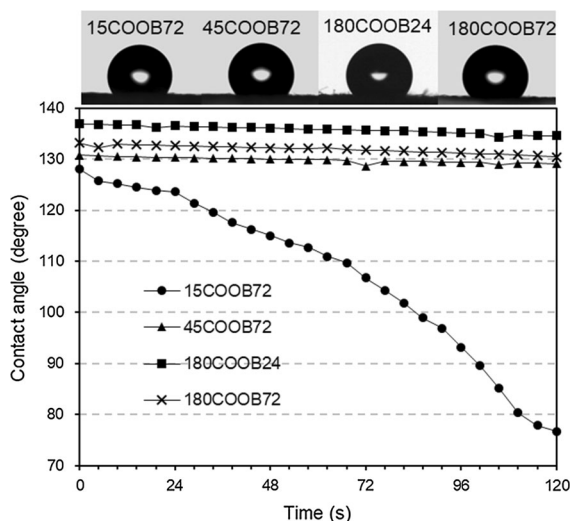


Fig. 3 Water contact angle of 45COOB72, 180COOB24, 180COOB72 and 15COOB72 as a function of time. XCOOBY stands for a sample prepared by X min of oxidation, followed by Y h of esterification

scale, can also contribute to hydrophobicity, since the combination of surface roughness and surface free energy affects the wettability of a surface (Cassie and Baxter 1944).

Antibacterial property

The antibacterial effect was evaluated by examining the number of viable bacteria after 18 h when inoculated with the tested textile sample, with and without added nutrients. The reduction of bacteria in nutrient-poor conditions is shown in Fig. 4a as the number of bacteria remaining after incubation and the percentage of bacterial removal compared to the positive reference. In Fig. 4a it can be seen that Ref. cotton, on which no betulin was grafted, showed no bacterial reduction, rather, it had an enhanced bacterial proliferation of 3.9 times for *E. coli* and 2.4 times for *S. aureus*. This indicated that in low-nutrient conditions, the polysaccharide-based textile was itself used as a nutrient source for both the Gram-negative and Gram-positive bacteria. A similar phenomena was also observed for cellulosic pulp fibres (Chen et al. 2017). Conversely, sample 180COOB24 showed an obvious reduction of both Gram-negative *E. coli* and Gram-positive *S. aureus*. It was particularly effective against *E. coli*, with a reduction of up to 99.94%. The other two samples, 45COOB72 and 15COOB24, also

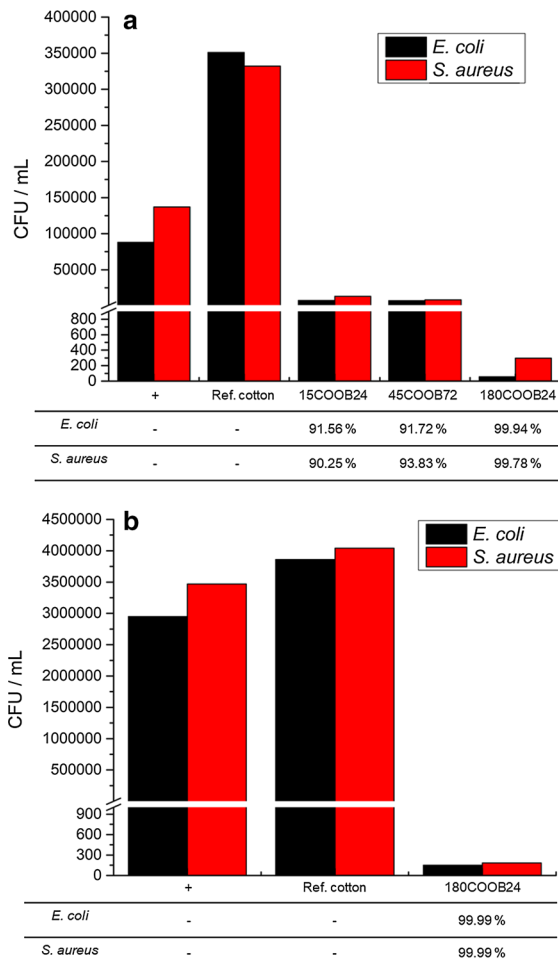
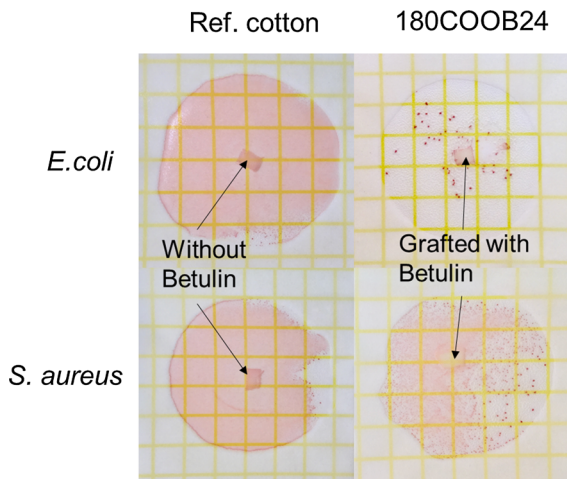


