

Supporting Information

Polycaprolactone/Sodium Alginate Coaxial Wet-Spun Fibers modified with Carbon Nanofibers and Ceftazidime for Improved Clotting and Infection Control in Wounds

Elina Marinho¹, Beatriz M. Silva¹, Catarina S. Miranda¹, Sonia L.C. Pinho^{2,3,4}, Helena P. Felgueiras^{1*}

¹Centre for Textile Science and Technology (2C2T), University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal; elinamarinho@2c2t.uminho.pt (E.M.); pg50248@alunos.uminho.pt (B.M.S.); catarina.miranda@2c2t.uminho.pt (C.S.M.); helenafelgueiras@2c2t.uminho.pt (H.P.F.).

²Center for Neuroscience and Cell Biology (CNC), Centre for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, 3004-517, Coimbra, Portugal

³ Research Centre for Natural Resources, Environment and Society (CERNAS), Coimbra Agriculture School, Polytechnic of Coimbra, 3045-601 Coimbra, Portugal

⁴ Vasco da Gama Research Center (CIVG), Vasco da Gama University School, 3020-210 Coimbra, Portugal (S.L.C.P.)

* Correspondence: helenafelgueiras@2c2t.uminho.pt; Tel.: +351-253-510-283; Fax: +351-253-510-293

Section 1. Brightfield Microscopy

The fiber outer layer thickness, core diameter and total diameter were determined using brightfield optical microscopy and listed in Table S1. It was possible to verify the production of fibers with a coaxial structure, whether in the form of controls or the complete formulations.

Table S1. Fiber diameters determined from 5 images at 5× magnification in which 5 measurements were performed per image, using ImageJ software (averaging 25 measurements).

Fibers	Shell diameter (μm)	Inner diameter (μm)	Total diameter (μm)
PCL	427 ± 4	-	427 ± 4
SA	-	111 ± 7	111 ± 7
SA/CZ	-	152 ± 4	152 ± 4
PCL - SA	432 ± 61	85 ± 14	517 ± 60
PCL - SA/CZ	528 ± 80	84 ± 22	613 ± 79
PCL/CNFs 50	428 ± 2	-	428 ± 2
PCL/CNFs 100	476 ± 5	-	476 ± 5
PCL/CNFs 150	495 ± 3	-	495 ± 3
PCL/CNFs 50 - SA	376 ± 33	71 ± 10	447 ± 35
PCL/CNFs 100 - SA	404 ± 45	109 ± 19	513 ± 44
PCL/CNFs 150 - SA	426 ± 88	129 ± 40	554 ± 120
PCL/CNFs 50 - SA/CZ	451 ± 32	137 ± 38	587 ± 40
PCL/CNFs 100 - SA/CZ	470 ± 93	140 ± 24	609 ± 95
PCL/CNFs 150 - SA/CZ	492 ± 63	137 ± 9	629 ± 62

Section 2. ATR-FTIR

ATR-FTIR spectra analysis was performed on all fiber typologies, in which several characteristic peaks were detected and listed in Table S2, confirming the presence of PCL and SA on the fibers.

Table S2. List of characteristic peaks of PCL and SA detected by ATR-FTIR technique.

Wavenumber (cm ⁻¹)	Description of vibrations
≈ 3302	-OH groups
2940, 2870	C-H stretching vibrations
1725	C=O stretching vibrations

1600, 1422	COO ⁻ group vibrations
1248, 1170	C-O-C vibrations

Figure S1 shows the ATR-FTIR data of the CNFs. This sample was completely ground together with 1% dry KBr and pressed into a pellet about 1 mm thickness. This pellet was then analyzed by IR transmission, noting that KBr is IR transparent, and the resulting spectra are shown in figure S1. The peak between 1580 and 1650 cm^{-1} can be attributed to the C=C stretching mode associated with surface defects of nanofibers

