Self-Assembling Peptides Forming Anti-Parallel β-sheet Nanostructures Cross-Linked with Genipin

Self-Assembled Peptide-Coated Fibers: Bilayer Materials for Sustainable Textile and Packaging Applications

Q. CHENIN[1,2], N. FOLLAIN[1], C. BELLO[2], L. ROSI[3], P. ROVERO[4], A.M. PAPINI[2]

[1] *Université de Rouen Normandie, INSA Rouen Normandie, Polymères Biopolymères Surface (PBS) UMR 6270 CNRS, 76000 Rouen, France*
[2] *Interdepartmental research Unit of Peptide and Protein Chemistry and Biology, University of Florence, 50019-Sesto Fiorentino (FI), Italy*
[3] *Department of Chemistry "Ugo Schiff", University of Florence, 50019-Sesto Fiorentino (FI), Italy*
[4] *Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, 50019-Sesto Fiorentino (FI), Italy*
https://doi.org/10.17952/38EPS.2026.P555
Quentin Chenin e-mail *: quentin.chenin@unifi.it*

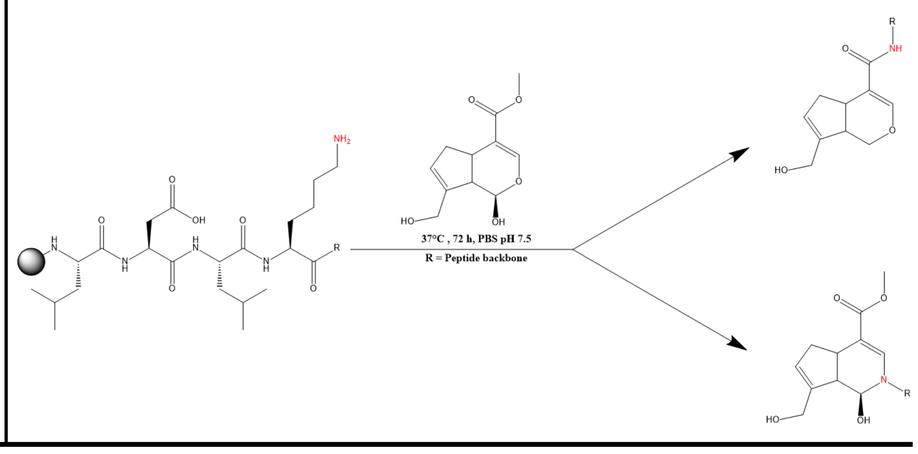
Self-assembling peptides are versatile biomolecular building blocks for hydrogel scaffolds in biomedical and technological applications. However, their limited mechanical stability often requires reinforcement. Here, two peptide sequences were designed from a parent sequence (1) identified in the N-terminal region of the *Helicobacter pylori* MutS2 protein and conserved among related *ε-proteobacteria* [1]. Peptides were synthesized by induction heating-assisted Solid-Phase Peptide Synthesis and used for hydrogel formation without chromatographic purification, reducing processing requirements while enabling mechanically reinforced ***materials for sustainable packaging and biomaterial applications***. The project leverages EUniWell's "infrastructure without walls," combining the University of Florence's expertise in sustainable peptide-based materials with the University of Rouen Normandie's advanced polymer characterization capabilities.

Peptide design, synthesis and characterization

Peptide	Mw (g/mol)	GRAVY score[2]	Isoelectric point	Average hydrophilicity	HPLC purity^{bp} (%)	HPLC purity^{ap} (%)	[M + H]+ calcd/found	Minimally processed yield (%)
LDLKLDLKLDLK-NH_2 (1)	1425.90	0.8375	9.68	0.6	77	88	1425.90/ 1427.58	84
DDLKLDLKLDLK-NH_2 (2)	1427.85	0.4917	7	1	68	83	1428.81/ 1427.85	80
LDLKLDLKLDLKFF-NH_2 (3)	1720.04	1.1179	9.68	0.16	70	88	1721.83/ 1720.04	52

bp : before purification; ap : after purification

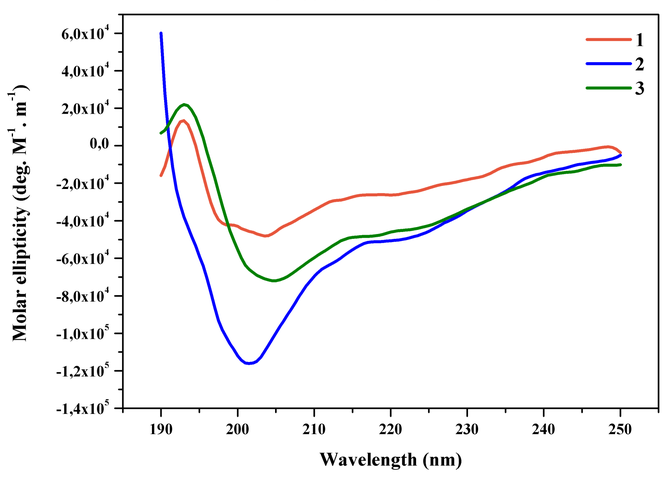
Genipin cross-linking



To enhance network stability, Genipin was investigated as a naturally derived cross-linker forming covalent networks through amino functionalities [3]. Genipin treatment improved the mechanical and thermal properties of hydrogels, increasing the storage modulus from 9 to 21 kPa and the residual mass from 1% to 26%. Notably, minimally processed peptides exhibited hydrogel-forming behavior comparable to purified materials, highlighting a more streamlined, cost-effective, and sustainable approach to peptide-based hydrogels.

