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Covalent Grafting of Antimicrobial Peptides onto Microcrystalline Cellulose

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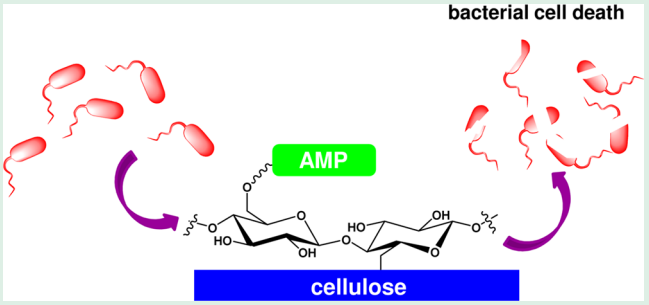
ABSTRACT: The purpose of this work is to set up a general protocol for the production of antimicrobial materials based on cellulose and peptides. We exploited the chemical ligation reaction to achieve the conjugation of peptides to cellulose; to this aim, we produced thioester peptides and cysteine-modified cellulose. As the thioester handle can be inserted at any position of the peptide, the peptide can be immobilized onto the cellulose through its N- or C-terminal end or through any other position within the sequence. Our experiments performed on *Escherichia coli* cultures show that the cellulose conjugated to the peptides lasioglossin-III and TBKKG6A causes a significant reduction in the concentration of viable cells as compared to unmodified cellulose. In conclusion, antimicrobial peptides bound to cellulose through a covalent bond retain their activity and therefore have the potential to be used as active ingredients in antimicrobial materials.



KEYWORDS: *peptide, cellulose, conjugation, chemical ligation, antimicrobial, E. coli*

INTRODUCTION

Cellulose is the most abundant naturally occurring polymer obtained from renewable sources, composed of repeating units of poly-(β-1,4-D-anhydroglucopyranose); it can be isolated from plant cell walls, or it can be produced by bacteria from the genera *Gluconacetobacter*, *Sarcina*, and *Agrobacterium* or by tunicates, algae, and fungi. Importantly, it can also be derived from agro-industrial wastes.[1]

The cellulose macromolecular structure presents both crystalline and amorphous regions; physical properties of cellulose such as crystallinity and polymerization degree are different depending on its origin and processing.[2−4]

Due to its biocompatibility and biodegradability, cellulose has various applications in different fields; as an example, in the food industry, it is used as a gelling or stabilizing agent, whereas in the cosmetic industry, it is employed as a scrub or as a water retainer, emulsifier, and suspension stabilizer in pastes and cream. Other applications are in the paper, fuel, and leather industries.[5] Composites made of bacterial cellulose (BC) and polymeric or non-polymeric compounds have been exploited to produce artificial bones or cartilages.[6−8]

The use of cellulose addressed to the preparation of antimicrobial materials is reported in an increasing number of publications. The combination of Ag nanoparticles and cellulose matrices results in nanocomposites, endowed with antimicrobial activity against Gram-positive and Gram-negative bacteria.[9−11] Immobilization of peptides on cellulose to produce antimicrobial materials is slightly explored. Antimicrobial peptides (AMPs) are natural molecules produced by organisms of all domains of life, which represent the weapon by which organisms defend themselves from external pathogens.[12] They show a wide spectrum of activity and are selective toward bacterial cells. Being natural molecules, they are well tolerated by the body, and importantly they are less prone to trigger resistance as compared to common antibiotics. For this reason, they are considered as a valid alternative to small molecules to combat infections caused by resistant bacteria; the use of AMPs to coat biodevices, such as implants, is widely explored.[13−16]

In a recent paper, Gonzalez et al. report the immobilization of an antimicrobial peptide on cellulose nanopapers; the peptide is adsorbed on nanopapers previously oxidized by 2,2,6,6-tetramethylpiperidinyloxy radical (TEMPO) and treated with alkyl ketene dimer to increase the hydrophobicity.[17] The release of the peptide upon contact with the culture medium results in antimicrobial activity.