Fig. 4 Antibacterial properties: **a** bacterial concentration after 18 h of incubation with textile samples in bacterial reduction tests. The reduction percentage is shown in the tables below; **b** bacterial concentration and inhibition percentage in the bacterial growth inhibition tests

showed greater than 90% bacterial reduction; however, they were much less efficient than 180COOB24, which had the highest hydrophobicity. Samples 15COOB24 and 45COOB72 did not show obvious bacterial growth inhibition capabilities. Only one of the betulin-grafted samples, 180COOB24, had a significant bacterial growth inhibitory effect (presented in Fig. 4b), with a bacterial reduction of up to 99.99% for both *E. coli* and *S. aureus*. Compared Fig. 4a with Fig. 4b, the Ref. cotton showed higher bacterial growth enhancement than in the bacterial reduction test due to the accessible nutrients that were in the bacterial solution.

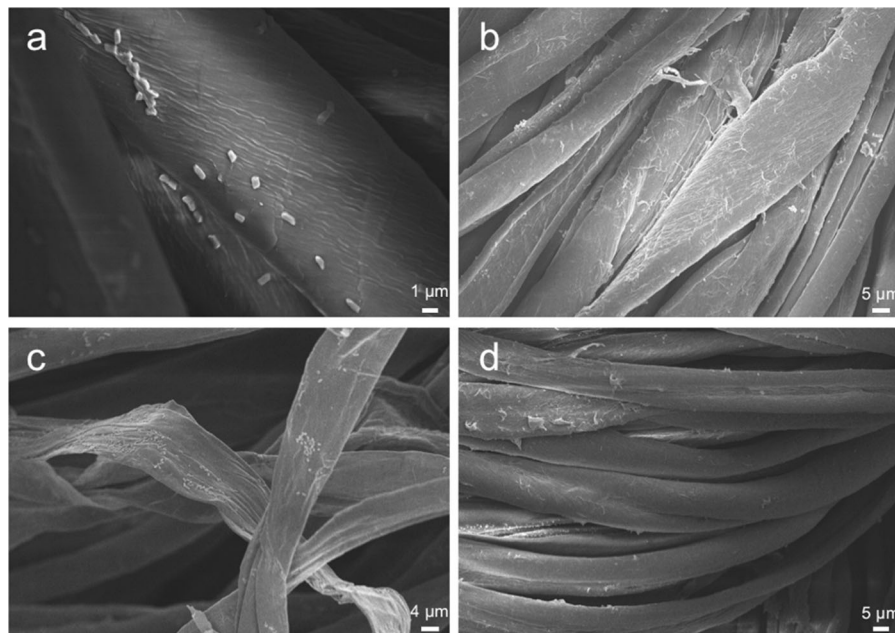
Table 4 Bacterial reduction of some textile after antibacterial treatment with the reagents indicated

Substrate	Antibacterial reagent	Bacterial type	Bacterial reduction	Reference
Cotton fabric	Silver Nanoparticles	<i>E. coli</i> / <i>S. aureus</i>	99.26%/99.01%	Zhang et al. (2009)
Cotton fabric	Silver Nanoparticles	<i>E. coli</i> / <i>S. aureus</i>	95%/96.4%	Hebeish et al. (2011)
Bamboo rayon fabric	CuO nanoparticle	<i>E. coli</i> / <i>S. aureus</i>	99.47%/100%	Teli and Sheikh (2014)
Cotton fabric	ZnO nanoparticles	<i>E. coli</i> / <i>S. aureus</i>	99.88%/99.98%	Singh et al. (2012)
Cotton fabric	Nanosized silver colloidal solution	<i>K. pneumoniae</i> / <i>S. aureus</i>	99.9%/99.9%	Lee et al. (2003)

**Fig. 5** Ref. cotton and 180COOB24 incubated on Petrifilms after an 18 h immersion in *E. coli* or *S. aureus* solution

These results from antibacterial assays are comparable to those reported (Table 4): the bacterial reduction (BR) of cotton fabric modified with silver nanoparticles were in the range of 95–99.26% (Hebeish et al. 2011; Zhang et al. 2009), while the BR is high as 99.9% by modification with nanosized silver colloidal solution (Lee et al. 2003). ZnO nanoparticles can also be used for rendering cotton fabric with antibacterial property, and the BR can exceed 99% as well (Singh et al. 2012). Additionally, by using CuO nanoparticle, the modified bamboo rayon fabric could even reach a BR of 100% against *S. aureus*, as reported (Teli and Sheikh 2014).