Peptide morphology

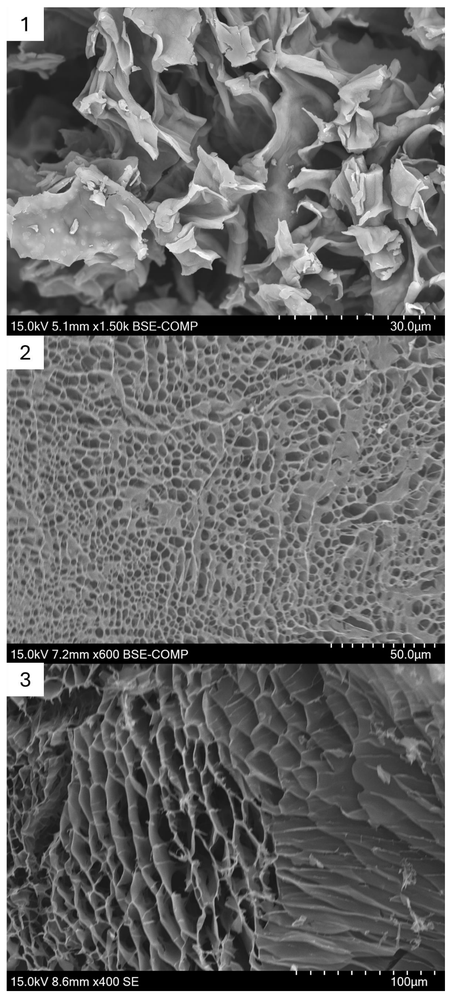
Circular dichroism



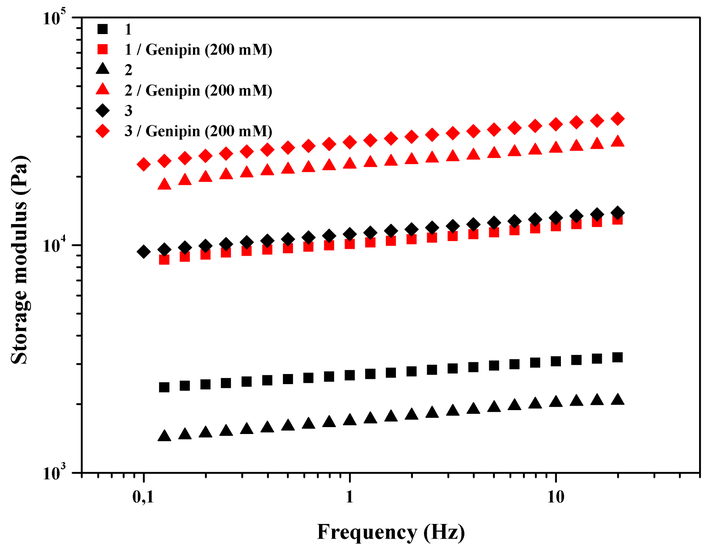
Secondary structures (%)	1	2	3
α-helix	0,8	6,4	2,3
β-sheets	0	3,1	1,7
Anti β-sheets	34,8	22,5	27,2
β-turns	16,1	14,2	15,3
Random coils	48,3	53,9	53,5

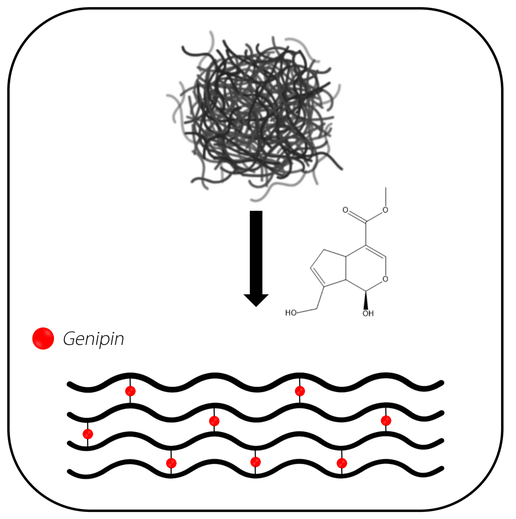
- ***Majority of anti-parallel β-sheets and random coil structures by CD measurements for all peptides.***
- ***Hydrogels showed a porous structure as made in evidence by scanning electron microscopy analysis.***

Scanning electron microscopy



Hydrogel rheology and peptide cross-linking





Conditions
0,1 – 100 Hz frequency – sweep at 25 °C, 1% strain,20 mm serrated parallel plate geometry

Improvement in the storage modulus G' after Genipin cross-linking (increase of G' up to 20-times).