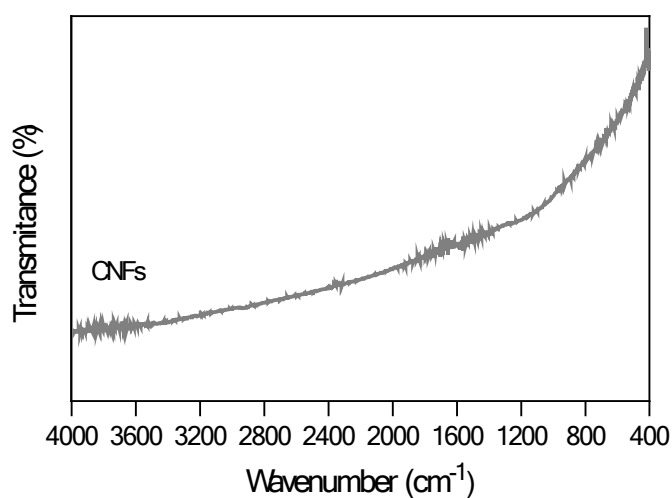


Figure S1. ATR-FTIR spectra of CNFs in the range of 4000-400 cm^{-1} .

Section 3. DSC

DSC measurements were conducted to evaluate the thermal behavior of the fibers. Various temperatures were recorded, along with the different enthalpies for each characteristic peak. The results are listed in Table S3.

Table S3. Main DSC thermal events (T_O onset temperature, T_P peak temperature, T_e endset temperature, and ΔH enthalpy).

Fibers	Endothermic I (°C)				Endothermic II (°C)				Endothermic III (°C)				Exothermic I (°C)				Exothermic II (°C)				Exothermic III (°C)			
	T_O	T_P	T_e	ΔH (J/g)	T_O	T_P	T_e	ΔH (J/g)	T_O	T_P	T_e	ΔH (J/g)	T_O	T_P	T_e	ΔH (J/g)	T_O	T_P	T_e	ΔH (J/g)	T_O	T_P	T_e	ΔH (J/g)
PCL	51.7 7	59.75	65.38	-62.74	379.3 6	413.3 0	444.2 1	- 559.4 9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
SA	61.5 9	105.3 9	147.4 1	-349.51	---	---	---	---	---	---	---	---	218.4 7	227.7 9	236.6 5	72.4 6	260.3 2	290.5 2	303.5 5	69.86	425.0 2	437.6 0	456.2 0	31.4 0
SA/CZ	61.5 4	108.5 5	152.8 0	-252.51	---	---	---	---	---	---	---	---	221.8 5	234.6 0	244.0 1	57.9 4	260.5 4	293.5 8	306.7 7	59.97	419.4 3	441.1 4	459.6 7	37.2 0
PCL - SA	44.1 9	51.70	55.59	-34.15	345.5 9	349.0 4	358.1 7	-56.76	---	---	---	---	234.1 4	244.5 6	248.2 7	21.7 0	447.1 0	450.1 1	453.9 9	1.18	---	---	---	---
PCL - SA/CZ	40.5 9	46.05	49.40	-25.48	78.55	101.1 5	122.0 8	-20.94	---	---	---	---	232.6 9	248.4 2	252.7 6	6.22	436.3 0	440.7 4	445.0 2	1.41	---	---	---	---
PCL/CNFs 50	52.4 1	58.65	63.85	-61.80	381.7 5	417.4 7	447.3 8	- 434.5 8	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PCL/CNFs 100	52.7 0	59.71	65.06	-57.10	382.3 9	417.6 0	446.8 2	- 393.3 2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PCL/CNFs 150	53.2 0	59.89	65.28	-52.66	393.9 9	419.0 6	444.6 9	- 268.0 2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
PCL/CNFs 50 – SA	44.3 2	51.99	55.76	-27.62	59.34	75.89	101.1 0	-9.12	346.5 9	354.0 3	379.6 1	- 113.6 9	229.0 0	248.6 9	263.4 8	48.1 9	396.1 3	420.9 0	433.2 4	97.23	---	---	---	---
PCL/CNFs 100 – SA	44.1 8	49.26	52.47	-27.32	71.71	103.0 4	123.8 1	- 198.8 7	319.1 7	342.6 7	383.1 4	- 118.4 3	233.4 3	250.9 1	258.1 5	14.8 6	383.9 2	391.3 2	395.4 3	3.58	---	---	---	---
PCL/CNFs 150 – SA	45.4 9	53.76	57.83	-43.78	63.06	85.20	108.6 3	-17.16	373.3 2	403.8 7	422.7 6	- 427.7 9	246.9 2	262.9 4	269.6 7	17.6 2	---	---	---	---	---	---	---	---
PCL/CNFs 50 - SA/CZ	40.6 5	49.22	53.00	-38.75	60.56	88.27	117.6 9	-22.69	398.5 8	401.0 3	414.6 4	-65.26	236.2 2	249.7 1	257.9 5	31.8 2	440.7 5	449.7 5	455.6 6	9.01	---	---	---	---

