Multifunctional Bioactive Cotton: Synergistic Antimicrobial and Regenerative Properties through Chemoselective Peptide Conjugation

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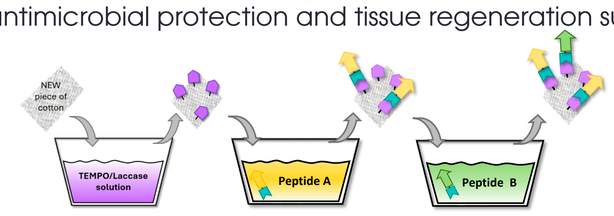
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SUMMARY

Skin wounds represent a significant public health challenge, requiring the development of active dressings that can prevent infections and support the healing process.

While cotton is the most widely used material for wound bandages, it lacks intrinsic bioactivity. In this work, we present chemoselective strategies to transform cotton into a versatile multifunctional platform by functionalizing it with bioactive peptides using 'click chemistry' approaches.

By conjugating two biological active peptides we developed a dressing that offers simultaneous antimicrobial protection and tissue regeneration support.



ACTIVE PEPTIDES

Antibacterial ①

from PMAP36(12-24) H-KRLKKIGKVLKWIH-NH_2

- Broad spectrum antibacterial properties and low cytotoxicity
- Membranolytic mechanism of action
- Its D-enantiomer is resistant to proteases
- High solubility in water

2a

H-**Cys**-KRLKKIGKVLKWI-NH_2

PMAP36(12-24)
Maintains the helicity and **antimicrobial activity** of the natural PMAP36(1-36)

MIC (μM)*					
E.coli ATCC 25922	*S.aureus* ATCC 25923	*A.baumanni* ATCC 19606	*K.pneumoniae* ATCC 700603	*P.aeruginosa* ATCC 27853	*S.epidermidis* ATCC 12228
4	>64	2	8	8	4

- Not cytotoxic against HaCaT
- Not hemolitic against red blood cells

Collagen Boosting ②

from pentapeptide 4 H-KTTKS-OH

- Fragment of procollagen sequence
- Collagen regenerative properties by inhibiting the collagenase activity and promote the ECM.
- Palmitic chain at the N-terminus is reported to give better stability and higher permeability through the skin.

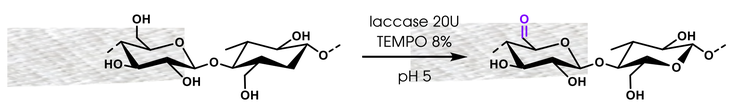
4d

H-**Cys**-WKTTKS-NH_2

THE METHOD

The cellulose backbone is modified with aldehyde groups through mild TEMPO/laccase/O_2 catalysed oxidation, which preserves the fibres' structural integrity. Then, thiazolidine was employed to covalently graft bioactive peptides.

This strategy was employed to produce dual-functional materials through consecutive ligations.

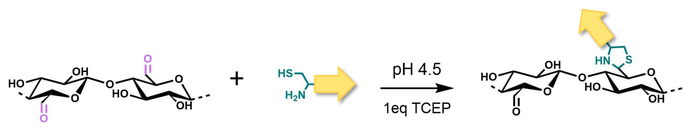


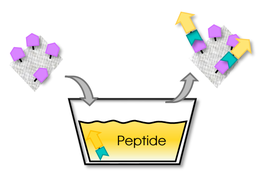


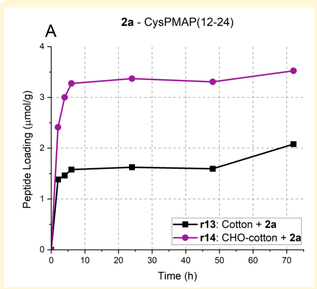
Colorimetric Schiff test to evaluate the presence of the aldehyde. From left to right cotton, 1st , 2nd and 3rd use of the oxidizing solution.

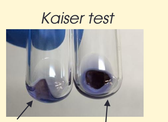
Titration with NaOH 0.01M to quantify the grafting

- Selective for primary OH
- Aqueous solution
- Room temperature
- Oxidizing solution can be re-used



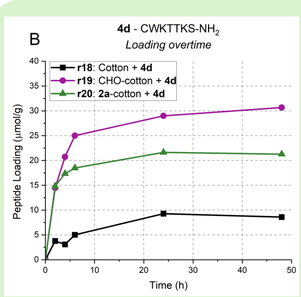


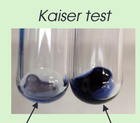




Peptide loading overtime (monitored by absorbance at 280nm) for the reaction with cotton.

