Supplementary data

Superabsorbent crosslinked bacterial cellulose biomaterials for chronic wound dressings

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Abbreviations

BC – bacterial cellulose; CAT – catalyst; CA – citric acid; M1 – modification with disodium phosphate as a catalyst (CAT); M2 – modification with sodium bicarbonate as a CAT; M3 – modification with mixture of disodium phosphate and sodium bicarbonate in the ratio 1:1 as a CAT; M4 – modification with ammonia as a CAT; M5 – modification with disodium phosphate and ammonia in the ratio 1:1 as a CAT; M6 – modification with sodium bicarbonate and ammonia in the ratio 1:1 as a CAT; M7 – modification with sodium hypophosphite as a CAT; SHP – sodium hypophosphite; SR – swelling ratio; WHC – water holding capacity; WPG – weight percent gain.

1. Optimization of BC cross-linking reaction

1.1. Percentage of citric acid (CA) and catalysts (CAT) solutions

Tab. S1. Swelling ratio (%) after 24 h of each BC modification depending on the percentage of citric acid (CA) and catalysts (CAT) solution.

Type of modification	CA30% + CAT15%	CA20% + CAT10%	CA10% + CAT5%	CA5% + CAT2,5%
M1	2449.16 ± 156.45	**2528.32 ± 192.08**	1826.14 ± 100.02	1517.57 ± 62.19
M2	**2232.43 ± 112.60**	**2201.56 ± 50.66**	1039.72 ± 46.89	933.58 ± 63.76
M3	3154.53 ± 124.08	**3377.09 ± 184.43**	1818.91 ± 57.98	1499.65 ± 112.31
M4	**1084.74 ± 80.41**	**1082.45 ± 131.98**	968.81 ± 68.64	966.31 ± 38.34
M5	2292.05 ± 141.70	**2324.66 ± 22.52**	1786.63 ± 60.10	1186.33 ± 50.10
M6	2247.87 ± 200.02	**2343.81 ± 43.81**	1556.29 ± 135.01	1176.79 ± 53.98
M7	2016.87 ± 152.37	**2033.25 ± 118.50**	1245.54 ± 162.51	1049.19 ± 84.81

Tab. S2. Statistical differences between SR [%] values after 24 h, depending on percentage of CA and CAT solutions.

	CA30% + CAT15%	CA20% + CAT10%	CA10% + CAT5%	CA5% + CAT2,5%	CA20%
CA30% + CAT15%	×	**ns**	****	****	****
CA20% + CAT10%	ns	×	****	****	****
CA10% + CAT5%	****	****	×	*	****
CA5% + CAT2,5%	****	****	*	×	****
CA20%	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

1.2. Citric acid and catalysts solutions mass ratio

Tab. S3. Swelling ratio [%] after 24 h of each BC modification depending on the CA:CAT ratio.

Type of modification	CA : CAT					
	1:1	1.5:1	2:1	2.5:1	3:1	1:2
M1	2012.14 ± 109.11	2228.20 ± 62.50	**2468.47 ± 34.69**	2083.67 ± 62.41	2074.08 ± 69.13	743.71 ± 109.19
M2	1887.34 ± 87.49	2010.88 ± 79.14	**2178.67 ± 61.31**	2036.58 ± 57.11	2073.20 ± 146.58	685.82 ± 85.04
M3	2400.17 ± 147.02	3060.65 ± 89.58	**3410.72 ± 146.97**	2561.84 ± 73.33	2468.16 ± 160.54	849.62 ± 99.14
M4	1001.73 ± 114.37	1031.81 ± 40.40	**1110.47 ± 33.42**	1101.47 ± 119.91	1069.14 ± 68.07	776.02 ± 105.40
M5	2114.87 ± 102.96	2178.25 ± 22.70	**2500.39 ± 47.87**	2361.61 ± 201.80	2239.19 ± 10.88	839.18 ± 83.96
M6	1944.51 ± 39.75	**2403.25 ± 60.18**	**2399.71 ± 121.64**	2172.80 ± 164.02	2090.97 ± 173.99	770.12 ± 48.43
M7	1888.74 ± 88.38	1945.37 ± 179.11	**2028.26 ± 57.11**	2020.59 ± 50.41	1985.56 ± 86.06	866.32 ± 108.60

Tab. S4. Statistical differences between SR [%] values after 24 h, depending on different CA and CAT mass ratio.

	1:1	1.5:1	2:1	2.5:1	3:1	1:2
1:1	×	***	****	ns	ns	****
1.5:1	***	×	*	**	***	****
2:1	****	*	×	****	****	****
2.5:1	ns	**	****	×	ns	****
3:1	ns	***	****	ns	×	****
1:2	****	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

1.3. Reaction temperature

Tab. S5. Swelling ratio [%] after 24 h of each BC modification depending on the reaction temperature.

Type of modification	Reaction temperature (°C)			
	120	140	160	180
M1	844.87 ± 141.14	1605.45 ± 230.53	**2479.11 ± 97.01**	1834.64 ± 78.82
M2	911.85 ± 81.53	1235.85 ± 75.58	**2193.29 ± 371.01**	1832.06 ± 133.83
M3	1051.64 ± 65.72	1871.51 ± 221.02	**3444.81 ± 262.25**	2367.43 ± 139.72
M4	680.51 ± 102.15	857.58 ± 75.31	**1101.29 ± 46.17**	961.62 ± 35.76
M5	1087.18 ± 150.58	1491.20 ± 49.00	**2223.27 ± 117.86**	2009.50 ± 93.58
M6	918.38 ± 95.86	1722.73 ± 134.25	**2265.81 ± 363.48**	1883.16 ± 89.70
M7	707.86 ± 92.64	802.36 ± 88.81	**2019.84 ± 75.91**	1315.95 ± 187.29

Tab. S6. Statistical differences between SR [%] values after 24 h, depending on different reaction temperature.

	120	140	160	180
120	×	**	****	***
140	**	×	****	*
160	****	****	×	***
180	***	*	***	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

1.4. Reaction time

Tab. S7. Swelling ratio [%] after 24 h of each BC modification depending on the time of reaction in 160 °C.

Type of modification	Reaction time (min)					
	5	15	30	60	75	90
M1	529.57 ± 28.20	632.38 ± 51.50	668.04 ± 50.72	928.64 ± 88.97	**2417.22 ± 122.38**	1621.78 ± 35.97
M2	629.98 ± 42.83	751.51 ± 30.88	816.66 ± 36.72	937.05 ± 154.54	**2037.51 ± 74.38**	1146.15 ± 51.36
M3	573.89 v 28.77	618.04 ± 89.14	662.94 ± 42.53	944.21 ± 176.28	**3410.32 ± 130.04**	2456.54 ± 98.43
M4	523.39 ± 44.48	602.04 ± 17.57	659.34 ± 40.00	712.83 ± 62.14	**989.52 ± 58.44**	915.05 ± 71.75
M5	568.39 ± 24.09	633.18 ± 58.49	628.67 ± 14.74	918.83 ± 128.36	**2229.65 ± 106.17**	1711.16 ± 137.43
M6	603.73 ± 46.82	611.33 ± 21.59	562.30 ± 73.09	1095.10 ± 12.13	**2340.193 ± 196.60**	1974.74 ± 101.48
M7	542.68 ± 48.27	630.32 ± 53.38	632.16 ± 98.74	879.46 ± 57.53	**2018.44 ± 166.23**	1508.25 ± 173.19

Tab. S8. Statistical differences between SR [%] values after 24 h, depending on different reaction time.