PCL/CNFs 100 SA/CZ	38.9 1	47.46	51.90	-29.04	58.15	84.32	111.4 7	-23.35	354.1 0	402.7 2	424.5 1	- 251.9 3	253.0 5	255.7 3	267.4 6	24.5 8	---	---	---	---	---	---	---	---
PCL/CNFs 150 SA/CZ	40.3 3	47.06	50.29	-25.39	106.8 8	128.9 5	159.2 5	- 221.6 7	402.1 2	420.0 7	430.3 8	- 123.6 7	240.5 2	256.2 4	262.8 3	20.5 2	---	---	---	---	---	---	---	---

Section 4. TGA

Figure S2 shows the TGA data of PCL, SA and CZ powder.

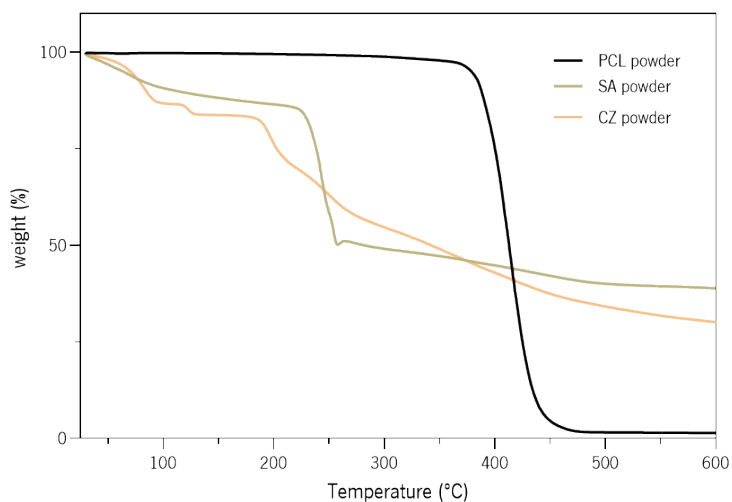


Figure S2. TGA thermograms obtained from 25 to 600 °C under a nitrogen atmosphere with a flow rate of 200 mL/min and temperature increase of 10 °C/min, for PCL, SA and CZ powders.

Section 5. Mechanical Performance

To complement the results of the mechanical test of the fibers, the maximum force and the tensile strength were also calculated and listed in Table S4.

Table S4. Mechanical examinations of the engineered wet-spun fibers. Data are reported as mean $\pm$ SD (n = 5).

Fibers	Maximum Force (N)	Maximum elongations at break (%)	Tensile Strength (kPa)
PCL	0.17 $\pm$ 0.02	169 $\pm$ 33	173 $\pm$ 18
SA	0.44 $\pm$ 0.14	4 $\pm$ 4	458 $\pm$ 61
SA/CZ	0.33 $\pm$ 0.14	5 $\pm$ 3	367 $\pm$ 25
PCL - SA	0.13 $\pm$ 0.01	234 $\pm$ 86	134 $\pm$ 11
PCL - SA/CZ	0.15 $\pm$ 0.03	269 $\pm$ 94	154 $\pm$ 30
PCL/CNFs 50	0.05 $\pm$ 0.00	187 $\pm$ 44	48 $\pm$ 4
PCL/CNFs 100	0.15 $\pm$ 0.02	313 $\pm$ 45	93 $\pm$ 16
PCL/CNFs 150	0.09 $\pm$ 0.02	347 $\pm$ 29	152 $\pm$ 17
PCL/CNFs 50 - SA	0.15 $\pm$ 0.03	207 $\pm$ 49	156 $\pm$ 29
PCL/CNFs 100 - SA	0.13 $\pm$ 0.02	331 $\pm$ 42	132 $\pm$ 22
PCL/CNFs 150 - SA	0.13 $\pm$ 0.04	355 $\pm$ 36	133 $\pm$ 35
PCL/CNFs 50 - SA/CZ	0.14 $\pm$ 0.02	292 $\pm$ 29	134 $\pm$ 16
PCL/CNFs 100 -	0.11 $\pm$ 0.02	348 $\pm$ 15	128 $\pm$ 19

