Colorimetric pH-Responsive Nanofibrous Hydrogels for In Vitro Monitoring of Wound Infection

Nadia Banitorfi Hoveizavi[a,1], Farzaneh Alihosseini[a,*], Sandro Lehner[b], Philipp Meier[c], Sabyasachi Gaan[b,*]

[a] *Department of Textile Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran*

[b] *Laboratory of Advanced Fibers, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland*

[c] *Laboratory of Nanomaterials in Health, Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland*

*Corresponding authors, Email: fhosseini@cc.iut.ac.ir, sabyasachi.gaan@empa.ch

Supporting Figures:

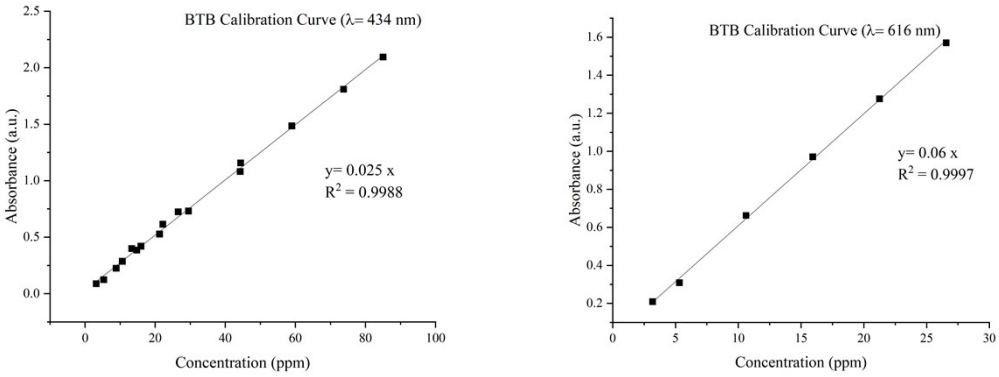

Figure S1- Calibration curve of BTB at two different maximum wavelengths.

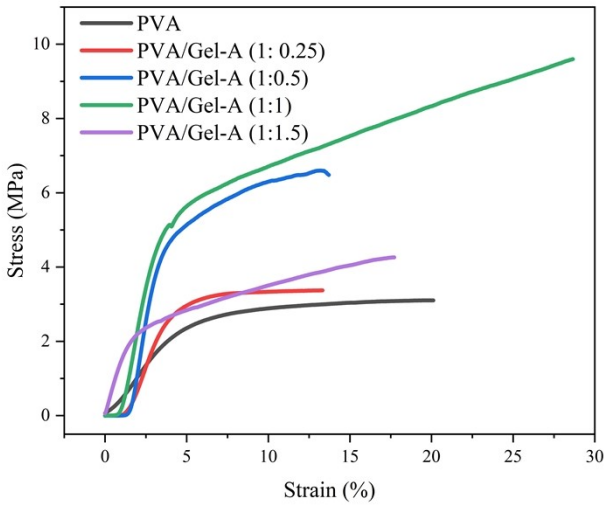

Figure S2- Representative stress (MPa)- strain (%) curves of PVA/Gel-A nanofibers with varying weight ratios.

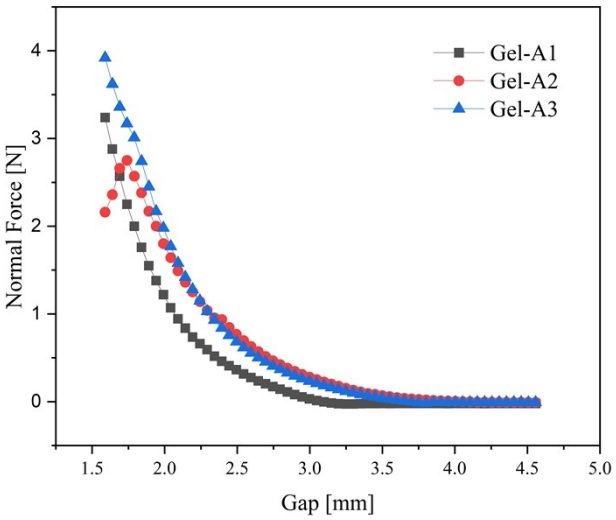

Figure S3. Graph illustrating the normal force (N) as a function of gap distance (mm) for Gel-A hydrogel, measured using a Rheometer (Physica MCR 301, Anton Paar, Austria) (n = 3). Compressive stress and strain values were calculated based on this data.

