

Electronic Supplementary Information for

Robust biopolymer based ionic-covalent entanglement hydrogels with reversible mechanical behaviour.

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S1. Mechanical Characterisation

Table S1. The compressive mechanical properties of gellan gum-gelatin hydrogels possessing various molar network ratios (\pm standard deviation). Hydrogels were prepared with the same concentrations of calcium and genipin cross-linkers but varying ratios of gellan gum and gelatin.

Gelatin / (gelatin + gellan gum) (%)	σ_c (MPa)	ϵ_c (%)	E_c (kPa)	U (kJ.m⁻³)
33	0.27 \pm 0.06	76 \pm 2	56 \pm 6	40 \pm 5
50	0.6 \pm 0.2	81 \pm 3	56 \pm 5	70 \pm 20
56	0.8 \pm 0.2	85 \pm 2	60 \pm 20	100 \pm 10
60	0.9 \pm 0.2	85 \pm 2	79 \pm 9	110 \pm 20
64	1.1 \pm 0.2	85 \pm 1	120 \pm 20	150 \pm 20
69	0.8 \pm 0.3	81 \pm 4	180 \pm 20	130 \pm 40
71	0.5 \pm 0.1	74 \pm 4	180 \pm 20	90 \pm 20

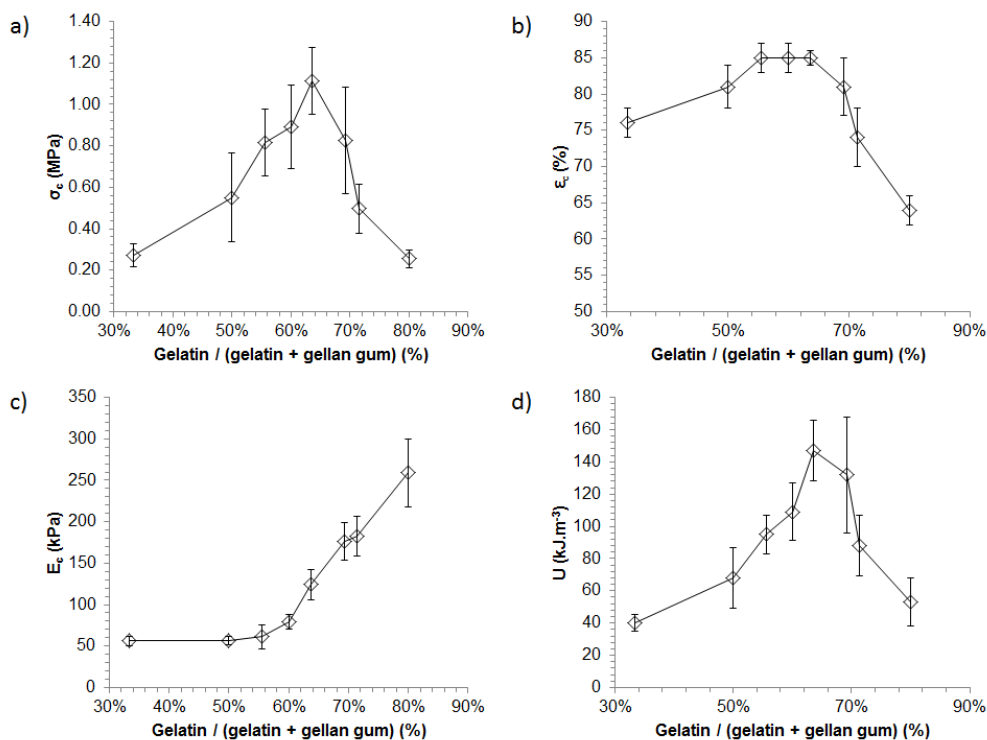


Figure S1. The polymer network ratio affects the a) maximum compressive stress, b) secant modulus, c) maximum compressive strain, and d) strain energy to failure (\pm SD).

Table S2. The compressive mechanical properties of gellan gum-gelatin hydrogels possessing various calcium concentrations (\pm SD). Hydrogels were prepared with the same concentrations of gellan gum and gelatin polymers and genipin cross-linker but concentrations of calcium.