SA/CZ			
PCL/CNFs 150 -	0.21 ± 0.04	376 ± 36	166 ± 27
SA/CZ			

The results obtained in mechanical tests confirmed the elasticity of the fiber shell, with the core breaking faster than the shell (Figure S3).



Figure S3. Visual confirmation of the presence of a shell and core (PCL - SA) in the fibers.

Section 6. Fibers Degradation

Fibers were visually evaluated after different periods of incubation in PBS over 28 days to test their structural integrity. The results confirmed that all wet-spun fibers (except SA and SA-CZ) maintained their structures until the 28th day of incubation, as shown in Figure S4.

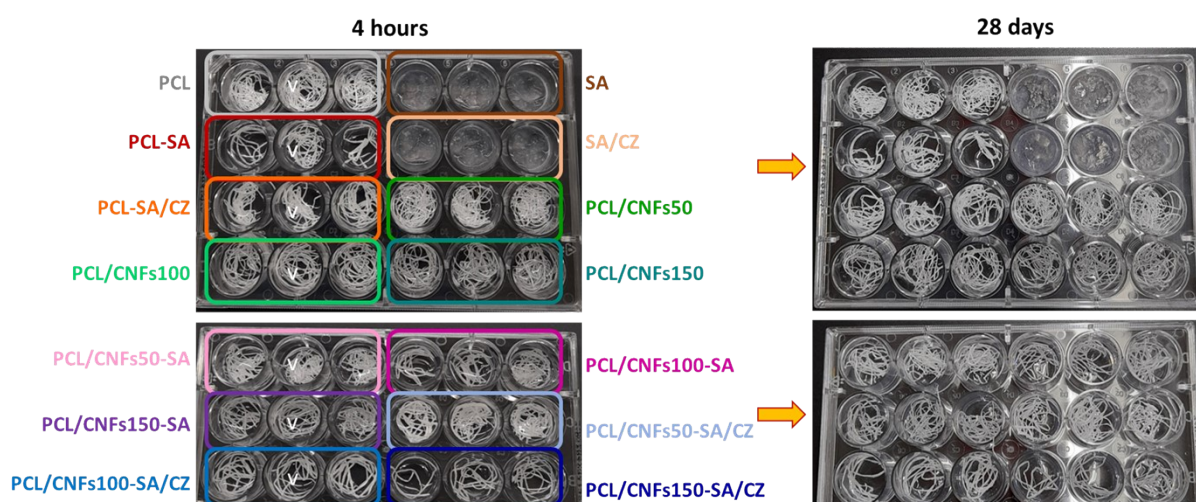


Figure S4. Wet-spun fibers before and after being incubated in PBS for 28 days.

Water retention (WR) was determined by assessing the ability of the fibers to retain water over different incubation periods in PBS (0.1 M) at 37 °C. The WR values for the various materials are presented in Table S5.

Table S5. Wet-spun fibers water retentions over 7 days of incubation in PBS. Data are presented as average percentage of water retention (WR) $\pm$ SD (n = 3).