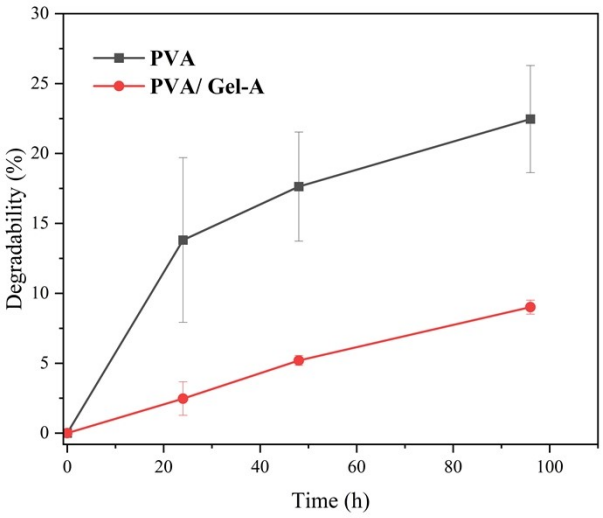

Figure S4- The degradation profile of both PVA and PVA/Gel-A (1:1) in PBS (pH 7.4) over 96 hours.

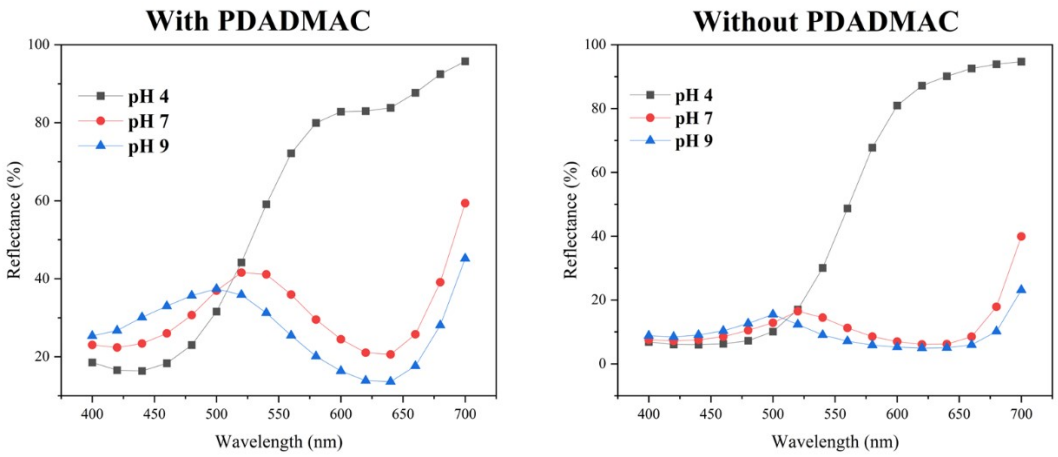

Figure S5- Reflectance Spectra of of PVA/Gel-A (1:1)/BTB nanofibrous hydrogels with and without PDADMAC.

Supporting Tables:

Table S1- Viscosity (mPas), shear stress (N/m^2), shear rate (1/s), and conductivity (mS/cm) of pure PVA and PVA/Gel-A (1:1) solutions prior to electrospinning.

