SUPPLEMENTARY INFORMATION

Raman Spectroscopy

Materials and Methods: Infrared spectroscopic analysis was complemented by capitalizing on the Raman module of the alpha300 RSA system (WITec, Germany). Single spectra were collected from three randomly selected regions of each sample using a 785 nm Toptica XTRA Laser (Germany, 40 mW, acquisition time = 0.2 sec) through a 50X objective (EC Epiplan NEOFLUAR, NA = 0.9, Zeiss). Spectra were composed of 10 accumulations at an integration time of 30 s and a laser power at the objective of 25 mW. Analysis of the Raman bands in the 700–1900 cm^{-1} region was carried out in OriginPro following the same processing described for the FT-IR analysis, with the additional use of the Savitzky-Golay smoothing filter before baseline subtraction. Raman peak assignment was carried out according to previous literature (**Table S1**). (1–4)

Results and Discussion: Spectra extracted from both treated samples showed an evident increase of the C-C stretching band at 934 cm^{-1} (R1) suggesting the presence of CA molecules. (1,2) **Figure S1B** (*left*) shows the normalized area increase relative to this band, which varied from an average of 1.1 for the NC condition to 2.2 and 2.5 for the C165 and C190 samples, respectively. Furthermore, the creation of covalent bonds due to the crosslinking process was demonstrated by the significant increase relative to the normalized area of the band at 1328 cm^{-1} associated with the C-N stretching vibration of the Amide III (R5), (3,4) which varied from 1.8 in NC samples to 4.3 and 4.1 for the C165 and C190 conditions, respectively (**Figure S1B,** *right*). The combined use of FTIR and Raman spectroscopy provided consistent information regarding the chemical arrangement resulting from the CA-based crosslinking process employed in this work. Both FTIR and Raman analysis of treated samples reflected the presence of CA molecules as well as the formation of new C-N covalent bonding, thus providing significant evidence to validate the successful crosslinking process.

Table S1. Raman vibrational modes.

RAMAN PEAK ASSIGNMENT		
Ref.	**Position (cm^{-1})**	**Description**
R1	934 ± 3	C-C (stretch.), C-H (def. out plane)
R2	1049 ± 1	C-O (stretch.), C-CH_2 (stretch.)
R3	1151 ± 3	C-O-C (stretch.)
R4	1261 ± 2	C-H (def. in plane), C-O (stretch.)
R5	1328 ± 3	C-N (stretch.); [*Amide III*]
R6	1371 ± 1	CH_3 (def. in plane), CH_2 (def. in plane)
R7	1411 ± 2	C-H (def. sym.)
R8	1592 ± 2	N-H; [*Amide II*]
R9	1656 ± 4	C=O; [*Amide I*]

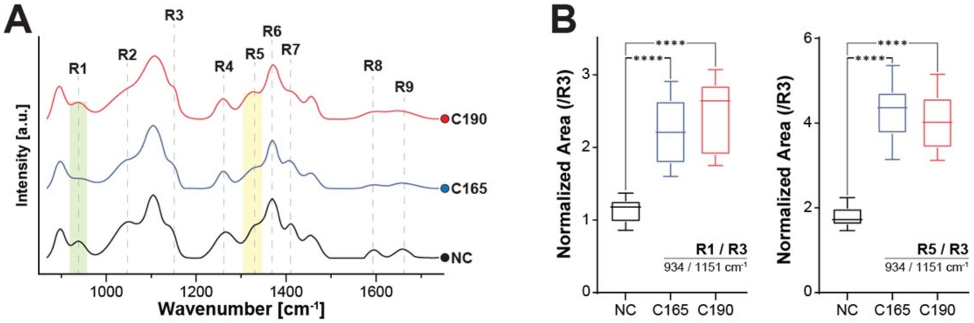

Figure S1. A) Representative Raman spectra for as-deposited (NC) and crosslinked chitosan treated at 165 °C (C165) and 190 °C (C190). For clarity, each band was labeled with a code ranging from R1 to R9, reported in Table S1. B) Quantification of R1/R3 and R5/R3 peak area ratios obtained from Raman spectra.

Supplementary Images

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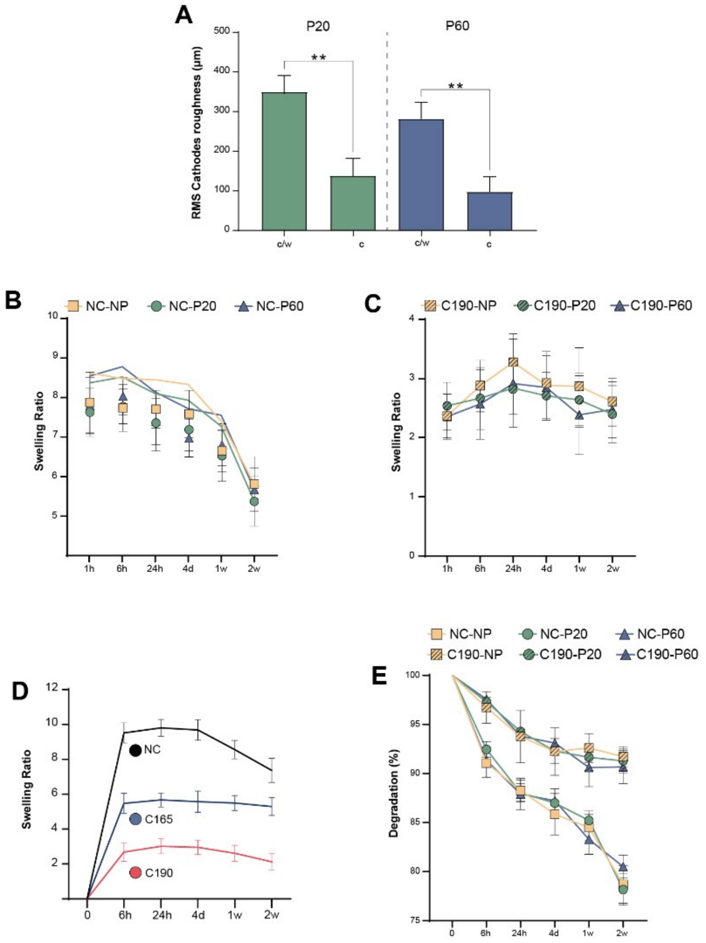