WR (%)						
Fibers	Hour 1	Hour 4	Day 1	Day 2	Day 3	Day 7
PCL	142.2 $\pm$ 33.1	201.1 $\pm$ 67.2	201.5 $\pm$ 61.1	226.5 $\pm$ 65.1	203.8 $\pm$ 37.8	204.2 $\pm$ 28.3
SA	1983.1 $\pm$ 329.6	2502.5 $\pm$ 158.2	2462.5 $\pm$ 148.1	2277.7 $\pm$ 186.5	1867.2 $\pm$ 321.5	517.7 $\pm$ 241.2
SA / CZ	2021.4 $\pm$ 94.4	2303.2 $\pm$ 46.6	2323.9 $\pm$ 15.1	2265.1 $\pm$ 41.6	2247.7 $\pm$ 159.6	1518.2 $\pm$ 704.0
PCL - SA	266.5 $\pm$ 93.3	154.0 $\pm$ 55.3	95.2 $\pm$ 28.6	92.9 $\pm$ 25.9	87.4 $\pm$ 31.4	84.4 $\pm$ 30.4
PCL - SA / CZ	398.4 $\pm$ 66.1	319.8 $\pm$ 75.7	181.8 $\pm$ 65.9	157.8 $\pm$ 42.5	134.5 $\pm$ 34.3	110.9 $\pm$ 15.2
PCL / CNFs50	211.9 $\pm$ 46.4	151.5 $\pm$ 25.4	152.9 $\pm$ 24.5	181.1 $\pm$ 19.1	204.6 $\pm$ 11.7	242.7 $\pm$ 38.5
PCL / CNFs100	159.2 $\pm$ 34.7	151.1 $\pm$ 12.4	175.6 $\pm$ 30.3	192.0 $\pm$ 16.2	199.9 $\pm$ 27.3	224.6 $\pm$ 28.9
PCL / CNFs150	131.1 $\pm$ 2.8	134.3 $\pm$ 5.1	151.9 $\pm$ 9.5	164.3 $\pm$ 13.6	187.1 $\pm$ 12.1	216.6 $\pm$ 20.6
PCL / CNFs50 - SA	324.1 $\pm$ 37.9	213.3 $\pm$ 29.3	181.9 $\pm$ 28.8	146.6 $\pm$ 31.8	129.0 $\pm$ 32.7	138.7 $\pm$ 35.5
PCL / CNFs100 - SA	442.6 $\pm$ 120.4	315.0 $\pm$ 137.8	259.0 $\pm$ 163.0	134.4 $\pm$ 78.0	152.4 $\pm$ 64.6	167.1 $\pm$ 47.8
PCL / CNFs150 - SA	203.6 $\pm$ 18.5	158.3 $\pm$ 5.0	155.1 $\pm$ 7.3	151.0 $\pm$ 15.6	149.5 $\pm$ 4.7	172.4 $\pm$ 10.4
PCL / CNFs50 - SA / CZ	437.1 $\pm$ 80.1	420.8 $\pm$ 73.4	329.4 $\pm$ 40.8	254.6 $\pm$ 13.9	233.2 $\pm$ 8.9	262.5 $\pm$ 4.4
PCL / CNFs100 - SA / CZ	343.1 $\pm$ 94.5	244.0 $\pm$ 56.5	125.6 $\pm$ 21.6	120.3 $\pm$ 19.6	110.2 $\pm$ 38.7	117.5 $\pm$ 52.4
PCL / CNFs150 - SA / CZ	232.7 $\pm$ 42.9	144.9 $\pm$ 24.2	121.8 $\pm$ 14.8	106.2 $\pm$ 15.0	104.6 $\pm$ 15.9	114.1 $\pm$ 0.7

Section 7. Bacterial Time-Kill Kinetics

The following figure presents representative images of bacterial colonies of *S. aureus* and *P. aeruginosa* after 2, 6 and 24 hours of incubation, illustrating the morphological changes over time, as shown in Figure S5.