Based on the observation that antimicrobial peptides retain their activity upon immobilization on a solid surface,[14,15,18] it may be envisaged that covalent immobilization of antimicrobial

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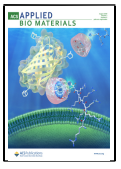

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peptides on cellulose may result in some advantages with respect to absorption. The main one is that peptides covalently bound to a surface are not released in culture media, which in turn implies the possibility to reuse the surface. This aspect is of particular relevance if we think of an application of peptide−cellulose conjugates to the production of textiles. For the covalent immobilization, the free hydroxyl of cellulose can be exploited.

Protocols for the grafting of peptides on cellulose have been described by Orlandin et al.[19,20] In these papers, cellulose was used as a solid support for the synthesis of peptides, yielding peptides connected to the cellulose through their C-terminal end.

In addition, Edwards et al. reported the conjugation of peptides to cellulose to produce sensors for elastase and antibacterial cotton fibers.[21−23] The immobilization of peptides was performed using glycine-modified cellulose. The reaction between the glycine amino group and a tripeptide bearing a succinic acid linker at the N-terminal end results in the formation of an amide bond and the immobilization of the peptide on the cellulose through its N-terminal end. This reaction affords a single conjugate product only when the peptide has one carboxylic acid function to react with the amine of glycine on the cellulose. As a matter of fact, coupling of unprotected mixed sequence peptides containing lysine, serine, or threonine residues in combination with aspartic and/or glutamic acid will result in a complicated mixture of products, which will lower the yield of immobilization.

It appears that a general protocol for the covalent immobilization of unprotected peptides on cellulose is lacking. Considering the strong potential in the use of this bioconjugated material with antimicrobial activity, we explored the chemical ligation reaction to achieve peptide immobilization. This reaction can be applied to conjugate peptides of any length and also proteins. Moreover, it allows for binding the peptide through its C- and/or its N-terminal end as well as any desired position within the peptide chain, therefore enabling the control of the orientation of the peptide with respect to the cellulose. It has been demonstrated that the activity of antimicrobial peptides grafted on solid supports may depend on the site of anchoring. For example, Bac2A derivatives exhibit stronger activity when the hydrophilic residues are placed close to the cellulose surface, whereas melimine shows the best antibacterial activity when hydrophobic residues at the N-terminal end are linked to glass surfaces.[24,25] Moreover, temporin-SH maximizes its antimicrobial potential when tethered to titanium surfaces through an amino acid inserted in the middle of the sequence.[26] In the present study, we modified Avicel PH-101, microcrystalline cellulose suitable for pharmaceutical applications.[4] We accomplished the functionalization of Avicel PH-101 with two antimicrobial peptides, namely, TBKKG6A and lasioglossin-III (Table 1). TBKKG6A is an analogue of the natural peptide temporin B, a peptide secreted by the skin of the *Rana temporaria* frog; lasioglossin-III was isolated from the venom of *Lasioglossum laticeps*.[27−30] Both peptides show activity at very low concentrations against Gram-positive and Gram- negative bacteria. The antibacterial activity of the new generated biopolymers was investigated.

Table 1. Name and Sequences of the Antimicrobial Peptides Immobilized on Cellulose

name (number)	sequence
lasioglossin-III (1)	VNWKKILGKIIKVVK
TBKKG6A (2)	KKLLPIVANLLKSLL
TBKKG6A-K (3)	KKLLPIVANLLKSLLK

RESULTS AND DISCUSSION

Peptide Synthesis and Conjugation to Cellulose. The immobilization of peptides on cellulose is achieved by a chemoselective reaction, the native chemical ligation, affording highly homogeneous products. The native chemical ligation reaction, described by Dawson et al., involves the reaction of an N-terminal cysteine with a thioester function and results in the formation of an amide bond between the N-terminal end of cysteine and the activated carboxyl group.[31] Importantly, no other residue participates in the reaction, granting therefore the formation of a single product. As reported above, two antimicrobial peptides have been chosen for immobilization purposes, TBKKG6A and lasioglossin-III (Table 1).

To achieve the conjugation, both cellulose and peptides need to be modified. We installed cysteine on the cellulose and thioester on the peptides.

First, the cellulose scaffold to be used for tethering peptides was prepared (Scheme 1).

