Supplementary Information

Functionalization of cellulose nanofibrils to develop novel ROS-sensitive biomaterials

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1. Experimental section

1.1. Cytotoxicity study

Adult human dermal fibroblasts (hDFs, European Collection of Authenticated Cell Cultures, EACC) were cultured in DMEM-F12 medium supplemented with 10% v/v FBS, 100 u/mL penicillin, and 100 ug/mL streptomycin. Cells were cultured at 37°C and 5% CO_2 in a humidified atmosphere and passaged at 80% confluency.

The cytotoxicity of $AcKP_5NH_2$-CNF and $AcKP_{10}NH_2$-CNF was investigated by exposing hDF monolayers to suspensions of the materials at concentrations ranging from 0.1 to 1 mg/mL.

hDF cells were seeded in a 96 well-plate at a density of 4.8 x 10^3 cells/well and after 24 h of culture, they were exposed to the oligoproline-CNF suspensions (200 µL/well) and cultured for another 24 h. Non-exposed cells were the negative control and cells exposed to 5% DMSO in cell culture medium were the positive control. Cellular metabolic activity was measured with the presto blue (PB) assay after 24 h of exposure to the oligoproline-CNF materials. Cell culture medium was removed from the cell culture wells and 200 µL of PB reagent diluted 1:10 in cell culture medium was added to each well and incubated for 90 min at 37 °C, and 5% CO_2 in a humidified atmosphere. Aliquots of 100 µL were transferred to a black 96-well plate and fluorescence was measured at 560 nm excitation and 590 nm emission wavelengths using a plate reader (Tecan infinite M200, Switzerland). Cell metabolic activity was used as an indicator of cell viability and the results were expressed as the percentage of the negative control.

1.2. Statistical analysis

Data are presented as mean ± standard error of the mean from at least three independent experiments, each with triplicate sampling. Statistical analysis was performed using GraphPad Prism software. One sample *t*-test and paired *t*-test were used for the evaluation of the cytotoxic effects of

the different treatment groups. The differences were considered statistically significant when $p < 0.05$.

2. Results section

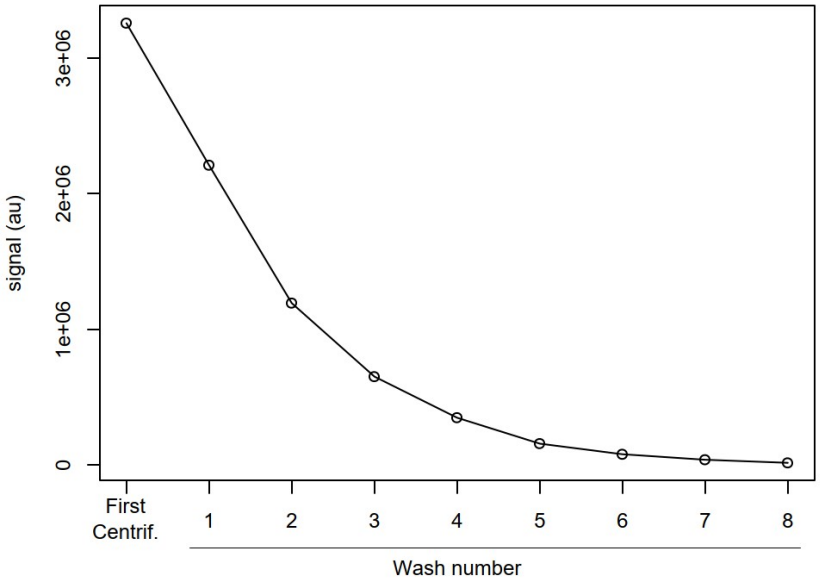


Figure S1. Liquid chromatography–mass spectrometry analysis of the washing supernatants after the consecutives washing steps. Free peptide in solution (unreacted peptide) is detected in the first centrifugation steps, with the amount of peptide decreasing with the subsequent washing steps. After the fifth wash with 0.1 M NaOH/ 0.05 M $NaHCO_3$ buffer (pH=11), the amount of free peptide is below the detection limit.