[Ca ²⁺]	σ_c	ϵ_c	E_c	U
% (w/w)	(MPa)	(%)	(kPa)	($\text{kJ}\cdot\text{m}^{-3}$)
0.0	0.30 ± 0.08	72 ± 4	120 ± 20	50 ± 10

0.4	0.5 ± 0.1	77 ± 3	160 ± 10	80 ± 10
0.8	0.6 ± 0.2	82 ± 2	130 ± 10	100 ± 20
2	1.1 ± 0.2	85 ± 1	120 ± 20	150 ± 20
4	0.9 ± 0.2	82 ± 2	140 ± 10	130 ± 20
8	0.5 ± 0.1	69 ± 4	290 ± 30	100 ± 20
20	0.29 ± 0.01	64 ± 1	350 ± 20	72 ± 4

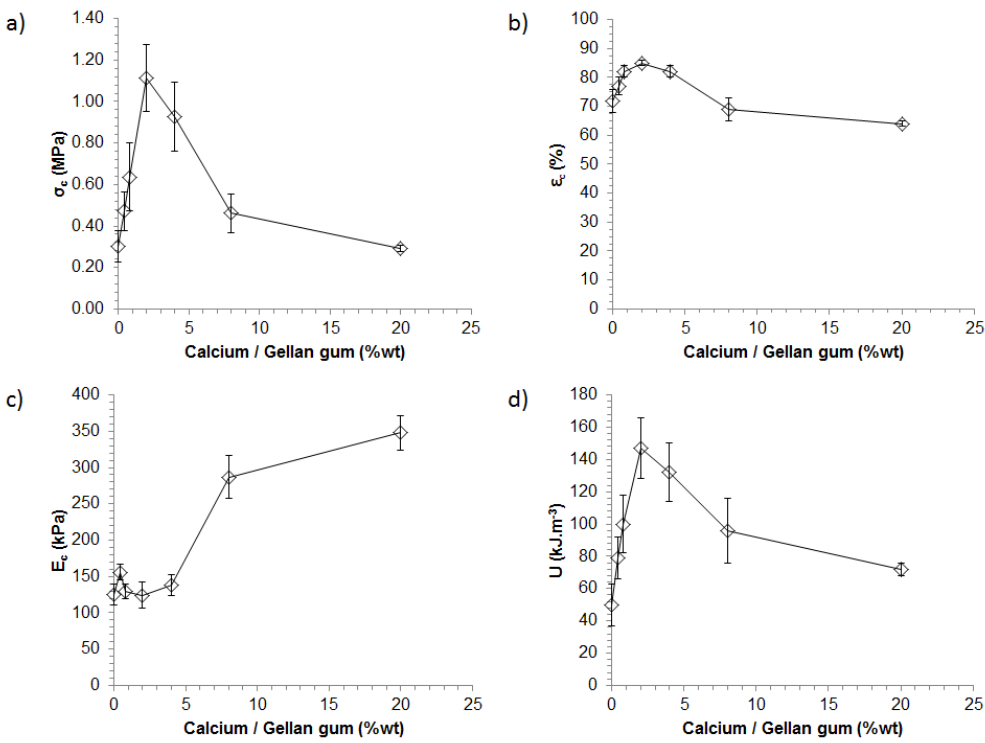


Figure S2. The concentration of calcium cross-linker affects the a) maximum compressive stress, b) secant modulus, c) maximum compressive strain, and d) strain energy to failure (\pm SD).

Table S3. The compressive mechanical properties of gellan gum-gelatin hydrogels with genipin concentrations (\pm SD). Hydrogels were prepared with the same concentrations of gellan gum and gelatin polymers and calcium cross-linker but concentrations of genipin.

[Genipin]	σ_c	ϵ_c	E_c	U
%(w/w)	(MPa)	(%)	(kPa)	(kJ.m⁻³)
0	0.19 \pm 0.04	57 \pm 3	190 \pm 10	38 \pm 7
4	0.5 \pm 0.1	74 \pm 4	150 \pm 10	80 \pm 20
8	0.64 \pm 0.09	78 \pm 2	150 \pm 20	100 \pm 10
12	0.7 \pm 0.2	80 \pm 3	120 \pm 20	100 \pm 30
16	1.0 \pm 0.2	84 \pm 2	100 \pm 20	130 \pm 20
20	1.1 \pm 0.2	85 \pm 1	120 \pm 20	150 \pm 20
24	1.1 \pm 0.2	85 \pm 1	110 \pm 20	140 \pm 20
28	1.0 \pm 0.1	86 \pm 2	100 \pm 10	140 \pm 20