Figure S2. A) RMS roughness of laser-ablated patterned cathodes. Measurements were taken at the edges of the engravings (*c/w*, mirroring the channel wall/interface on the patterned chitosan samples) and in the central plane between engravings (*c*, mirroring the channel's central region on the patterned chitosan samples). B) Swelling behavior of non-crosslinked chitosan samples with different surface micro-topographies. C) Swelling behavior of CA-crosslinked samples with different surface micro-topographies. D) Adjusted swelling behavior to account for mass loss. E)

Degradation profiles of both non-crosslinked and CA-treated chitosan accounting for all topographies.

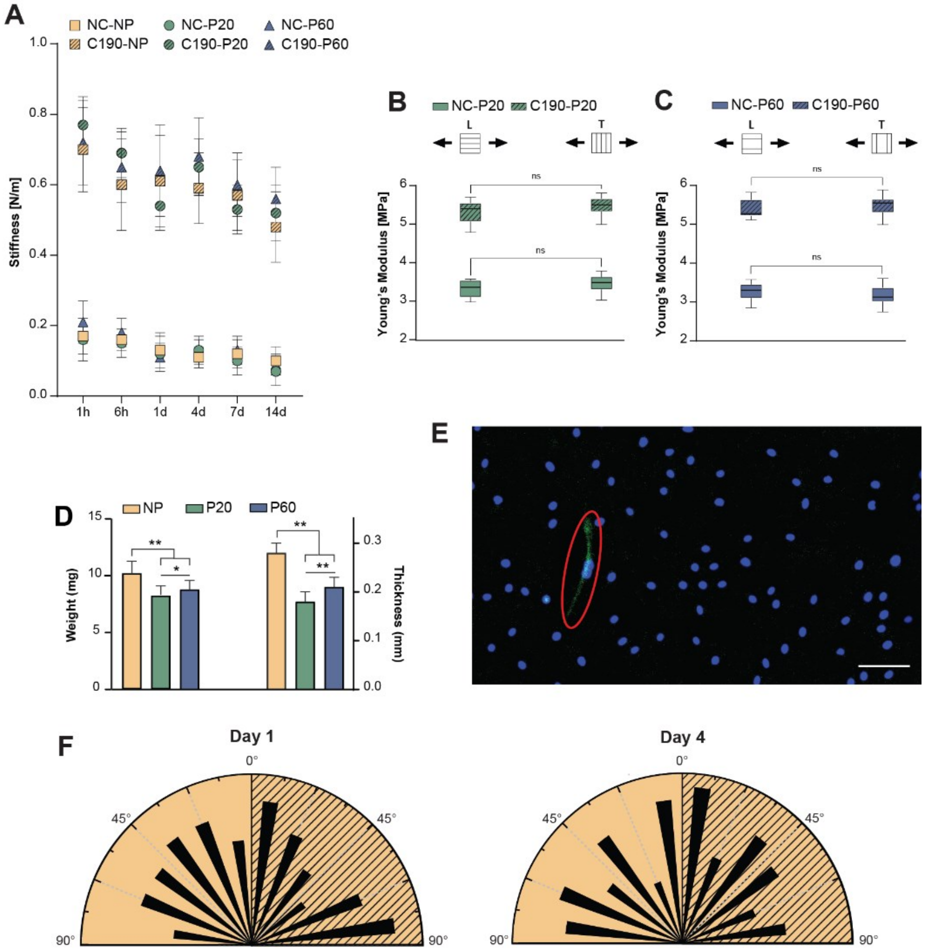

Figure S3. A) Surface stiffness obtained via AFM indentations of un-patterned (NP) and patterned (P20/P60) substrates, both as-deposited (NC) and CA-crosslinked at 190 °C (C190). B) P20 average Young's modulus for both non-crosslinked (*bottom*) and crosslinked (*top*) conditions, obtained by applying longitudinal (*left*) and transversal (*right*) load during the axial testing. C) P60 average Young's modulus for both non-crosslinked (*bottom*) and crosslinked (*top*) conditions, obtained by applying longitudinal (*left*) and transversal (*right*) load in axial testing. D) Weight and thickness of electrodeposited chitosan films. E) hMSCs tubulin expression after 24h. The red circle underlines cells expressing tubulin. Scale bar: 100 µm. F) hMSCs body orientation at 1 day (*left*) and 4 days (*right*) on non-patterned samples with respect to a randomly selected preferential direction.

References

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