	5	15	30	60	75	90
5	×	ns	**ns**	*	****	****
15	ns	×	**ns**	*	****	****
30	ns	ns	×	ns	****	****
60	*	*	**ns**	×	****	****
75	****	****	****	****	×	****
90	****	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S9. Adjusted p-values and appropriate marks for each variant of optimization

	*	**	***	****
Percentage of CA and CAT solutions	0.0412	-	-	< 0.0001
CA and CAT solutions mass ratio	0.0444	0.0039	0.0003 – 0.009	< 0.0001
Reaction temperature	0.0481	0.0031	0.0001 – 0.0005	< 0.0001
Reaction time	0.0111 – 0.0261	-	-	< 0.0001

3. Morphological characteristics

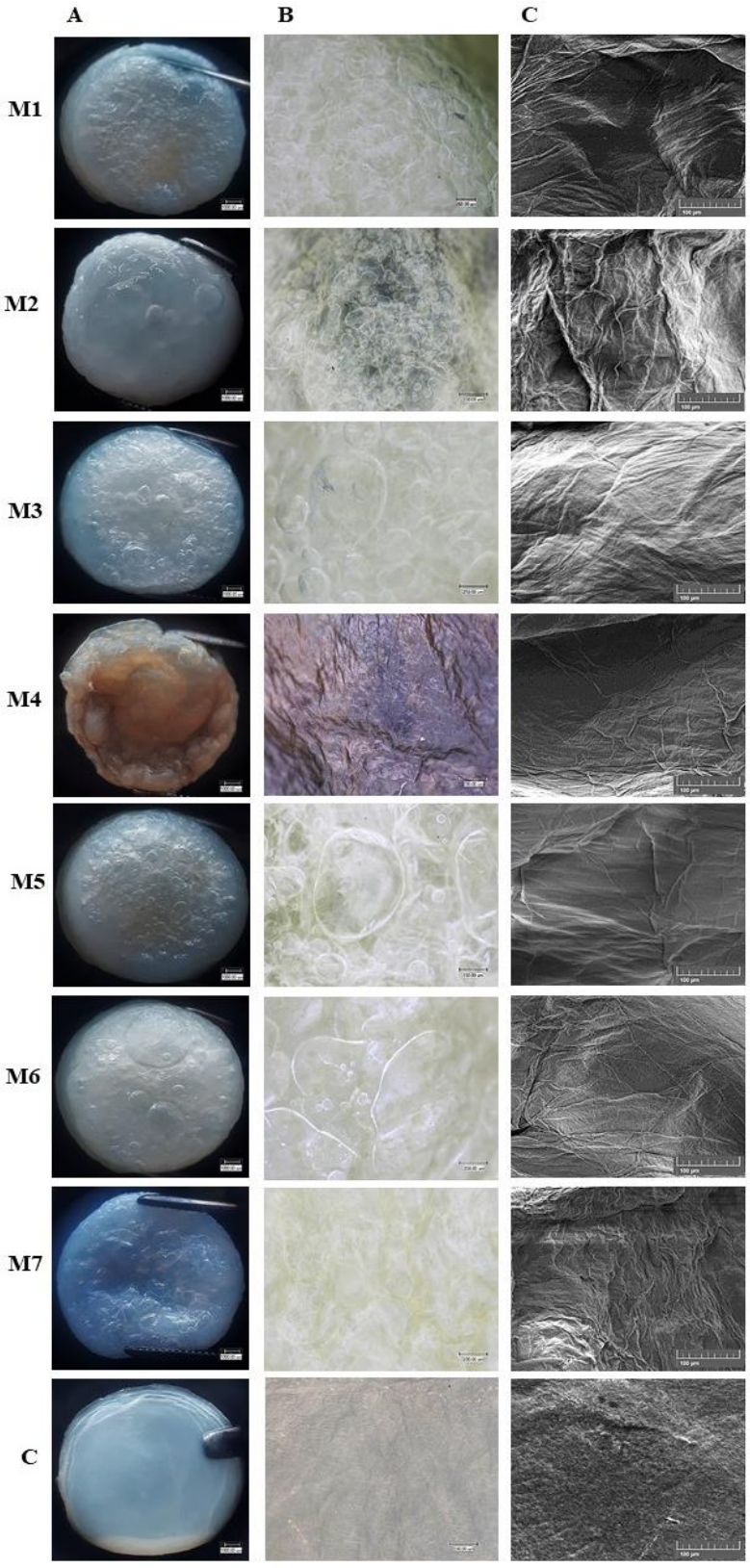


Fig. S1. The structure of modified and unmodified BC. A, B, C - magnification 20, 150 and 300 x, respectively.