- G' > G" for all tests, proving that the materials **exhibit solid-like viscoelastic behavior.**
- Peptide 3 shows higher initial storage modulus before cross-linking, as it binds less to water through hydrogen bonds, relying more on ***hydrophobic interactions.***
- Cross-linked hydrogels: storage modulus (G') ranging between 9-21 kPa.
- Unreacted hydrogels: G' values ranging between 1-10 kPa.

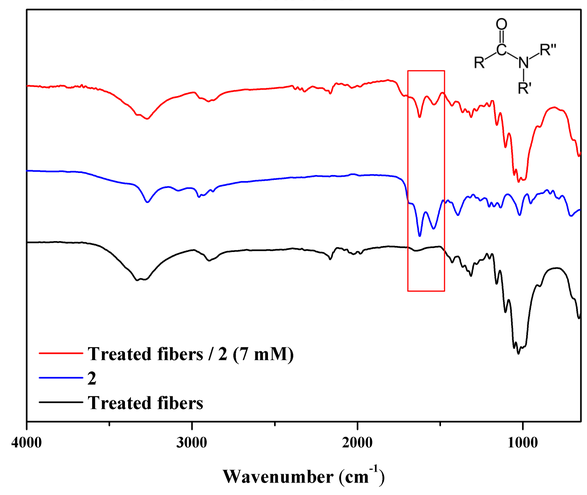
Conclusions and ongoing researches

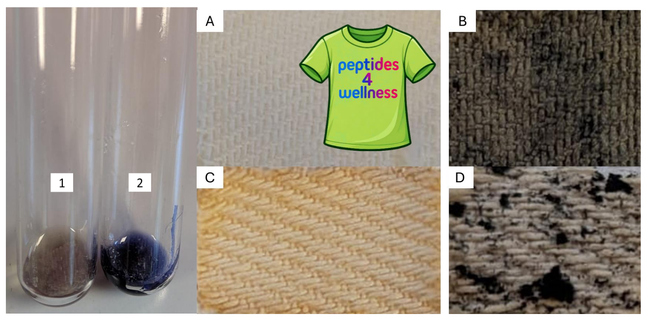
- ***Minimally processed peptides*** were obtained, employed for hydrogel formation, and subsequently cross-linked with Genipin.
- ***Thermal, mechanical and self-assembling properties*** of all peptides (1-3) in solution revealed chemical cross-linking with Genipin in the hydrogels.
- ***Grafting peptide DDLKLDLKLDLK-NH_2 (2) onto cellulose fibers*** paves the way for the development of stronger, environmentally friendly and ***biodegradable materials*** in textiles.

- Grafting ***RYamide*** onto fabrics could activate ***NPYLR7 signaling*** in mosquitoes, potentially inducing satiety and reducing biting behavior [4].
- Antiviral peptide-functionalized fibers could pave the way for next-generation protective masks designed to inhibit SARS-CoV-2 and reduce viral transmission.

Self-assembled peptide-fibers bilayer material

FTIR-ATR





(1) Kaiser test of raw cotton treated with peptide. (2) Kaiser test of oxidized cotton treated with peptide. (A) Untreated cotton fibers. (B) Oxidized cotton fibers treated with a Genipin solution, showing a homogeneous cross-linking distribution. (C) Oxidized cotton fibers treated with oxidized hyaluronic acid. (D) Unoxidized cotton fibers treated with a Genipin solution, showing a heterogenous cross-linking distribution.

Following oxidative treatment of textile cellulose fibers, a bilayer cellulose–peptide matrix is obtained through **Schiff base formation**. This complex can undergo further cross-linking with molecules such as Genipin via the amino groups of lysine residues.

Acknowledgments

This work is financially supported by XL-CHEM, PBS, PeptLab and the Italian Ministry of Health for funding the "borsa di ricerca" under project code RF-2021-12374177.
This project was funded in part by EUniWell: 5th seed funding call for the TTPep project and 3rd Well-Being Research Incubator Call for the project "Sustainable and green upscaling processes for peptide(-based) in vitro diagnostics and cosmeceuticals" and the Champion program "Peptides4Wellness".
Quentin Chenin gratefully acknowledges the European Peptide Society for the registration grant to attend the 38th European Peptide Symposium.

[1] P. P. Damke, R. Dhanaraju, S. Marsin, J. P. Radicella and D. N. Rao, Molecular Microbiology, 2015, 96, 1240–1256.
[2] J. Kyte and R. F. Doolittle, Journal of Molecular Biology, 1982, 157, 105–132.
[3] M. F. Butler, Y. Ng and P. D. A. Pudney, J. Polym. Sci. A Polym. Chem., 2003, 41, 3941–3953.
[4] C. Greppi, K. Frank, V. Saltz and L. B. Duvall, *Current Biology*, 2026, **36**, 1702-1715.e6.