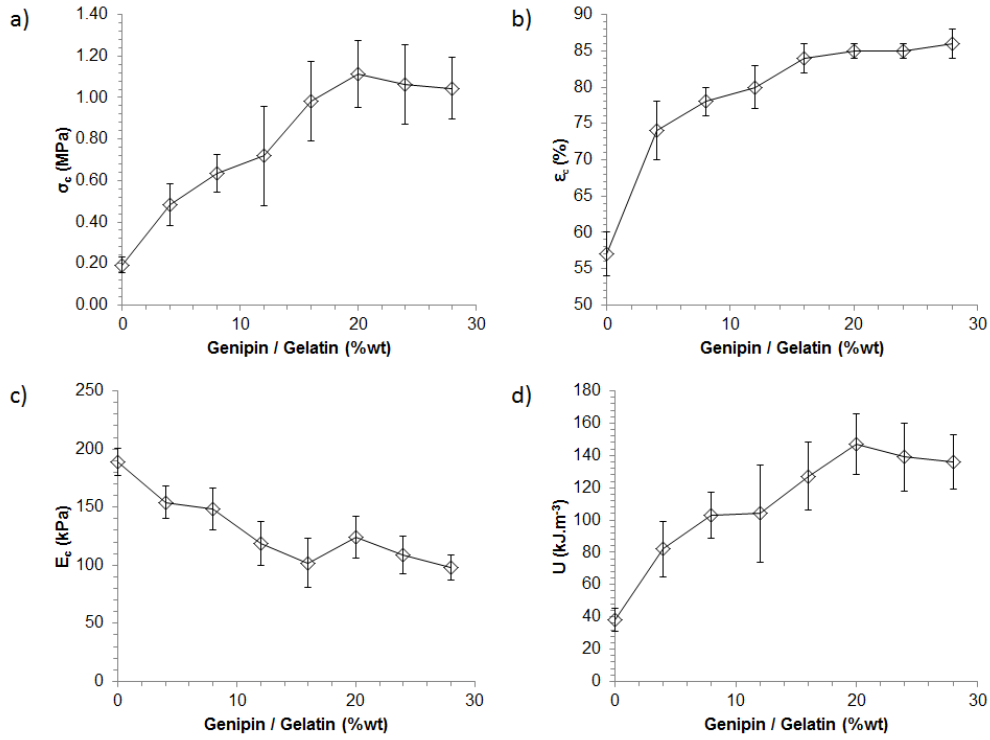


Figure S3. The concentration of genipin cross-linker affects the a) maximum compressive stress, b) secant modulus, c) maximum compressive strain, and d) strain energy to failure (\pm SD).