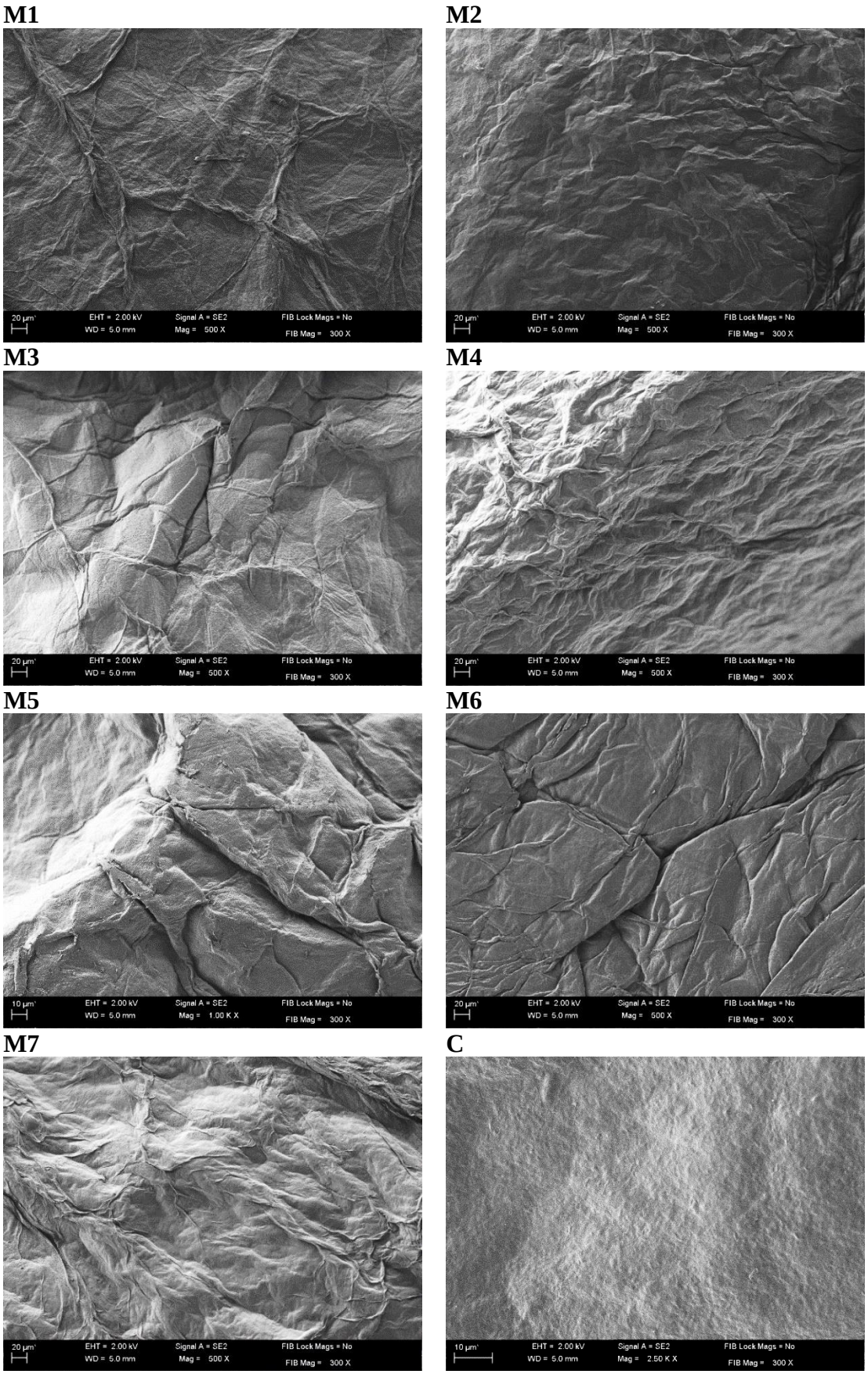


Fig. S2. SEM images of modified and unmodified BC (magnification 500x).

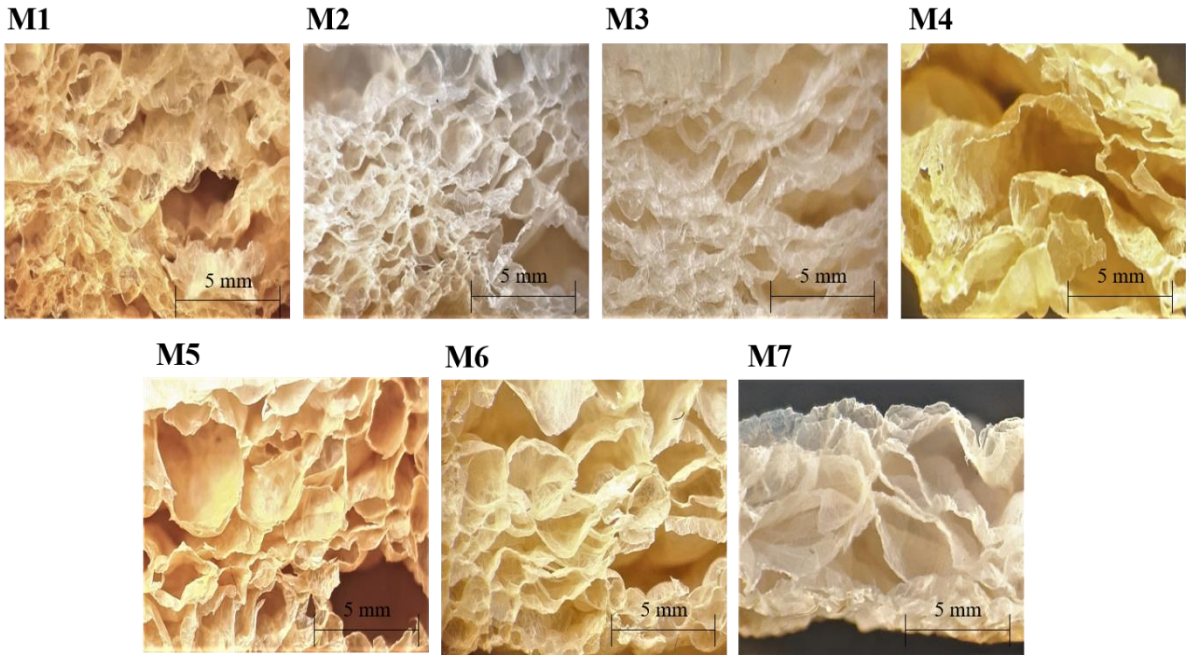


Fig. S3. Cross-section of modified BC samples visualized by stereoscopic microscope (magnification 25x).

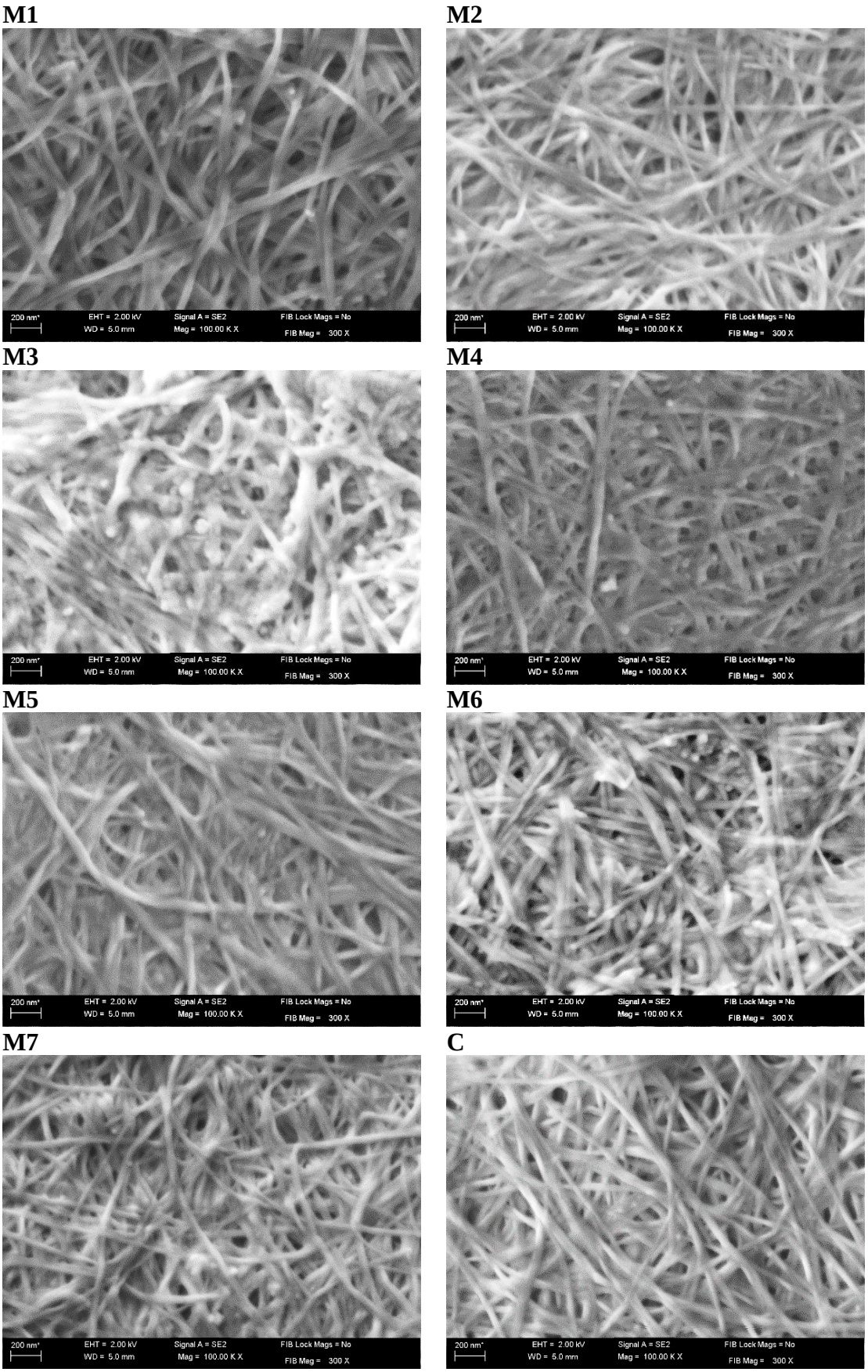


Fig. S4. SEM microscopy of the surface of modified and unmodified BC (magnification 10 000x).