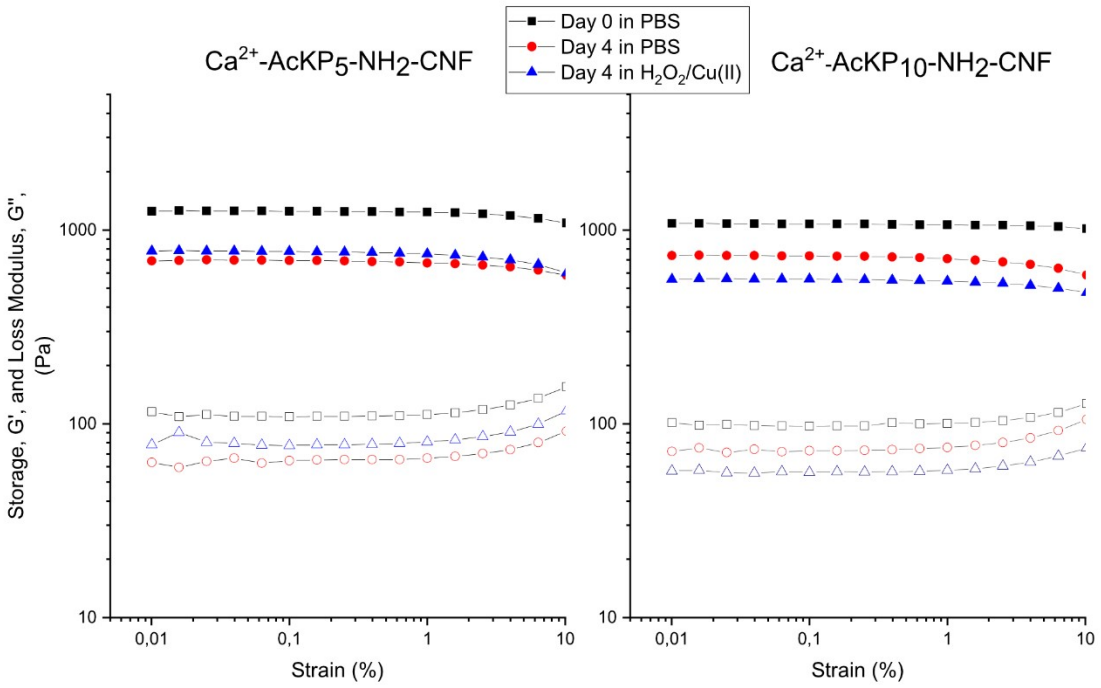


Figure S2: Amplitude sweep measurements of the double crosslinked Ca^{2+}-oligoproline-CNF hydrogels exposed to PBS and to an oxidative environment (H_2O_2) on day 0 and day 4. Filled symbols correspond to storage modulus G' and empty symbols to loss modulus G''. Data corresponds to average values with the standard deviation. A strain value within the LVR of 0.25% was selected for all samples for further evaluation of the materials with the frequency sweep.