	Sample	2a loading (μmol/g_{cot})	4d loading (μmol/g_{cot})	Time of Reaction
r13	Cotton + 2a	1.5	-	3 days
r14	CHO-Cotton + 2a	3.5	-	3 days

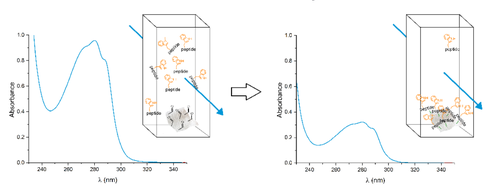




Peptide loading overtime (monitored by absorbance at 280nm) for the reaction with cotton.

	Sample	2a loading (μmol/g_{cot})	4d loading (μmol/g_{cot})	Time of Reaction
r18	Cotton + 4d	-	8.6	2 days
r19	CHO-cotton + 4d	-	30.7	2 days
r20	r14 (CHO-cotton+2a)+ 4d	3.5	21.3	2 days

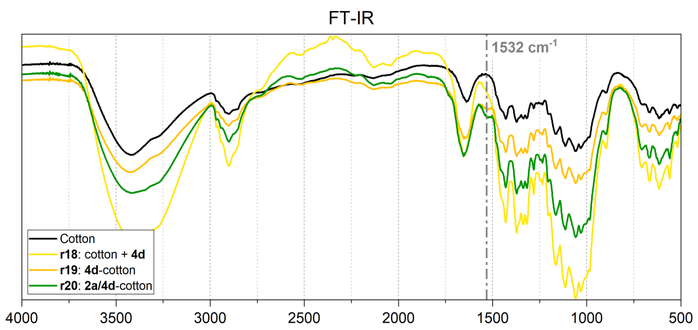
- One-pot reactions monitored by the decrease of the peptide from the reaction solution by UV-vis



- TCEP to avoid formation of dimers through disulfide bonds

RESULTS

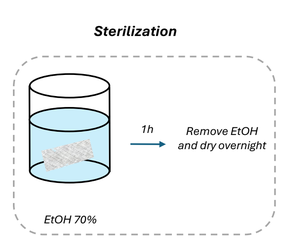
Successful conjugation was confirmed via FT-IR, amino acid analysis, and XPS spectroscopy.

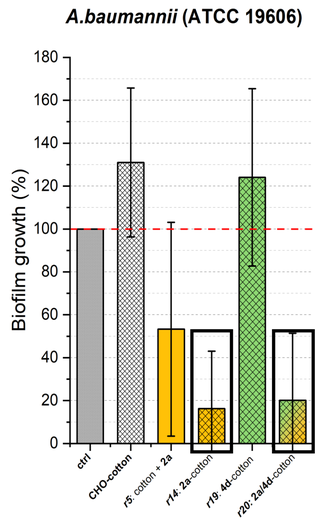


1532 cm-1, that can be attributed to the amide II stretching signal of the amides present in the peptide backbone

Bioactivity was assessed by biological assays against *Acinetobacter baumannii*.

The results demonstrate that chemoselective functionalization can effectively turn simple cotton into a high-performance, biocompatible medical textile with tailored multifunctional properties.





CONCLUSIONS

- With this approach all **reactions are performed in water**.
- In a **one-pot** reaction vessel
- All reaction steps can be followed by simple **colorimetric tests**.
- FT-IR and XPS (not shown) confirmed the **presence of peptides** on cotton.
- Varying the reaction time, peptide concentration and peptide excess, we can **modulate the cotton loading**: from 0.02-0.20 mmol/g cotton, quantified by UV.
- Thiazolidine bond allowed to bind **multiple peptides.**
- The **antibacterial** properties of PMAP(12-24) are maintained in the conjugate with CHO-cotton.
- Tests are undergoing to evaluate the **collagen regenerative properties** of 2a+4d-cotton.

REFERENCES

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UNI IMPRESA

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