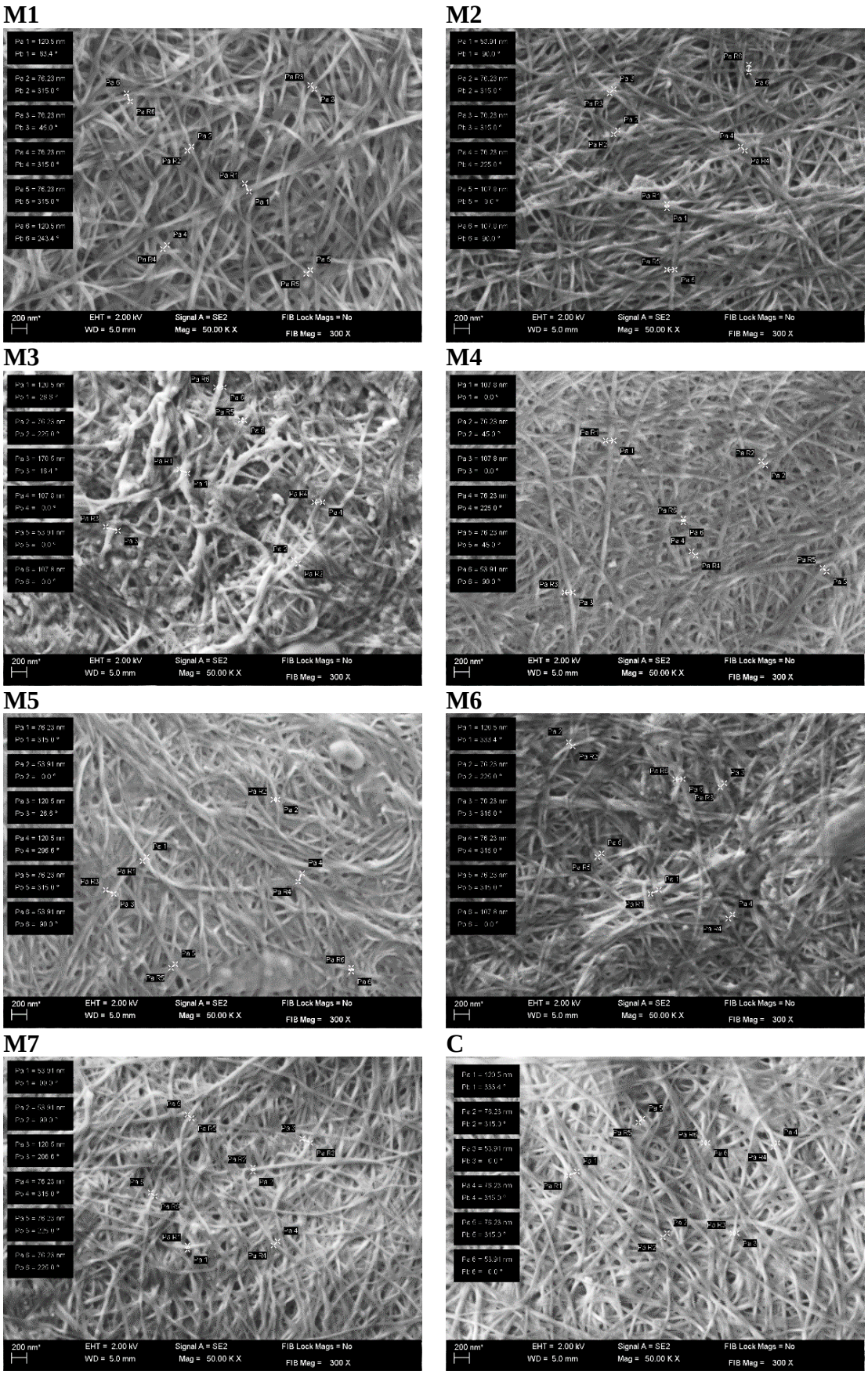


Fig. S5. SEM images of modified and unmodified BC with microfibrils diameters marked (magnification 5 000x).

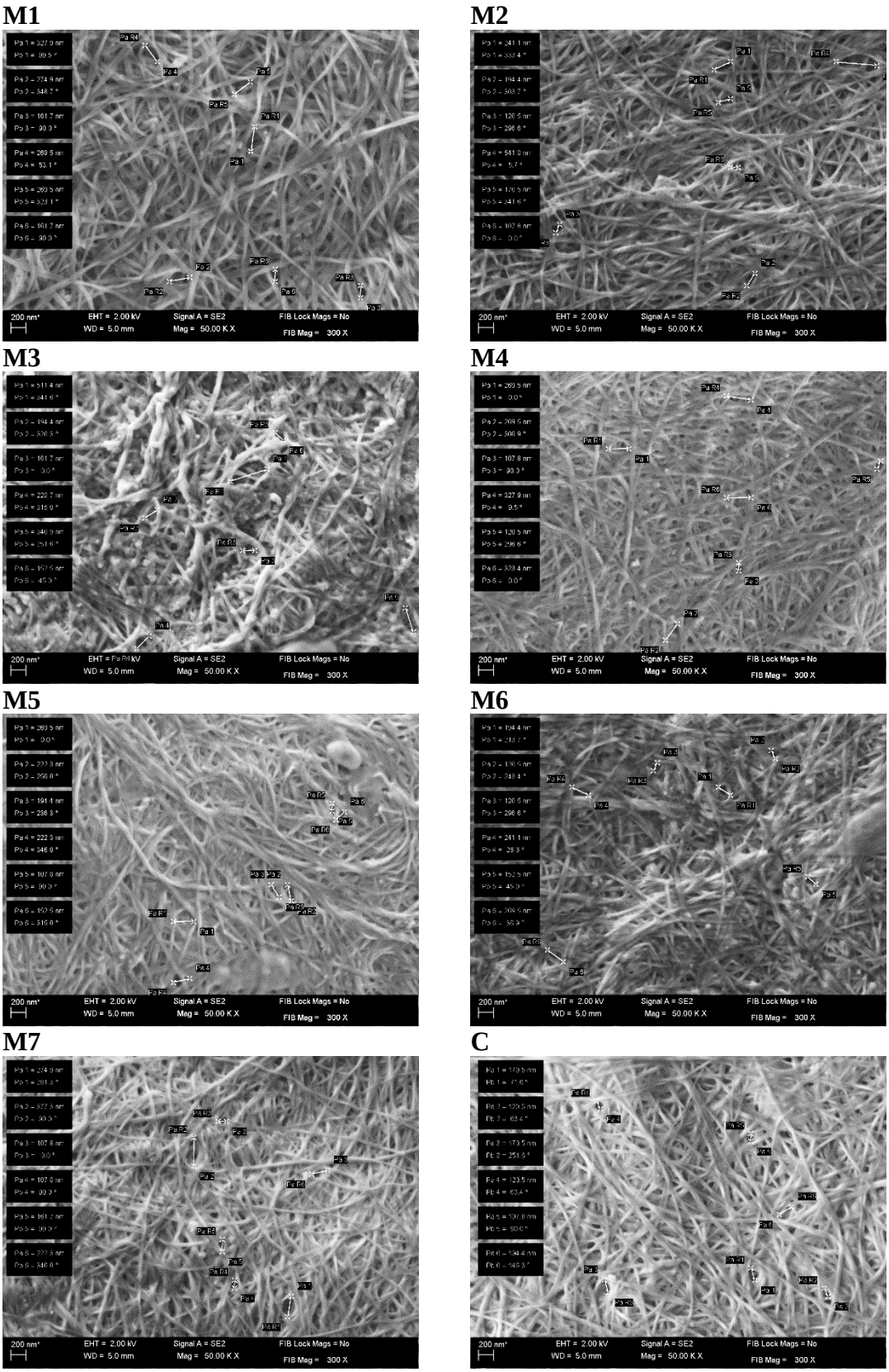


Fig. S6. SEM images of modified and unmodified BC with pore diameters marked (magnification 5 000x).