To preserve the antimicrobial activity of the peptide and prevent adhesion of the peptide to the cellulose, we immobilized on the cellulose a long linker composed of suberic acid and ethylenediamine. An Fmoc-cysteine, protected at sulfur with a trityl group was successively reacted with the free amine. The main advantage of linking Fmoc-cysteine on cellulose is that it is possible to calculate the loading of cellulose with the amino acid by quantifying the Fmoc group released by cysteine. We produced cellulose with a final loading of cysteine ranging from 0.050 to 0.075 mmol/g. The protecting group on cysteine was removed by standard protocols to afford a scaffold ready for the chemical ligation reaction with thioester peptides.

Next, we obtained thioester peptides. The thioester handle that is needed to perform ligation may be linked either at the C- or at the N-terminal end of the peptide and also at any desired position within the peptide chain, enabling all possible orientations of the peptide with respect to the cellulose to be tested.

We produced two different derivatives of TBKKG6A by solid-phase synthesis: one with a thioester linker, *i.e.*, benzyl thiosuccinic moiety, at the C-terminal end and the other with the thioester linker at the N-terminal end. In the case of lasioglossin-III, we produced the N-terminal modified peptide only as it has been demonstrated that, when immobilized in this orientation, the peptide keeps its antimicrobial activity.[16]

C-Terminal Derivatization (Scheme 2). To obtain the TBKKG6A peptide modified at the C-terminal end, we prepared peptide **3** by solid phase synthesis. This peptide bears lysine as the C-terminal amino acid, protected on the epsilon amine with a methyltrityl group that can be selectively removed when the peptide is still anchored to the resin. After acylation of the N-terminal residue, the methyltrityl group on lysine is removed; then, the benzyl thiosuccinic linker is covalently linked, affording the C-terminal thioester peptide. Cleavage from the resin and deprotection of the peptide occur simultaneously, treating the resin with a concentrated TFA solution. The peptide is obtained as a single product and then purified by reversed-phase high-pressure liquid chromatography (RP-HPLC) and characterized by mass spectrometry

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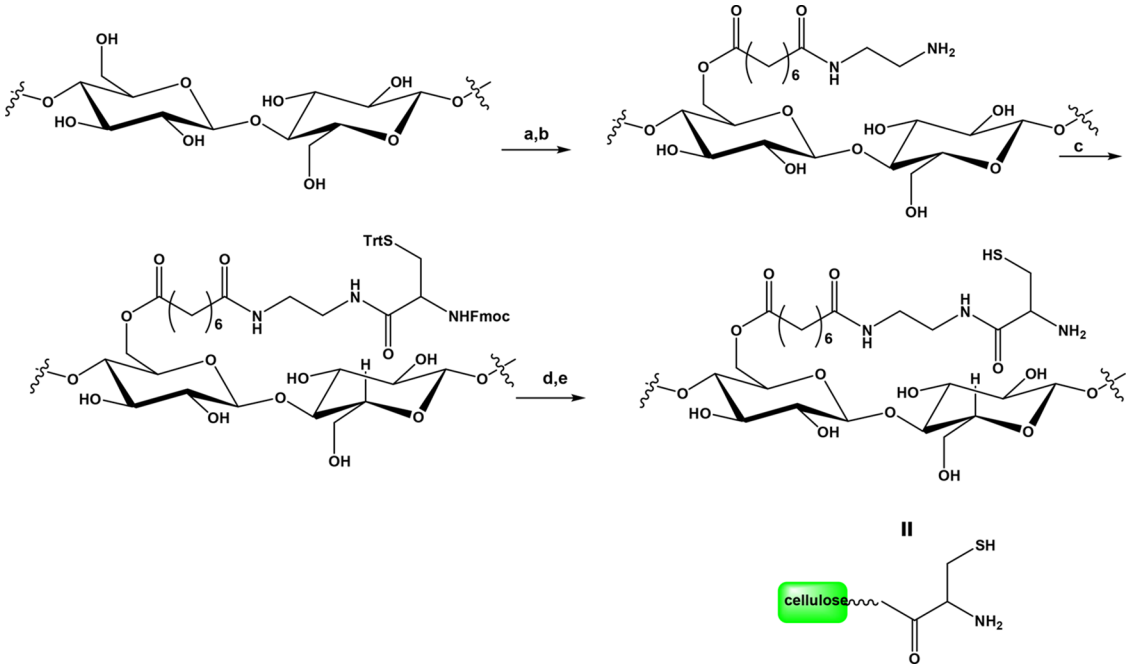
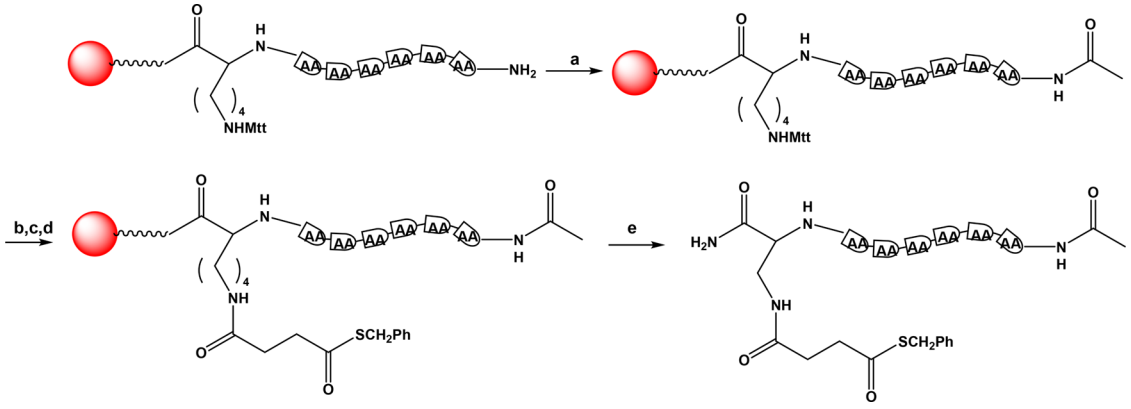
Scheme 1. Synthesis of the Cysteine−Cellulose Conjugate[a]