Few systematic studies of antibacterial properties of betulin have been reported, especially regarding betulin-functionalized materials. Based on our investigation, the textiles modified by betulin with more

**Fig. 6** SEM images of *E. coli* growth on **a** Ref. cotton, **b** 180COOB24; *S. aureus* growth on **c** Ref. cotton, **d** 180COOB24

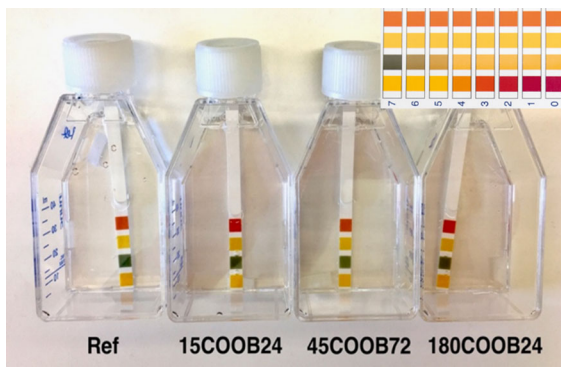


Fig. 7 The pH of 4 different samples incubated in $\frac{1}{4}$ Ringer's solution after 18 h at 37 °C

carboxylic acid groups, followed by 24 h of esterification, showed good antibacterial effects against both Gram-positive *S. aureus* and Gram-negative *E. coli*, including bacterial reduction in poor nutrient conditions and bacterial growth inhibition in the presence of nutrients. In Fig. 5, the small square of textiles are located in the middle of each Petrifilm, and it can be observed that the Ref. cotton were covered by bacteria (red colour), whereas the sample textile 180COOB24 was not “invaded” (original white colour). The textile samples were further examined under SEM, as shown in Fig. 6. It can be observed that many more bacteria grew on the Ref. cotton than on the 180COOB24 sample.

A strong antibacterial effect against both Gram-positive *S. aureus* and Gram-negative *E. coli* was seen in both the quantitative results of the bacterial solutions and in the qualitative microscopic images. The results indicate that the irreversibly grafted betulin textiles can have not only a large impact on

the bacterial activities in the surrounding solutions but can also deactivate bacterial proliferation on the surface. To verify this, the pH was examined for all samples that were incubated in Ringer's solution at 37 °C after 18 h, since the pH of the aqueous environment could interfere with the bacterial viability, i.e., lower viability at lower pH (Conner and Kotrola 1995; Leyer et al. 1995). However, in the current study, no pH change was observed, as shown in Fig. 7, which indicates that the pH was consistently 7. It has been reported that betulin is able to suppress the inflammation caused by *E. coli* and *S. aureus* (Guo et al. 2015; Wu et al. 2014). Combined with our studies, the decreased expression of inflammatory proteins could be due to the decreased bacterial viability caused by contact with betulin. It has also been reported that a series of triterpenoid chemical compounds were found to have antibacterial activity against *E. coli* and *S. aureus* (Dzubak et al. 2006). However, the specific antibacterial mechanisms remain unclear, and an important role for betulin use in possible medical applications could be discovered in future studies.

Mechanical properties

Mechanical properties of the textiles, such as tensile strength, dramatically decreased as a result of oxidation (Table 5). It has been reported that the degree of polymerization (DP) of cellulose oxidized in a TEMPO/NaClO/NaClO₂ system decreases with an extension of the oxidation time due to the cleavage of glycoside bonds (Shibata and Isogai 2003). A reduced DP will result in an inevitable reduction of mechanical properties (Saito et al. 2009). The tensile strength of

Table 5 Tensile properties including breaking tensile strength and elongation at break of Ref. cotton, oxidized textiles and betulin-grafted textiles

Sample	Tensile strength (MPa)	Elongation at break (%)
Ref. cotton	30.5 ± 2.0	21.6 ± 8.0
15COOH	19.9 ± 1.8	14.0 ± 0.5
45COOH	7.8 ± 0.6	24.8 ± 0.6
180COOH	6.6 ± 0.3	22.2 ± 0.5
15COOB24	5.7 ± 0.5	22.6 ± 1.3
15COOB72	4.5 ± 0.7	21.6 ± 0.7
45COOB24	5.7 ± 0.7	10.1 ± 0.4
45COOB72	2.9 ± 0.5	8.3 ± 0.6
180COOB24	4.2 ± 0.8	19.3 ± 0.9
180COOB72	2.2 ± 0.2	17.9 ± 0.6

betulin-grafted textiles also decreased upon increased esterification times (Table 5) due to either depolymerization of cellulose at high temperatures or the hydrolysis of cellulose caused by tartaric acid.

Conclusions

Hydrophobic and antibacterial cellulosic textile fibres were successfully prepared by grafting betulin onto carboxyl-functionalized cellulose via esterification. Different oxidation and esterification time durations were performed. The material prepared through 180 min of oxidation followed by 24 h of esterification exhibited the highest static water contact angle of 136° and exceeded a bacterial reduction of 99%. This new created functional textile would be a potential alternative for fabricating sportswear or medical mattress and bandages where waterproof or antibacterial properties are needed. However, at the current stage, this study just aims to provide an idea for the simple fabrication of functional materials and more importantly, the exhibited method can be envisaged to be applied to other cellulose sources (e.g., microfibrillated cellulose) to achieve the goal of functionalization. It is also true, however, that further research involving both optimization of the fastness of such properties and establishment of new cellulose-betulin system should be considered if the product is expected to bear a commercial viable application potential.

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