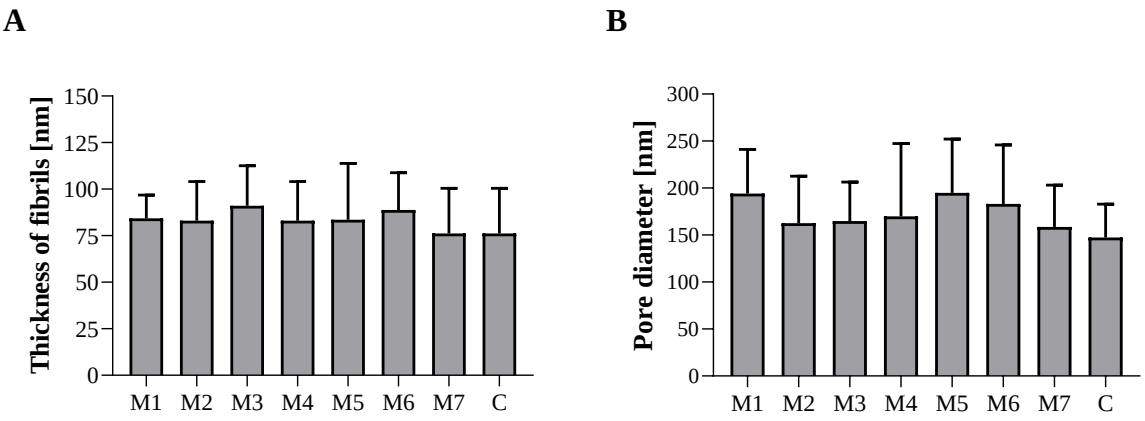
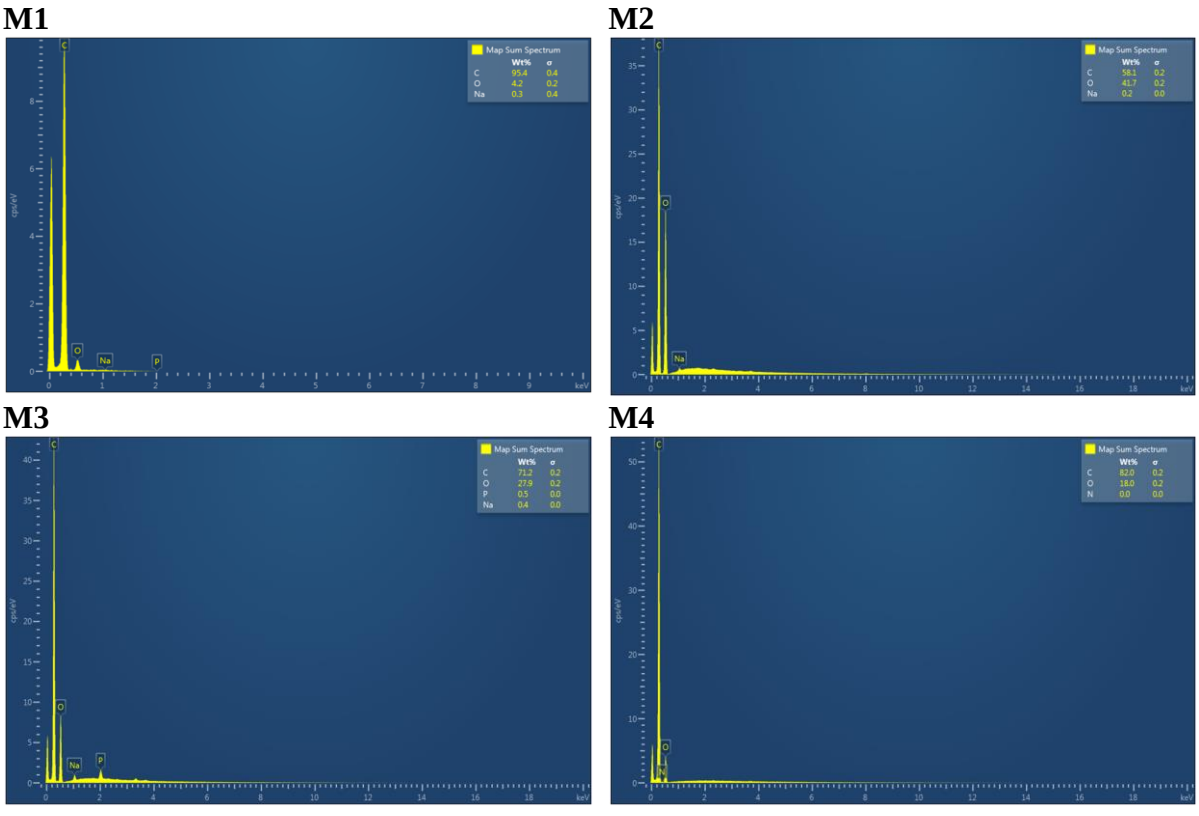


Fig. S7. The thickness and pore diameter measured on the surface of modified and unmodified BC using SEM. Data are presented as mean± standard error of the mean (SEM). There were no statistically significant differences between the samples.