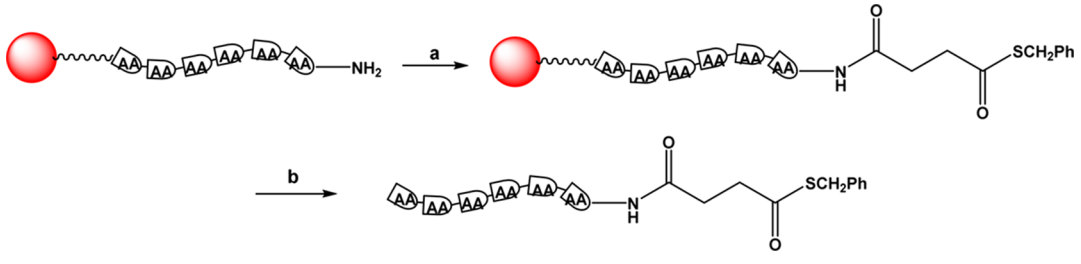
[a]Reagents and conditions: (a) suberic acid bis(hydroxysuccinimide), DIPEA, DMF, 2 h, r.t.; (b) ethylediamine, DMF, 2 h, r.t.; (c) Fmoc-Cys(Trt)-OH, HOBT/HBTU, *N*-methylmorpholine, DMF, overnight, r.t.; (d) TFA (30%), DCM, 30 min (x2); (e) piperidine 30%, DMF, 5 min (x2).

Scheme 2. Derivatization of the Peptide at the C-Terminal End[a]



[a]: Rink amide resin; AA block chain represents a peptide, in this case the peptide linked to the resin is TBKKG6A-K. Reagents and conditions: (a) acetic anhydride/DIPEA/DMF (15/15/70, v/v/v), 10 min, r.t.; (b) TFA (1%), TIS (5%), DCM, 2 min (x10); (c) DIPEA (10%), DMF, 10 min, r.t.; (d) benzyl thiosuccinic acid, HATU, DIPEA, DMF, 2 h, r.t.; (e) TFA, benzylmercaptan, phenol, H_2O (90/5/2.5/2.5, v/v/v/v), 2 h, r.t.

Scheme 3. Derivatization of Peptides at the N-Terminal End[a]



[a]: Rink amide resin; AA block chain represents a peptide, in this case TBKKG6A or lasioglossin-III. Reagents and conditions: (a) benzyl thiosuccinic acid, HATU, DIPEA, DMF, 2 h, r.t.; (b) TFA, benzylmercaptan, phenol, H_2O (90/5/2.5/2.5, v/v/v/v), 2 h, r.t.