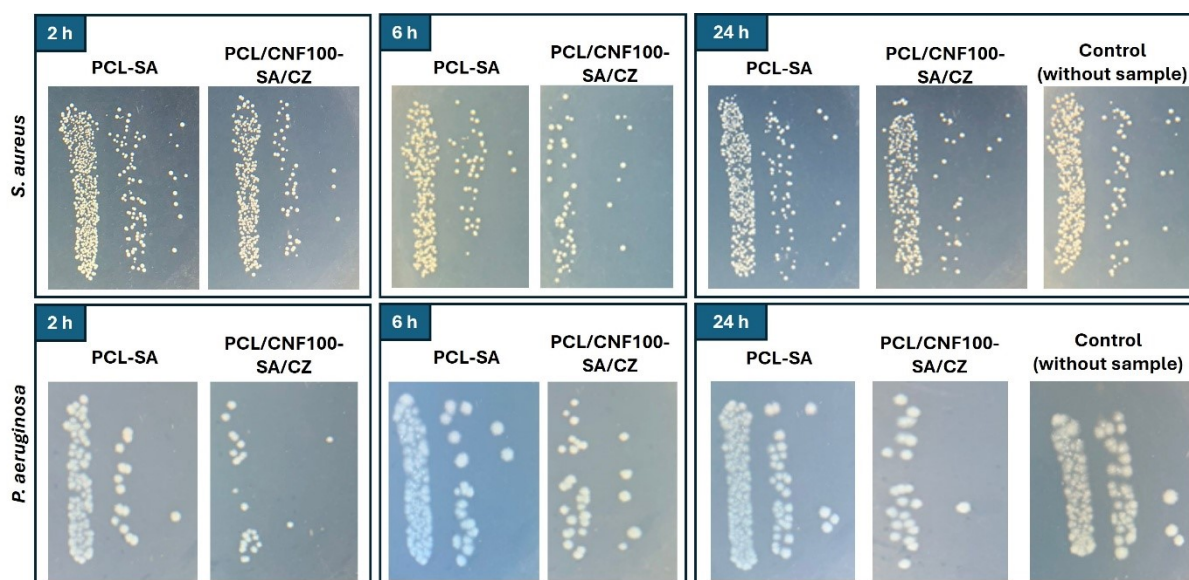


Figure S5. Representative images of bacterial colony morphology for *S. aureus* and *P. aeruginosa* at three time points: 2 hours, 6 hours and 24 hours of incubation. Presence and absence of the additives CBFs and CZ did not alter the bacteria colonies morphologies.

Section 8. Cytocompatibility

The following images, presented in Figure S6, illustrate the cell morphology observed during the cytocompatibility evaluation, corresponding to the incubation periods and conditions described.

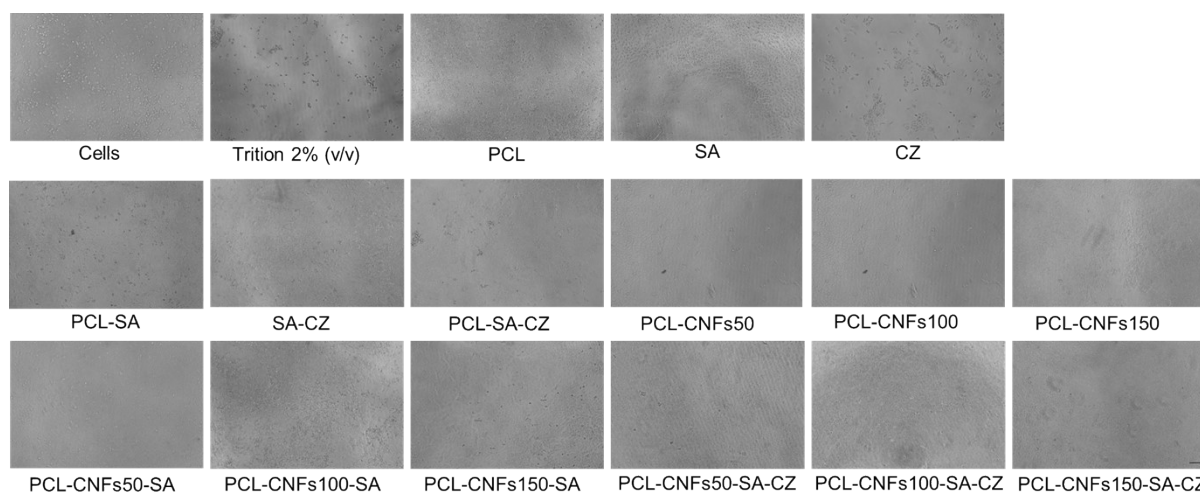


Figure S6: Images of cell morphology corresponding to the cytocompatibility evaluation, showing the effects of different incubation periods and conditions.