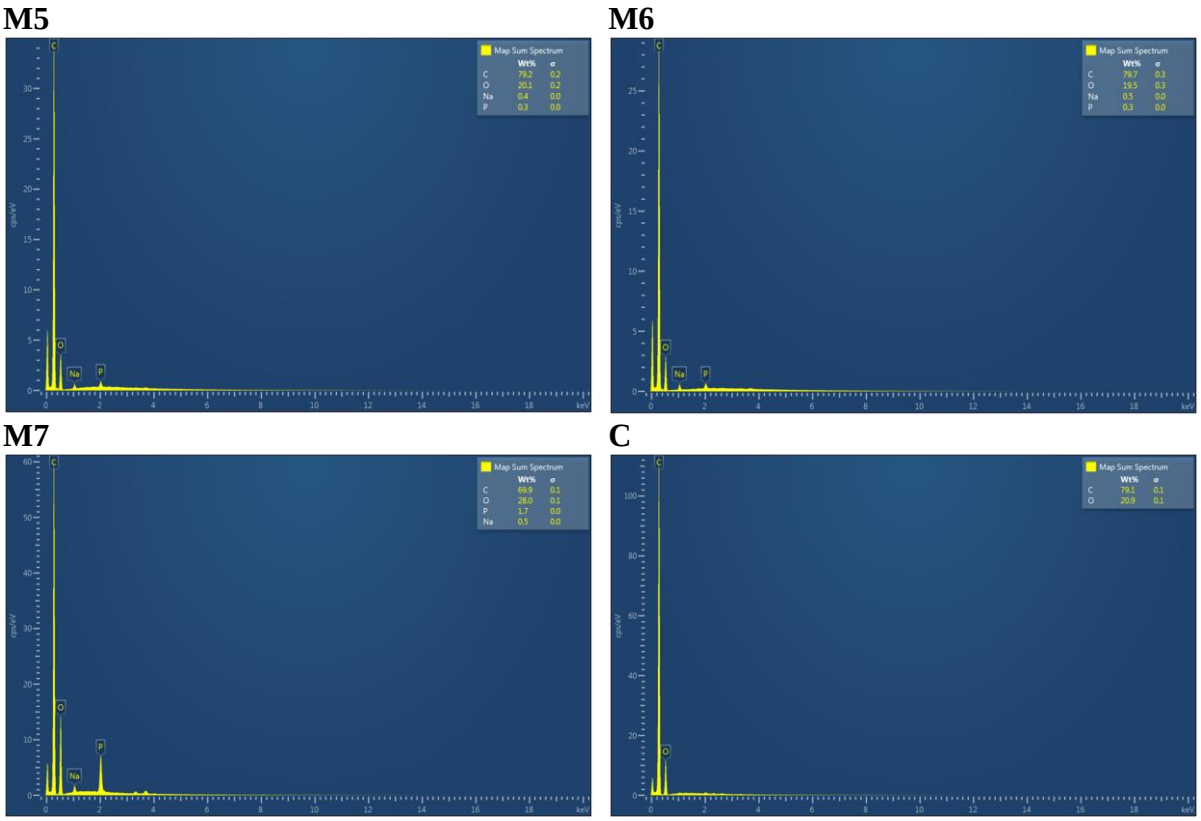


Fig. S8. EDX spectra of modified and unmodified BC.

Table S10. Results of EDX analysis.

		M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**C**
W_t%	C	73.7	58.1	71.2	82.0	79.2	79.7	69.9	79.1
	O	25.4	41.7	27.9	18.0	20.1	19.5	28.0	20.9
	Na	0.4	0.2	0.4	-	0.4	0.5	0.5	-
	P	0.5	-	0.5	-	0.3	0.3	1.7	-
	N	-	-	-	-	-	-	-	-

W_t% - Elemental composition (%)

4. Principle Component Analysis of ATR-FTIR spectra

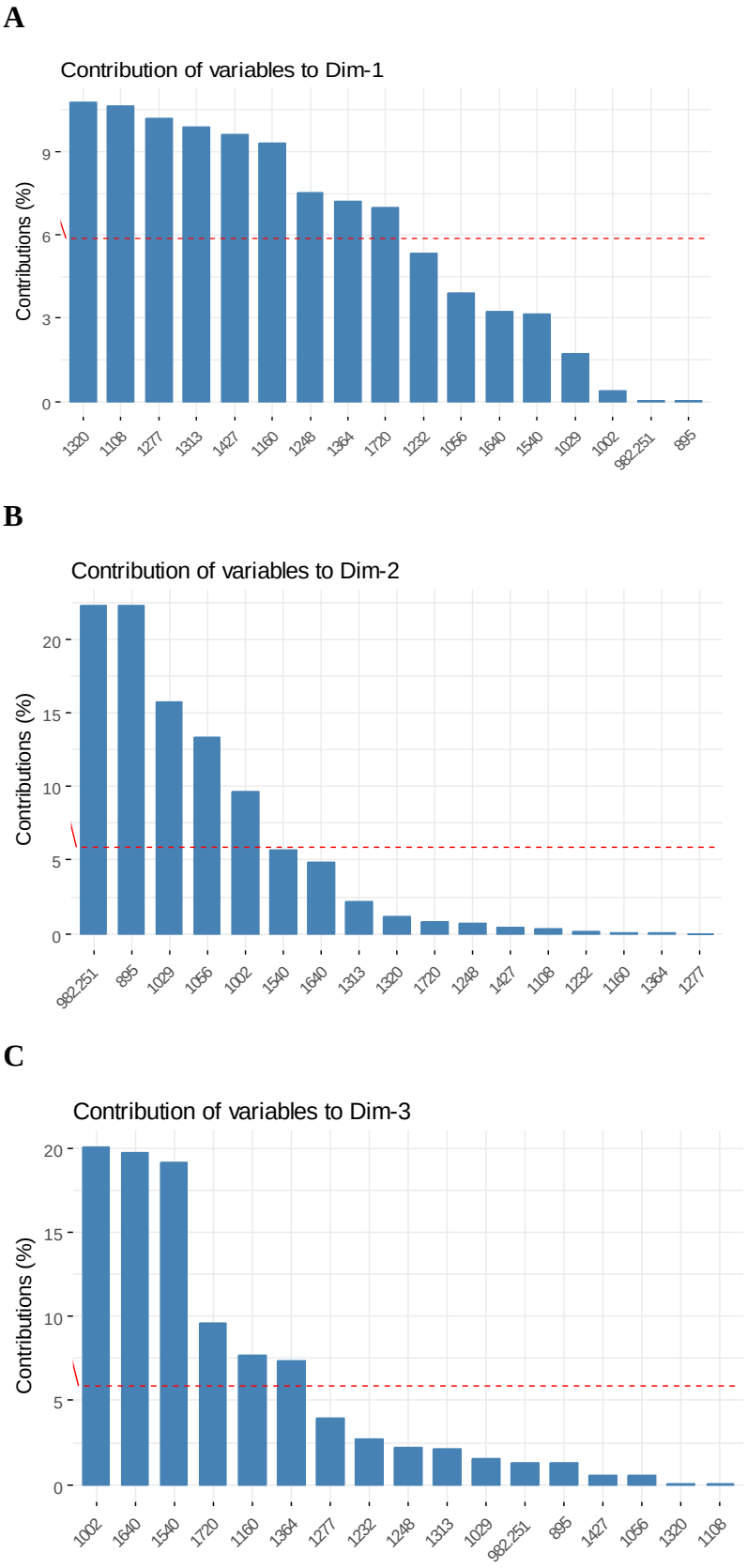


Fig. S9. Contribution of variables according to the results from ATR-FTIR analyses.

5. Water related properties of BC samples crosslinked under optimal conditions using various CATs

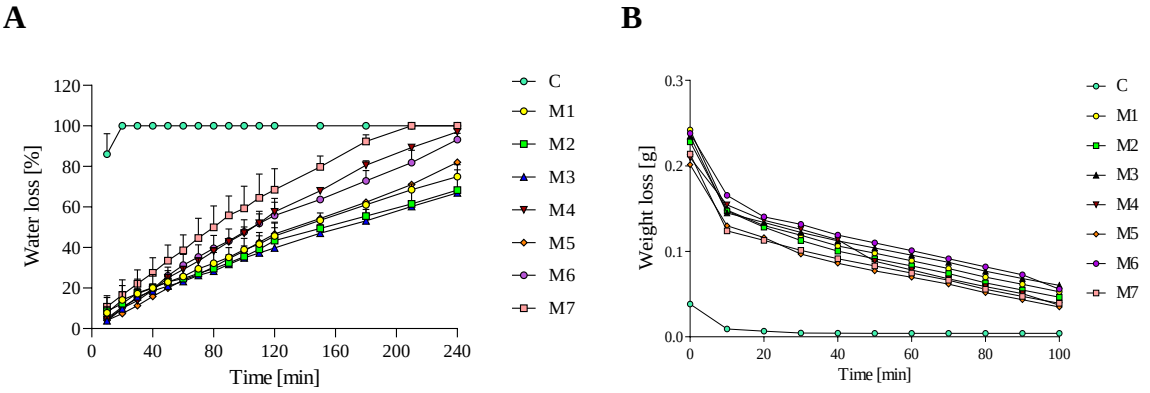


Fig. S10. Water related properties of BC samples crosslinked under optimal conditions using various CATs. **A)** % of water loss from BC samples during incubation at 37°C, **B)** Weight loss during centrifugation at 200g.