	Viscosity (rpm= 5) (mPas)	Shear stress (N/m^2)	Shear rate (1/s)	conductivity (mS/cm)
PVA	183.0	1.21	6.60	2.056
PVA/Gel-A (1:1)	264.5	1.75	6.60	4.80

Table S2- Elemental analysis of Carbon, Nitrogen, Oxygen, and Phosphorus in nanofibrous hydrogels obtained via EDX.

	C	N	O	P
PVA	57.59	0	42.41	0
PVA-Gel A (1: 0.25)	58.14	2.0	37.06	2.81
PVA-Gel A (1: 0.5)	58.45	4.36	33.36	3.84
PVA-Gel A (1: 1)	59.68	5.70	28.44	6.19
PVA-Gel A (1: 1.5)	56.08	11.48	26.97	5.48

Table S3- Nitrogen adsorption/desorption isotherm data for pure PVA nanofibers.

No	pe/ kPa	p0/ kPa	p/p0	$Va/cm^3(STP)\ g^{-1}$
ADS				
1	0.00	95.92	0.00	0.00
2	1.18	95.94	0.01	0.79
3	3.17	95.92	0.03	1.41
4	5.30	95.93	0.06	1.72
5	7.54	95.92	0.08	1.98
6	9.98	95.92	0.10	2.21
7	12.41	95.91	0.13	2.38
8	14.83	95.90	0.15	2.49
9	17.27	95.92	0.18	2.54
10	19.65	95.92	0.20	2.71
11	22.09	95.90	0.23	2.79
12	24.51	95.90	0.26	2.85
13	26.89	95.85	0.28	2.97
14	29.27	95.88	0.31	3.10
15	31.73	95.88	0.33	3.19
16	34.08	95.86	0.36	3.36
17	36.52	95.85	0.38	3.46
18	38.92	95.86	0.41	3.56
19	41.34	95.81	0.43	3.67
20	43.70	95.85	0.46	3.82
21	46.13	95.82	0.48	3.95

22	48.53	95.82	0.51	4.11
23	50.92	95.84	0.53	4.28
24	53.34	95.83	0.56	4.43
25	55.74	95.84	0.58	4.55
26	58.13	95.82	0.61	4.71
27	60.52	95.83	0.63	4.89
28	62.93	95.80	0.66	5.07
29	65.31	95.79	0.68	5.24
30	67.69	95.79	0.71	5.44
31	70.10	95.77	0.73	5.63
32	72.47	95.76	0.76	5.91
33	74.86	95.78	0.78	6.17
34	77.22	95.78	0.81	6.51
35	79.62	95.76	0.83	6.85
36	81.97	95.78	0.86	7.26
37	84.32	95.79	0.88	7.78
38	86.68	95.80	0.90	8.41
39	88.94	95.74	0.93	9.27
40	91.11	95.73	0.95	10.44
41	92.66	95.71	0.97	11.99
42	93.97	95.68	0.98	14.54
43	95.06	95.70	0.99	19.31
DES				
1	92.64	95.68	0.97	12.22
2	91.91	95.65	0.96	11.41
3	90.20	95.66	0.94	10.18
4	88.22	95.67	0.92	9.24
5	86.12	95.65	0.90	8.53
6	83.93	95.70	0.88	7.97
7	81.70	95.66	0.85	7.52
8	78.30	95.62	0.82	6.94
9	76.00	95.64	0.79	6.65
10	73.70	95.65	0.77	6.35
11	71.37	95.68	0.75	6.12
12	69.05	95.65	0.72	5.87
13	66.70	95.63	0.70	5.69
14	64.37	95.62	0.67	5.48
15	62.01	95.66	0.65	5.35
16	59.65	95.63	0.62	5.19
17	57.28	95.63	0.60	5.03
18	54.93	95.66	0.57	4.86
19	52.57	95.68	0.55	4.71
20	50.20	95.69	0.52	4.59
21	47.84	95.66	0.50	4.46
22	45.44	95.72	0.47	4.38