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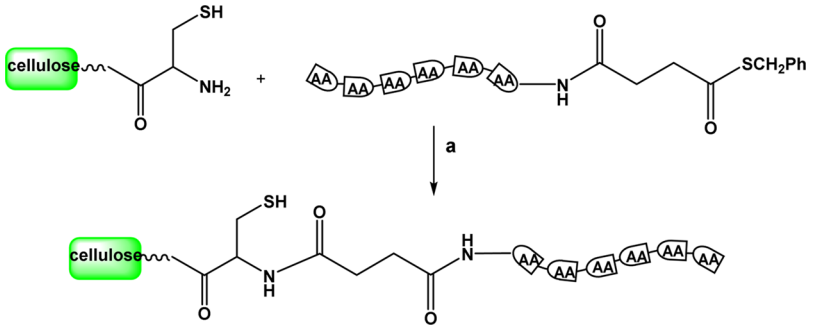
Scheme 4. Immobilization of a Thioester Peptide on the Cellulose Support by the Chemical Ligation Reaction[a]



[a]AA block chain represents any peptide modified by a thioester linker. Reagents and conditions: (a) 100 mM NaH_2PO_4, 50 mM Na ascorbate, 20 mM TCEP, pH 7.5, 48 h, r.t.

(see the Supporting Information for the HPLC profile and mass spectrum).

N-Terminal Functionalization (Scheme 3). Peptides **1** and **2**, obtained by solid-phase synthesis, were reacted with the benzyl thiosuccinic linker to give N-terminal modified peptides. Cleavage from the resin and deprotection of the peptide occur simultaneously, as described earlier. The peptides are obtained as single products and then purified by RP-HPLC (see the Supporting Information for HPLC profiles and mass spectra).

Grafting of the peptides on the cellulose was achieved by the chemical ligation reaction; to this aim, peptides were dissolved in buffer at pH 7.5 and incubated with the functionalized cellulose (Scheme 4).

Progress of the reaction was monitored by checking the disappearance of the peptide from the solution incubated with the cellulose by HPLC. As the peptide peak disappears, we assume that peptides quantitatively react with cysteine on the cellulose; therefore, the loading of the peptide on the cellulose is identical to the loading of the cysteine.

The peptide−cellulose conjugates were characterized by Fourier transform infrared (FT-IR) spectroscopy (Figure 1 and Supporting Information, Figures S1 and S2). Appearance of the band ∼1650 nm is a diagnostic of amide bonds and therefore confirms the conjugation of peptides to cellulose. Interestingly, the spectra recorded for lasioglossin−cellulose conjugates are very similar, in the amide region, to that reported in the literature for the same peptide conjugated to a silicon surface.[32] Dry peptide−cellulose conjugates are stored at −20 °C.

Antimicrobial Activity. The MIC (minimal inhibitory concentration) of the TBKKG6A-K peptide against *Escherichia coli* was evaluated to be 50 μg/mL; the MIC values of TBKKG6A and lasioglossin-III are reported in the literature to be 5 and 2.5 μg/mL, respectively.[27,29,30,33] The antimicrobial properties of the functionalized crystalline cellulose were evaluated against *Escherichia coli* MG1655, a reference strain for Gram-negative bacteria, as described in the Experimental Section. The antibacterial activity was determined by calculating the percent reduction of bacterial concentration (%*R*) after 6 h of contact of *E. coli* cells with different concentrations of functionalized or non-functionalized crystalline cellulose. The results representative of at least two independent experiments are reported in Table 2.

Cellulose functionalized with peptides **1**−**3** showed significant reduction (%*R* = 100%) in the concentration of viable cells relative to the initial inoculum. The %*R* values