Tab. S11. Statistical differences between SR [%] obtained after 60 min incubation in water.

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	ns	ns	***	****	****	ns	****
M2	ns	×	ns	ns	****	****	ns	*
M3	ns	ns	×	**	****	****	ns	***
M4	***	ns	**	×	****	****	ns	ns
M5	****	****	****	****	×	ns	***	****
M6	****	****	****	****	ns	×	ns	****
M7	ns	ns	ns	ns	****	****	×	**
C	****	*	***	ns	****	****	**	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S12. Statistical differences between SR [%] obtained after 24 h incubation in water.

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	*	ns	****	ns	ns	**	****
M2	*	×	***	**	ns	ns	ns	****
M3	ns	***	×	****	*	*	****	****
M4	****	**	****	×	****	****	**	ns
M5	ns	ns	*	****	×	ns	ns	****
M6	ns	ns	*	****	ns	×	ns	****
M7	**	ns	****	**	ns	ns	×	***
C	****	****	****	ns	****	****	***	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S13. Statistical differences between WHC% values obtained after 60 min of the analyses.

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	ns	ns	ns	ns	ns	ns	****
M2	ns	×	ns	ns	ns	ns	ns	***
M3	ns	ns	×	ns	ns	ns	ns	****
M4	ns	ns	ns	×	ns	ns	ns	****
M5	ns	ns	ns	ns	×	ns	ns	****
M6	ns	ns	ns	ns	ns	×	ns	****
M7	ns	ns	ns	ns	ns	ns	×	****
C	****	***	****	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

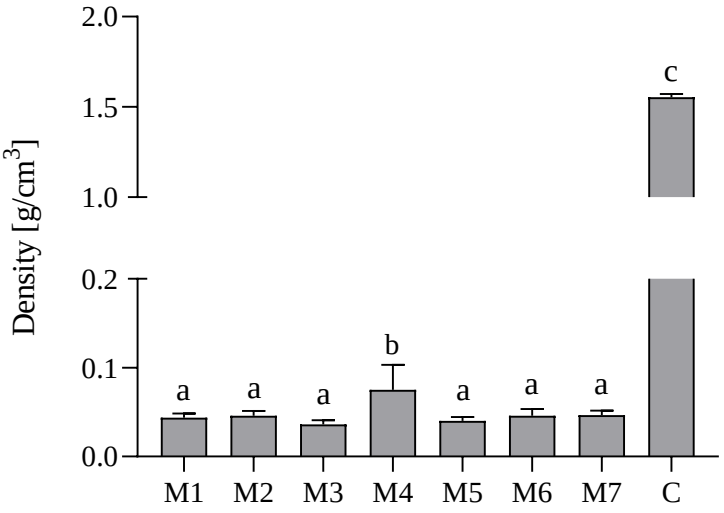


Fig. S11. Density of BC samples.

Data are presented as mean± standard error of the mean (SEM); values with different letters are significantly different (P<0.05): a, b, c, d – statistically significant differences between the analyzed parameters.

Tab. S14. Statistical differences between the values of the density of BC samples.

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	ns	ns	**	ns	ns	ns	****
M2	ns	×	ns	*	ns	ns	ns	****
M3	ns	ns	×	***	ns	ns	ns	****
M4	**	*	***	×	**	*	*	****
M5	ns	ns	ns	**	×	ns	ns	****
M6	ns	ns	ns	*	ns	×	ns	****
M7	ns	ns	ns	*	ns	ns	×	****
C	****	****	****	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S15. Adjusted p-values and appropriate marks for each test.

	*	**	***	****
SR% after 30 min	0.0711 – 0.9997	0.0149	0.0032 – 0.0061	< 0.0001
SR% after 24 h	0.0107 – 0.0254	0.0018 – 0.0074	0,0002 – 0.0003	< 0.0001
WHC % after 60 min	-	-	-	< 0.0001
Density	0.0176 – 0.0215	0.0028 – 0.0090	0.0006	< 0.0001