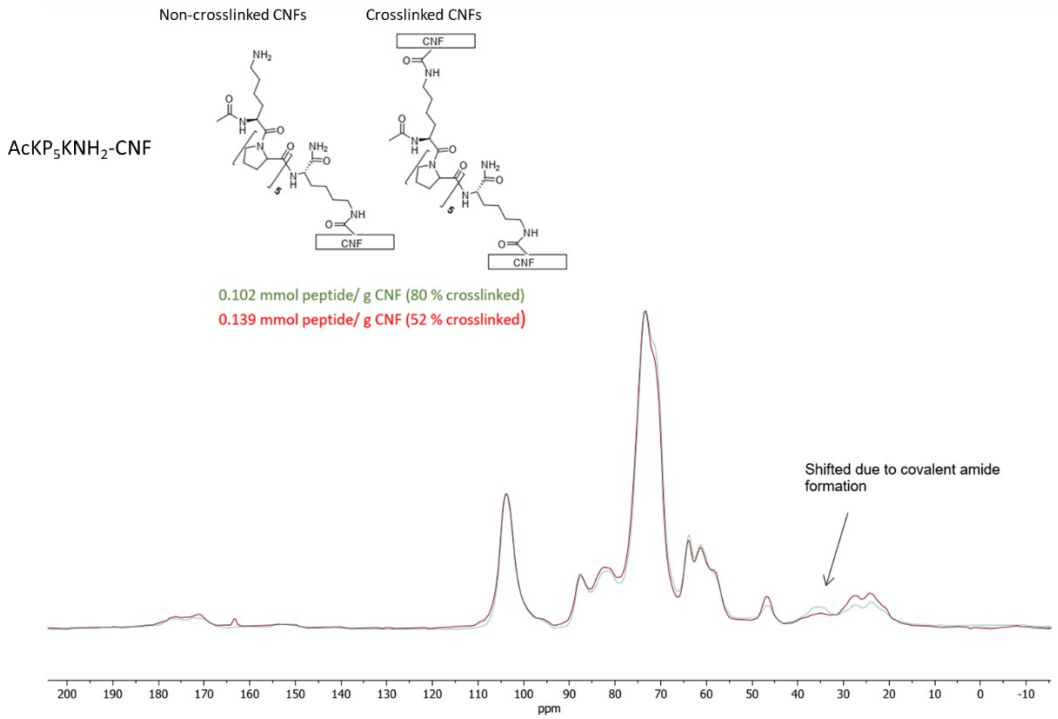


Figure S3. Stacked solid-state nuclear magnetic resonance (CP/MAS ^{13}C-NMR) spectrum of AcKP$_5$KNH$_2$-CNF materials**.** Two AcKP$_5$NH$_2$-CNF materials with different degree of substitution and crosslinking were chosen for this analysis (0.102 mmol peptide/g CNF, 80% degree of crosslinking vs 0.139 mmol peptide/g CNF, 52% degree of crosslinking). The sifted signal observed around 36 ppm is assigned to the formation of amide bonds. Such signal presents higher intensity for the material with higher degree of crosslinking even though the amount of peptide incorporated is lower in this case. Thus, the increase in the signal corresponds to an increase in amide formation due to crosslinking rather than an increase in peptide substitution.

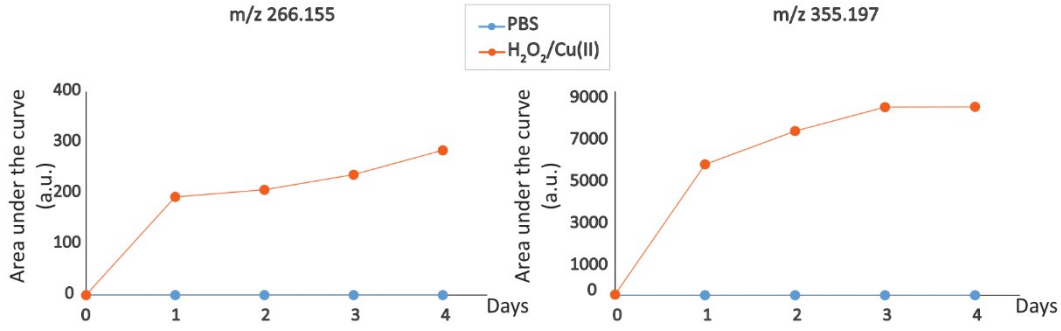


Figure S4. Oxidation of the KP$_5$K-CNF material (day 0-day 4) detected by the increase of the oxidation by-products with m/z 226.155 (left panel) and m/z 355.197 (right panel). Non-oxidative conditions (KP$_5$K-CNF in PBS) in blue; oxidative conditions in orange (KP$_5$K-CNF in H_2O_2/Cu(II)). The oxidation by-products increased with time in the oxidative conditions, but were not detected in the non-oxidative environment. The signals of the products were quantified by the area under the curve (AUC, arbitrary units) by using TargetLynx (MassLynx V4.1 SCN871, Waters).