23	43.06	95.65	0.45	4.26
24	40.67	95.69	0.43	4.18
25	38.26	95.67	0.40	4.10
26	35.86	95.70	0.37	4.01
27	33.45	95.70	0.35	3.89
28	31.03	95.70	0.32	3.81
29	28.63	95.63	0.30	3.68
30	26.20	95.67	0.27	3.61
31	23.75	95.72	0.25	3.55
32	21.32	95.77	0.22	3.44
33	18.91	95.77	0.20	3.31
34	16.45	95.81	0.17	3.27
35	13.99	95.83	0.15	3.14
36	11.57	95.80	0.12	2.97
37	9.17	95.83	0.10	2.81
38	4.45	95.82	0.05	2.36

Table S4- Nitrogen adsorption/desorption isotherm data for PVA/Gel-A (1:1) nanofibers.

No	pe/ kPa	p0/ kPa	p/p0	Va/cm^3(STP) g^{-1}
ADS				
1	0.00	95.92	0.00	0.00
2	1.93	95.93	0.02	0.30
3	4.93	95.94	0.05	0.43
4	7.74	95.93	0.08	0.50
5	10.18	95.92	0.11	0.56
6	12.61	95.90	0.13	0.60
7	15.02	95.90	0.16	0.63
8	17.43	95.92	0.18	0.67
9	19.87	95.91	0.21	0.66
10	22.23	95.88	0.23	0.71
11	24.67	95.89	0.26	0.72
12	27.07	95.91	0.28	0.72
13	29.49	95.90	0.31	0.74
14	31.88	95.87	0.33	0.78
15	34.29	95.86	0.36	0.81
16	36.70	95.88	0.38	0.81
17	39.11	95.88	0.41	0.82
18	41.51	95.87	0.43	0.85
19	43.91	95.86	0.46	0.87
20	46.32	95.86	0.48	0.91
21	48.73	95.84	0.51	0.92
22	51.12	95.80	0.53	0.96
23	53.48	95.79	0.56	1.02
24	55.91	95.80	0.58	1.04
25	58.28	95.81	0.61	1.10
26	60.72	95.82	0.63	1.11
27	63.15	95.82	0.66	1.11

28	65.51	95.80	0.68	1.17
29	67.93	95.84	0.71	1.19
30	70.34	95.78	0.73	1.25
31	72.71	95.80	0.76	1.30
32	75.11	95.79	0.78	1.38
33	77.52	95.76	0.81	1.45
34	79.86	95.77	0.83	1.57
35	82.28	95.78	0.86	1.67
36	84.65	95.77	0.88	1.82
37	87.02	95.77	0.91	1.99
38	89.38	95.78	0.93	2.20
39	91.70	95.77	0.96	2.55
40	94.24	95.74	0.98	3.47
41	95.41	95.72	1.00	5.47
DES				
1	92.39	95.71	0.97	2.81
2	91.39	95.73	0.95	2.58
3	88.09	95.70	0.92	2.20
4	85.84	95.71	0.90	2.01
5	83.53	95.70	0.87	1.88
6	81.21	95.73	0.85	1.79
7	78.91	95.67	0.82	1.67
8	76.58	95.71	0.80	1.57
9	74.23	95.68	0.78	1.54
10	71.90	95.69	0.75	1.50
11	69.55	95.71	0.73	1.47
12	67.19	95.65	0.70	1.42
13	64.83	95.69	0.68	1.38
14	62.45	95.67	0.65	1.36
15	60.08	95.68	0.63	1.34
16	56.60	95.64	0.59	1.29
17	54.23	95.66	0.57	1.27
18	51.86	95.67	0.54	1.24
19	49.49	95.64	0.52	1.21
20	47.11	95.66	0.49	1.20
21	44.74	95.64	0.47	1.17
22	42.36	95.66	0.44	1.16
23	39.97	95.64	0.42	1.16
24	37.59	95.69	0.39	1.12
25	35.21	95.63	0.37	1.11
26	32.79	95.65	0.34	1.14
27	30.44	95.66	0.32	1.12
28	28.05	95.66	0.29	1.13
29	25.65	95.70	0.27	1.13
30	23.26	95.70	0.24	1.14
31	20.91	95.70	0.22	1.11
32	18.49	95.70	0.19	1.11
33	16.13	95.67	0.17	1.08
34	13.73	95.68	0.14	1.07
35	11.38	95.68	0.12	1.03