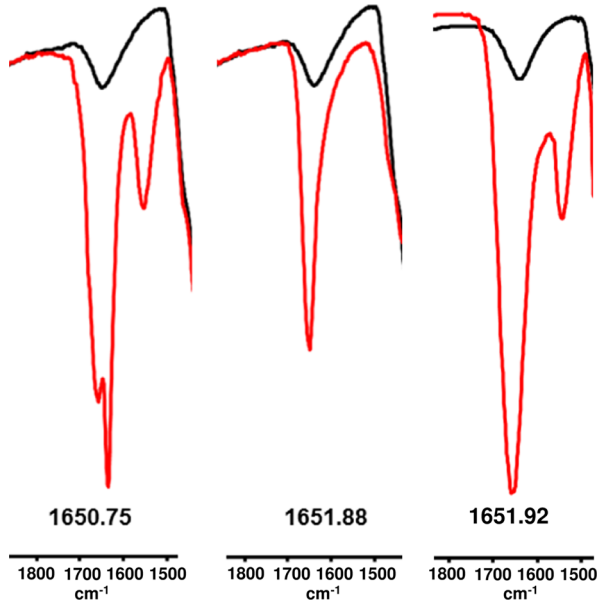


Figure 1. Details of the FT-IR spectra recorded for cellulose (black) and peptide−cellulose conjugates (red). On the left is the lasioglossin-III−cellulose conjugate, in the middle is the TBKKG6A-K Link−cellulose conjugate, and on the right is the Link TBKKG6A−cellulose conjugate.

displayed by peptide-functionalized cellulose samples correspond to a $\log_{10}$ reduction ranging approximately from 3 to 4. The highest potency is observed when peptides are immobilized through the N-terminal end at concentrations of 20 mg/mL in the case of lasioglossin-III and 50 mg/mL in the case of TBKKG6A.

These results confirm that both peptides maintain their antimicrobial activity after immobilization as cellulose functionalized with peptides not only prevents bacterial growth relative to control cellulose at the same (or even higher) concentration but also determines a significant decrease in viable cell number with respect to the initial inoculum. This result suggests that conjugation with cellulose does not interfere with the mechanism of action of the peptides, providing them with enough flexibility to interact with bacterial membranes. The peptide TBKKG6A exhibits a stronger antimicrobial activity when immobilized onto the cellulose through its N-terminal.

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Table 2. Antibacterial Properties of Functionalized Crystalline Cellulose

sample[a]	CFU/mL $t = 0$[b]	CFU/mL $t = 6$ h[c]	%R[d]
bacteria only	4×10^6	1.6×10^{10}	nr[e]
cellulose only 50 mg/mL	2×10^6	3×10^9	nr[e]
cellulose only 100 mg/mL	8×10^6	8×10^{10}	nr[e]
Link TBKKG6A 10 mg/mL	2×10^6	1×10^9	nr[e]
Link TBKKG6A 20 mg/mL	1.6×10^6	4×10^8	nr[e]
Link TBKKG6A 50 mg/mL	2×10^6	$<1 \times 10^3$	100
Link TBKKG6A 100 mg/mL	4×10^6	$<1 \times 10^3$	100
TBKKG6A-K Link 10 mg/mL	1.6×10^6	2.5×10^9	nr[e]
TBKKG6A-K Link 20 mg/mL	4×10^6	2.9×10^9	nr[e]
TBKKG6A-K Link 40 mg/mL	4×10^6	4×10^8	nr[e]
TBKKG6A-K Link 80 mg/mL	3×10^6	$<1 \times 10^3$	100
TBKKG6A-K Link 100 mg/mL	4×10^6	$<1 \times 10^3$	100
Link lasioglossin-III 10 mg/mL	4×10^6	3.1×10^9	nr[e]
Link lasioglossin-III 20 mg/mL	8×10^6	$<1 \times 10^3$	100
Link lasioglossin-III 100 mg/mL	8×10^6	$<1 \times 10^3$	100

[a] *E. coli* MG1655 cells alone (bacteria only) or in contact with nonfunctionalized (cellulose only) or crystalline cellulose functionalized with the indicated peptides. [b] $t = 0$: bacterial concentration at the time of contact. [c] $t = 6$ h bacterial concentration after 6 h of contact with the indicated sample. [d] % of bacterial reduction (%R) calculated as described in the Experimental Section. [e] nr = no reduction.

CONCLUSIONS

A protocol based on chemical ligation for the immobilization of peptides on cellulose through the C- or the N-terminal end has been set up. Following this general protocol, the amount of peptide immobilized on cellulose may be modulated. In fact, the loading of cellulose with cysteine can be increased or decreased, depending on the need. Antimicrobial activities suggest that peptides maintain their activity upon immobilization, paving the way to studies with different peptides. The cellulose−peptide conjugates could have potential applications in the formulation of antibacterial cream and pastes or as preservative agents to protect cosmetic or pharmacological formulation by microbial contamination.