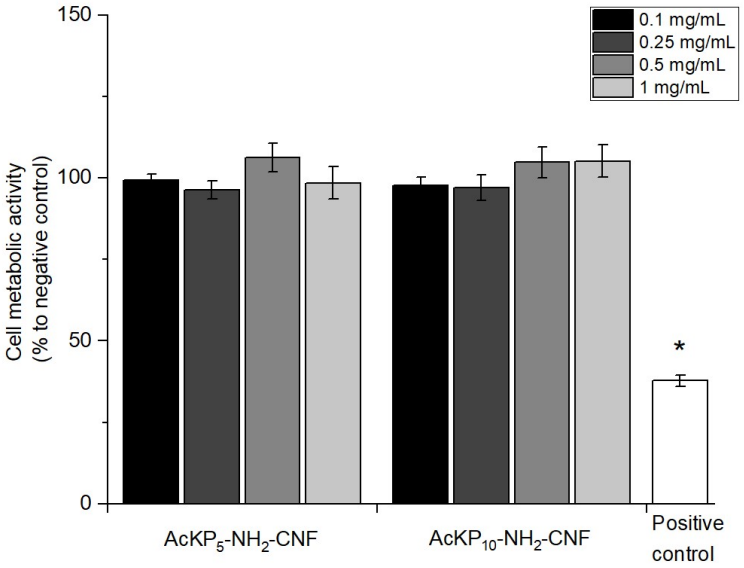


Figure S5. Metabolic activity of human dermal fibroblasts exposed to increasing concentrations of Ac-KP_5K-NH_2-CNF and Ac$KP_{10}K$-NH_2-CNF (0.1-1 mg/ml) during 24 h. Data are expressed as percentage relative to the negative control (non-exposed cells) and presented as mean ± standard error of the mean of three independent experiments. The materials did not significantly affect the metabolic activity of the cells, with all values above the 70% cytotoxicity limit [1] and not significant different from the negative control. Significant differences as compared to the positive control and the 70% cytotoxicity limit are marked with * ($p<0.05$).

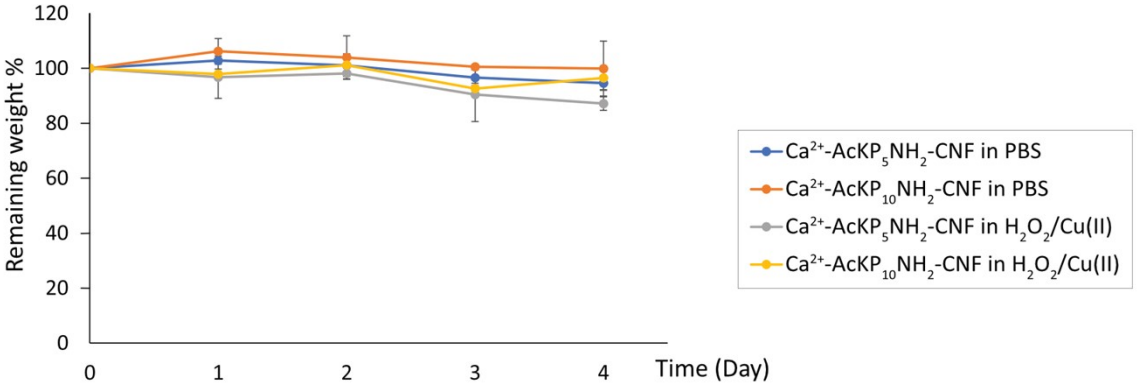
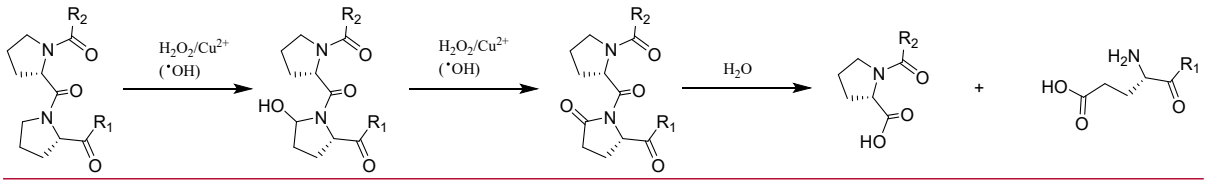


Figure S6. Remaining weight percentage of the Ca^{2+}-oligoproline-CNF hydrogels after incubation in PBS and in an oxidative environment (H_2O_2/Cu(II) in PBS).

3. Proposed mechanisms for the ROS-mediated degradation of oligoproline.



Scheme S1. Feasible scheme for the degradation of oligoproline peptides mediated by hydroxyl radicals. Formation of pyrrolidone derivative followed by tertiary amide cleavage.[2]

References

1. Iso 10993-5: Biological evaluation of medical devices - part 5: Test for in vitro cytotoxicity. Geneva, Switzerland.
2. R. Kawasaki, K. Tsuchiya, Y. Kodama and K. Numata, *Biomacromolecules*, 2020, **21**, 4116–4122.