36	9.00	95.67	0.09	1.00
37	4.34	95.65	0.05	0.92

Table S5- *Zeta potential* (mV) of pure PVA and PVA/Gel-A (1:1) nanofibers as a function of *pH.*

pH	Zeta Potential (mV)	
	PVA	PVA/Gel-A (1:1)
3.03	-2.88	12.18
3.03	-3.18	9.28
3.03	-3.07	7.54
3.03	-3.02	5.90
3.30	-3.03	0.56
3.30	-3.13	0.74
3.30	-3.28	0.80
3.31	-3.30	0.81
3.56	-3.42	0.06
3.56	-3.35	0.13
3.56	-3.37	0.20
3.56	-3.24	0.29
3.84	-3.66	0.08
3.83	-3.36	0.15
3.83	-3.65	0.17
3.82	-3.65	0.13
4.14	-4.31	0.03
4.13	-4.16	0.06
4.12	-4.16	0.06
4.11	-4.14	0.07
4.53	-4.86	-0.17
4.50	-4.84	-0.14
4.48	-4.87	-0.12
4.46	-4.86	-0.13
4.82	-4.99	-0.33
4.77	-4.72	-0.30
4.73	-4.54	-0.29
4.70	-4.26	-0.24
4.97	-5.00	-0.42
4.91	-4.99	-0.39
4.88	-4.90	-0.33
4.83	-4.77	-0.34
5.14	-4.78	-0.52
5.07	-4.52	-0.46
5.02	-4.44	-0.44
4.99	-4.41	-0.42

5.29	-4.44	-0.60
5.21	-4.97	-0.55
5.17	-5.29	-0.52
5.11	-5.64	-0.47
5.38	-5.21	-0.64
5.30	-5.27	-0.58
5.25	-5.25	-0.55
5.21	-5.15	-0.50
5.54	-5.25	-0.71
5.47	-5.16	-0.65
5.40	-5.91	-0.62
5.34	-5.72	-0.55
5.79	-5.72	-0.82
5.69	-5.83	-0.77
5.61	-5.83	-0.72
5.54	-5.74	-0.70
6.13	-6.11	-0.94
6.02	-6.26	-0.92
5.94	-6.18	-0.90
5.86	-6.23	-0.86
6.59	-6.26	-0.97
6.44	-6.16	-0.99
6.33	-6.19	-0.99
6.23	-6.11	-1.00
6.96	-6.27	-1.11
6.82	-6.26	-1.09
6.71	-6.40	-1.06
6.61	-6.40	-1.06
7.18	-6.45	-1.25
7.05	-6.46	-1.22
7.05	-6.32	-1.21
7.05	-6.34	-1.20
7.36	-6.49	-1.42
7.24	-6.49	-1.38
7.14	-6.55	-1.35
7.06	-6.65	-1.34
7.68	-6.77	-1.64
7.58	-6.50	-1.61
7.48	-6.69	-1.60
7.39	-7.16	-1.56
7.88	-7.18	-1.84
7.80	-7.18	-1.82
7.73	-7.17	-1.80