EXPERIMENTAL SECTION

Peptide Synthesis. Peptides were obtained by solid-phase synthesis on an automated synthesizer (Liberty Blue CEM) on the Rink amide resin (loading: 0.69 mmol/g). Derivatization of peptides **1** and **2** at the N-terminal end was performed on the resin by coupling the linker benzyl thiosuccinic acid to the N-terminal amino acid, following procedures reported in the literature.[34] Briefly, the linker is activated with HATU in DMF in the presence of DIPEA and reacted with the free amine. The synthesis of TBKKG6A modified by the thioester linker at the C-terminal end was performed as follows. Resin bound peptide **3** was acetylated at the N-terminal end by treatment with acetic anhydride/DIPEA/DMF (15/15/70, v/v/v) for 5 min; after selective deprotection of the epsilon amino group on the C-terminal lysine by treatment with 1% TFA, 5% TIS in DCM for 2 min, 10 times, the resin was treated with a solution of DIPEA (10%) in DMF for 10 min. Then, the linker was reacted to this amino group as described above. The peptide was cleaved off the resin and deprotected by treatment of the resin as previously reported.[34]

Peptides were purified by RP-HPLC (Phenomenex Jupiter 10 μ Proteo, 90 Å, 100 × 21.2 mm) and analyzed by mass spectrometry on a Thermo Scientific LCQ Fleet ion trap LC/MS. Peptide sequences and mass spectrometry data for peptide characterization are reported below. Link indicates the benzyl thiosuccinic linker. HPLC profiles and mass spectra of the peptides are provided in the Supporting Information (Figures S3−S8).

Link TBKKG6A. Sequence: Link-KKLLPIVANLLKSLL. Calculated mass: (Da) 1868.16; found $[M + H]^+$ 1869.76; $[M + 2H]^{2+}$ 935.67; $[M + 3H]^{3+}$ 623.99.

Purification gradient: from 30 to 80% of CH_3CN (0.1% TFA) in H_2O (0.1% TFA) in 20 min.

TBKKG6A-K Link. Sequence: KKLLPIVANLLKSLLK(Link). Calculated mass: (Da) 2037.28; found: $[M + 2H]^{2+}$ 1019.71; $[M + 3H]^{3+}$ 680.39; $[M + 4H]^{4+}$ 510.24.

Purification gradient: from 15 to 50% of CH_3CN (0.1% TFA) in H_2O (0.1% TFA) in 20 min.

Link Lasioglossin-III. Sequence: (Link)VNWKKILGKIIKVVK. Calculated mass: 1971.34; found: $[M + H]^+$ 1972.77; $[M + 2H]^{2+}$ 987.03; $[M + 3H]^{3+}$ 658.68.

Purification gradient: from 30 to 80% of CH_3CN (0.1% TFA) in H_2O (0.1% TFA) in 20 min.

Cellulose Derivatization. In a reaction vessel for peptide synthesis, microcrystalline cellulose (150 mg, Avicel PH-101) was suspended in DMF. Suberic acid bis(hydroxysuccinimide) (0.06 mmol), dissolved in DMF (1.0 mL) and DIPEA (0.6 mmol) was added. The solution was incubated for 2 h at r.t., then reagents were filtered out, and cellulose was rinsed with DMF. An excess of ethylenediamine (2.0 mmol) was dissolved in DMF (860 μL) and reacted with the cellulose for 2 h. After filtering reagents and washing the cellulose with DMF, a solution of Fmoc-Cys(Trt) (0.3 mmol), HOBT/HBTU (0.27 mmol), and *N*-methylmorpholine (0.48 mmol) in DMF (0.8 mL) was added. The reaction was performed overnight at r.t.

The cellulose was rinsed. The cysteine residue was then deprotected either on the side chain (by treatment with a solution of TFA/DCM/TIS (30/69/1, v/v/v) for 30 min two times) or on the amino terminal (by using a solution of piperidine in DMF (30/70, v/v)).