6. Analyses of cytotoxicity of modified BC

6.1. Extract assay of cellulose discs

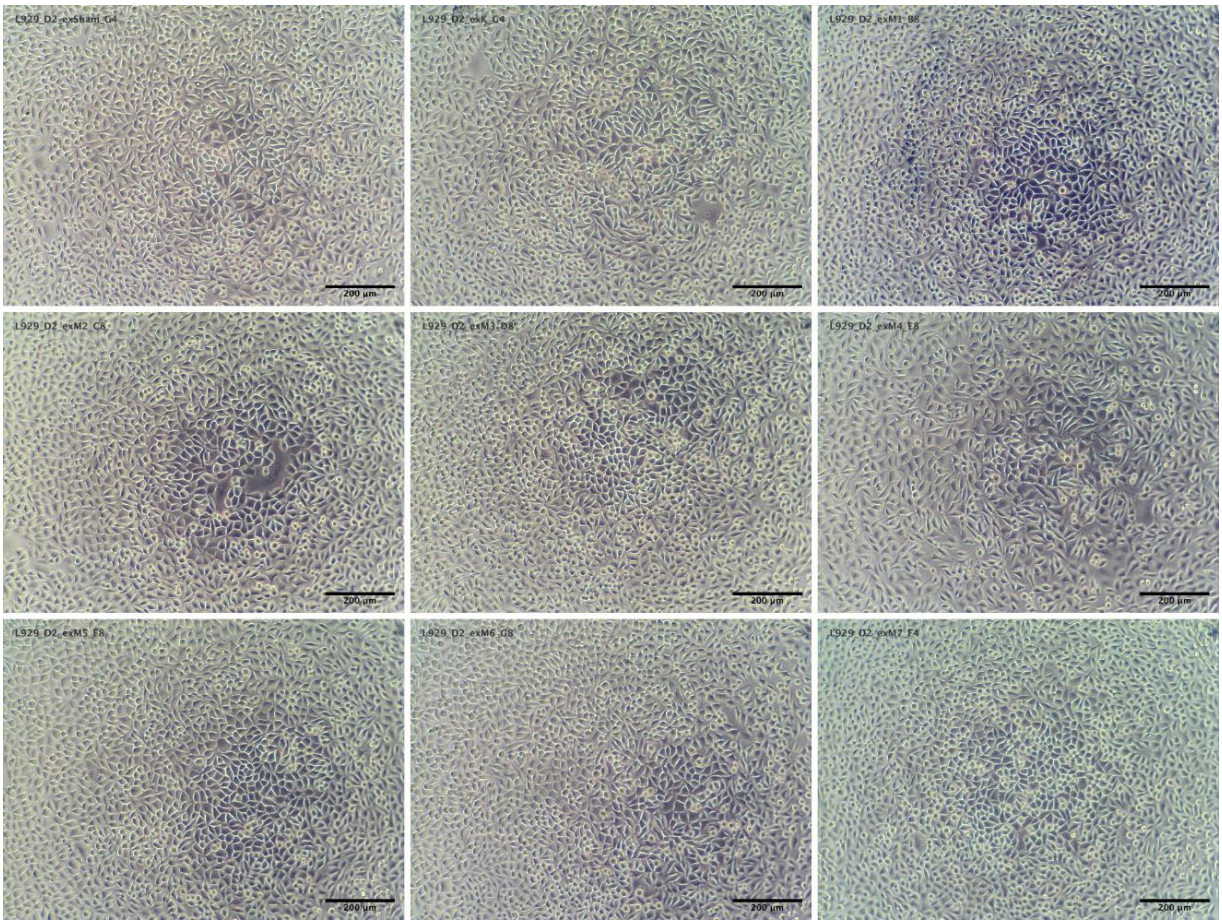


Fig. S12. Representative micrographs of L929 cells after 24 h of culture with BC extracts. Sequentially from top left to bottom right: Sham, K, M1-M7. Scale bar represents 200 μm.

6.2. Direct contact assay of cellulose discs

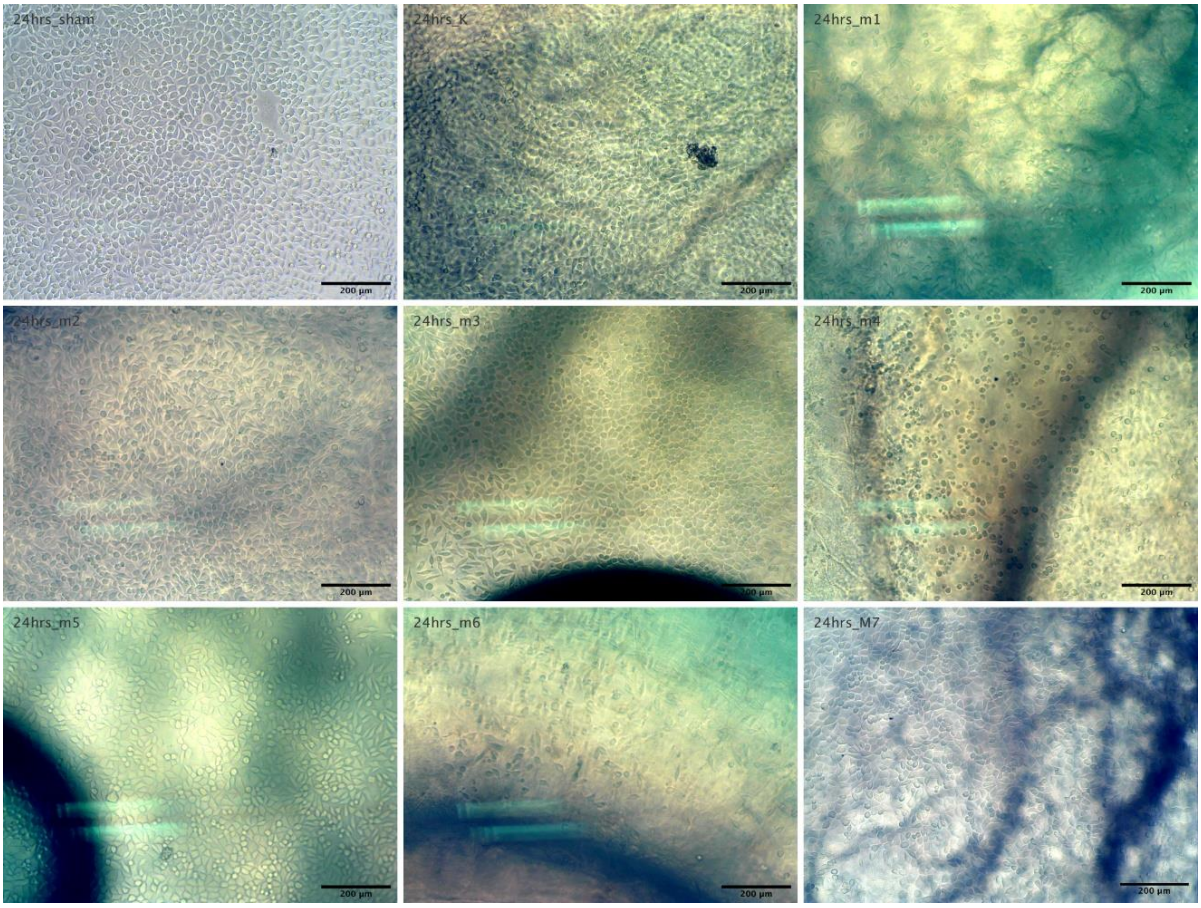


Fig. S13. Representative micrographs of L929 cells after 24 h of culture beneath sham (CellCrown insert alone, top left panel) cellulose discs, sequentially from top left to bottom right: K, M1-M7. Scale bar represents 200 µm. Overall, the CellCrown insert made visualization at the edges of the well difficult, thus only the center was imaged. To avoid any disruption to the monolayer, discs were not removed, thus image quality is reduced due to the effect of the sample on the transmitted light.

Tab. S16. Statistical differences between normalized cell viability (% of Sham) in extract assay

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	ns	ns	*	ns	ns	**	****
M2	ns	×	**	****	ns	*	ns	****
M3	ns	**	×	ns	ns	ns	****	****
M4	*	****	ns	×	*	ns	****	****
M5	ns	ns	ns	*	×	ns	**	****
M6	ns	*	ns	ns	ns	×	***	****
M7	**	ns	****	****	**	***	×	****
C	****	****	****	****	****	****	****	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S17. Statistical differences between normalized cell viability (% of Sham) in direct contact assay

	M1	M2	M3	M4	M5	M6	M7	C
M1	×	ns	ns	*	ns	ns	ns	ns
M2	ns	×	ns	*	ns	ns	ns	ns
M3	ns	ns	×	ns	ns	ns	**	ns
M4	*	*	ns	×	ns	**	****	***
M5	ns	ns	ns	ns	×	ns	**	ns
M6	ns	ns	ns	**	ns	×	ns	ns
M7	ns	ns	**	****	**	ns	×	ns
C	ns	ns	ns	***	ns	ns	ns	×

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Tab. S18. Adjusted p-values and appropriate marks for each cytotoxicity test

	*	**	***	****
Contact assay	0.0140 – 0.0198	0.0045 – 0.0070	0.0005	< 0.0001
Extract assay	0.0173 – 0.0231	0.0017 – 0.0074	0.0002	< 0.0001

Tab. S19. Comparison of SR [%] values after 24 h for commercial dressings and M3 sample.

	SR[%] after 24 h	
Polyacrylate fiber superabsorbent commercial dressing	1432.23 ± 37.25	
Hydrofiber superabsorbent commercial dressing	2590.53 ± 235.33	
M3	3334.21 ± 353.54	

Tab.S20. Statistical differences between SR [%] of commercial dressings and M3 sample.

	Fiber dressing	Hydrofiber dressing	M3
Fiber dressing	×	**	***
Hydrofiber dressing	**	×	*
M3	***	*	×

* p<0.05, ** p<0.01, *** p<0.001

Tab. S21. Adjusted p-values and appropriate marks for SR [%] values comparing commercial dressings and M3 sample.

	*	**	***	****
SR[%]	0.0319	0.0041	0.0001	-