7.64	-7.18	-1.79
8.11	-7.19	-2.01
8.05	-7.18	-2.03
8.00	-7.18	-2.00
7.93	-7.18	-2.00
8.31	-7.18	-2.24
8.26	-7.18	-2.24
8.23	-7.18	-2.24
8.19	-7.18	-2.26
8.53	-7.20	-2.47
8.49	-7.20	-2.52
8.45	-7.20	-2.53
8.43	-7.20	-2.53
8.77	-7.30	-2.82
8.74	-7.29	-2.99
8.72	-7.28	-2.93
8.70	-7.30	-2.94
9.01	-7.31	-3.31
8.98	-7.32	-3.41
8.98	-7.32	-3.45
8.95	-7.32	-3.42
9.25	-7.62	-3.92
9.22	-7.62	-4.00
9.21	-7.62	-4.02
9.20	-7.62	-4.09
9.62	-7.82	-4.72
9.60	-7.82	-4.80
9.58	-7.82	-4.87
9.57	-7.82	-4.91
9.93	-7.92	-5.46
9.91	-7.92	-5.47
9.90	-7.92	-5.51
9.89	-7.92	-5.54
10.21	-7.82	-5.76
10.20	-7.82	-5.78
10.20	-7.82	-5.76
10.19	-7.82	-5.77

Table S6- In vitro release data of BTB from PVA/Gel-A (1:1) nanofibrous hydrogels without PDADMAC at pH 5.0. The table displays sampling time (hours), absorbance (A), concentration of dye released (C, mg/L), amount of dye released (Q, mg), percentage release (R%), and cumulative release percentage over time.

Time (h)	A	C (mg/L)	Q (mg)	R (%)	Cumulative Release (%)
0.3	0.526	21.04	0.021	7.87	7.87
1	0.363	14.51	0.015	5.42	13.30
2	0.423	16.93	0.017	6.33	19.63
4	0.349	13.98	0.014	5.23	24.86
8	0.456	18.28	0.018	6.83	31.70
48	0.289	11.59	0.012	4.33	36.03

Table S7- In vitro release data of BTB from PVA/Gel-A (1:1) nanofibrous hydrogels without PDADMAC at 8.0. The table displays sampling time (hours), absorbance (A), concentration of dye released (C, mg/L), amount of dye released (Q, mg), percentage release (R%), and cumulative release percentage over time.

Time (h)	A	C (mg/L)	Q (mg)	R (%)	Cumulative Release (%)
0.3	0.855	14.26	0.014	4.22	4.22
1	0.636	10.61	0.011	3.14	7.36
2	0.794	13.29	0.013	3.92	11.28
4	0.872	14.55	0.015	4.31	15.59
8	1.143	19.07	0.019	5.64	21.23
48	0.982	16.37	0.016	4.84	26.08

Table S8- In vitro release data of BTB from PVA/Gel-A (1:1) nanofibrous hydrogels with PDADMAC at pH 5.0. The table displays sampling time (hours), absorbance (A), concentration of dye released (C, mg/L), amount of dye released (Q, mg), percentage release (R%), and cumulative release percentage over time.

Time (h)	A	C (mg/L)	Q (mg)	R (%)	Cumulative Release (%)
0.3	0.121	4.85	0.005	1.25	1.25
1	0.083	3.33	0.003	0.86	2.11
2	0.096	3.83	0.004	0.98	3.09
4	0.119	4.79	0.005	1.24	4.33
8	0.119	4.74	0.005	1.22	5.54
48	0.098	3.96	0.004	1.02	6.56

Table S9- In vitro release profile of BTB from PVA/Gel-A (1:1) nanofibrous hydrogels with PDADMAC at pH 8.0. The table displays sampling time (hours), absorbance (A), concentration of dye released (C, mg/L), amount of dye released (Q, mg), percentage release (R%), and cumulative release percentage over time.

Time (h)	A	C (mg/L)	Q (mg)	R (%)	Cumulative Release (%)
0.3	0.27	4.50	0.005	1.12	1.12
1	0.154	2.56	0.003	0.64	1.76
2	0.208	3.46	0.004	0.86	2.62

4	0.257	4.29	0.005	1.07	3.69
8	0.308	5.13	0.006	1.28	4.97
48	0.395	6.59	0.006	1.64	6.61