Resin loading was calculated by the Fmoc test (~0.075 mmol/g for cellulose + TBKKG6A-K Link and cellulose + Link TBKKG6A and 0.05 mmol/g for cellulose + Link lasioglossin-III).[35]

For the chemical ligation reaction, 1 equiv of peptide was dissolved at a 4×10^{-4} M concentration in a buffer composed of NaH_2PO_4 (100 mM), sodium ascorbate (50 mM), and TCEP (20 mM), pH 7.5. A 6 M solution of guanidinium hydrochloride was added to the buffer to dissolve Link TBKKG6A. The peptides were incubated with the functionalized cellulose. Aliquots of the supernatant were collected and analyzed by RP-HPLC to follow the immobilization reaction. After 48 h of incubation, the reaction was complete. The cellulose was further rinsed with buffer, DMF, and DCM and dried under vacuum. The conjugated cellulose was analyzed by ATR FT-IR on a PerkinElmer Spectrum One FT-IR spectrometer equipped with a universal ATR sampling accessory.

Antimicrobial Activity. We determined the MIC of the peptides according to the standard broth microdilution method by preparing twofold serial dilutions in Mueller-Hinton Broth (MHB) in 96-well microtiter plates.[27] A stationary phase culture of the *Escherichia coli* K12 MG1655 strain grown overnight at 37 °C with aeration was diluted to an optical density at 600 nm (OD_{600}) of 0.05 in the same medium. A stock solution of antibacterial peptides was made in water at an initial concentration of 5 mg/mL and used to perform serial dilutions in a ratio of 1:2 from 200 μg/mL in a final volume of 100 μL of water in the wells of the microtiter plate. A 100 μL solution of bacterial inoculum at $OD_{600} = 0.05$ was added in each well. Inoculated microtiter plates were incubated at 37 °C for 16−20 h. The MIC was calculated as the minimal concentration of the peptide that inhibits bacterial growth, as assessed by measuring the absorbance at 600 nm in a microtiter plate reader (EnSpire Multimode Plate reader, PerkinElmer). Lasioglossin-III was used as a positive control for the MIC determination of TBKKG6A-K. Untreated culture was used as a negative control.

To assess the antimicrobial activity of peptide-functionalized cellulose powder in a concentrated bacterial suspension under a fixed contact time (6 h), we used the standard method ASTM E2149-

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10 (Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions) with slight modifications. Briefly, bacterial cultures of MG1655 strain were grown in LB broth (Bacto triptone 10 g/L; yeast extract 5 g/L; NaCl 10 g/L) at 37 °C under aeration until the optical density OD_{600} reached ∼0.2 and adjusted to an OD_{600} of 0.003 (corresponding approximately to a concentration of $1-5 \times 10^6$ CFU/mL) in the same medium.[36] We resuspended the bacteria in the medium, instead of buffer, to explore not only the bactericidal but also bacteriostatic activity of peptide-functionalized cellulose powder. The bacterial concentration of the diluted suspension was determined by serial dilution and considered as contact time "0" ($t = 0$) concentration. Peptide-functionalized cellulose powder was mixed with bacterial cultures at the concentration ranging from 10 to 100 mg/mL, and the suspensions were incubated in Petri dishes at 37 °C for 6 h under static conditions. As negative controls, the bacterial suspensions incubated with microcrystalline cellulose at a concentration of 50 and 100 mg/mL (untreated sample control) or alone (inoculum only control) were used. To enumerate bacterial cells treated with cellulose or not treated, 10 volumes of sterile 0.9% NaCl solution were added to the Petri dishes and bacteria were resuspended by pipetting. The resulting suspensions were used for the determination of CFU/mL using serial dilutions prepared in sterile 0.9% NaCl solution and plated on LB agar plates. The plates were incubated at 37 °C for 18 h. The antibacterial activity was expressed by the percentage of the reduction of bacterial concentration (%R) according to the formula

$$\%R = (A - B)/A \times 100$$

where A is the CFU/mL of the sample at $t = 0$ and B is the CFU/mL of the treated sample at $t = 6$ h.

The antibacterial activity of the tested sample is considered significant if %R is higher than 90%.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.0c00412.

HPLC profiles and mass spectra of peptides and FT-IR spectra of cellulose−peptide conjugates in full (PDF)

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Notes

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