S2. Immersion studies

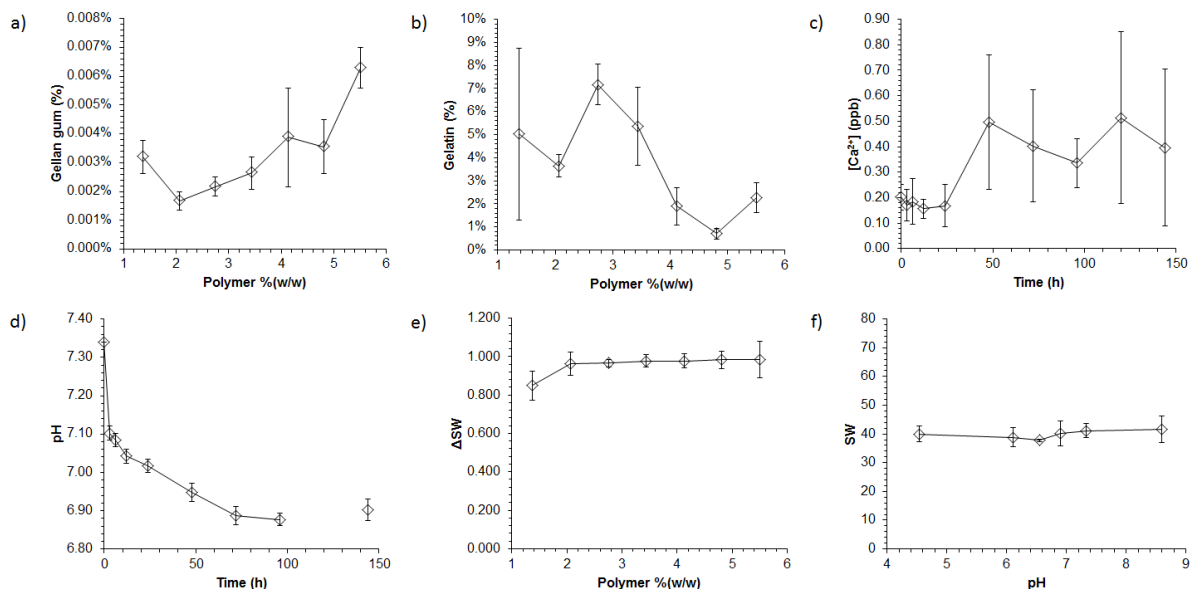


Figure S4. a) The percentage of gellan gum leached from hydrogels of different polymer concentrations after 144 hours immersion in SBF, b) the percentage of gelatin leached from hydrogels of different polymer concentrations after 144 hours immersion in SBF, c) the concentration of calcium in SBF containing a 2.75% (w/w) polymer hydrogel at different immersion intervals, d) The pH of SBF containing a 2.75% (w/w) polymer hydrogel at different immersion intervals, e) the change in swelling ratio of hydrogels of different polymer concentrations after 144 hours immersion in SBF, f) the swelling ratio of 2.75% (w/w) polymer hydrogels after being immersed in 0.1 M PBS solutions for 24 h of different pHs (\pm SD).

Table S4. Gellan gum and gelatin concentration and pH of simulated body fluid and the corresponding swelling ratio of hydrogels with different polymer concentrations with respect to immersion time.

Polymer %(w/v)	Immersion time (h)	Gellan gum leached (%)	Gelatin leached (%)	pH	Swelling ratio
1.375	0	0	0	7.46 ± 0.01	75 ± 3
	3		0.0 ± 0.1	7.54 ± 0.01	70 ± 2
	6		1 ± 1	7.55 ± 0.02	68 ± 4
	12		0.8 ± 0.9	7.49 ± 0.03	66 ± 3
	24	0.002 ± 0.001	2 ± 2	7.55 ± 0.02	66 ± 3
	48		2 ± 2	7.54 ± 0.02	65 ± 4
	72	0.0024 ± 0.0007	2 ± 1	7.50 ± 0.03	65 ± 3
	96		2 ± 1	7.48 ± 0.02	65 ± 3
	120		3 ± 3	7.44 ± 0.03	64 ± 2
	144	0.0032 ± 0.0006	5 ± 4	7.46 ± 0.03	64 ± 4

Polymer %(w/v)	Immersion time (h)	Gellan gum leached (%)	Gelatin leached (%)	pH	Swelling ratio
2.062	0	0	0.0	7.39 ± 0.01	53 ± 2
	3		0.5 ± 0.1	7.12 ± 0.02	53 ± 2
	6		1.0 ± 0.3	7.10 ± 0.02	53 ± 2
	12		1.6 ± 0.3	7.08 ± 0.02	53 ± 2
	24	0.0026 ± 0.0009	2.5 ± 0.3	7.12 ± 0.02	52 ± 2
	48		3.3 ± 0.4	7.04 ± 0.02	51 ± 2
	72	0.002 ± 0.001	3.6 ± 0.7	7.04 ± 0.04	52 ± 2
	96		3.3 ± 0.5	6.99 ± 0.01	51 ± 2

120					3.3 ± 0.4	6.96 ± 0.01	52 ± 2
144		0.0017 ± 0.0003			3.6 ± 0.5	7.00 ± 0.03	51 ± 2

Polymer %(w/v)	Immersion time (h)	Gellan gum (%)	leached	Gelatin leached (%)	pH	Swelling ratio
2.750	0	0.0000		0.0	7.34 ± 0.01	40.9 ± 0.6
	3			0.5 ± 0.2	7.10 ± 0.02	40.5 ± 0.6
	6			1.6 ± 0.7	7.09 ± 0.02	40.7 ± 0.6
	12			3.4 ± 0.9	7.04 ± 0.02	40.1 ± 0.6
	24	0.0018 ± 0.0009		5.9 ± 0.8	7.02 ± 0.02	39.9 ± 0.6
	48			9 ± 2	6.95 ± 0.2	40.0 ± 0.6
	72	0.002 ± 0.001		8 ± 2	6.89 ± 0.2	39.7 ± 0.5
	96			8 ± 2	6.88 ± 0.2	39.7 ± 0.6
	120			6 ± 1	7.00 ± 0.3	39.9 ± 0.7
	144	0.0022 ± 0.0003		7.2 ± 0.9	6.90 ± 0.3	39.5 ± 0.5

Polymer %(w/v)	Immersion time (h)	Gellan gum (%)	leached	Gelatin leached (%)	pH	Swelling ratio
3.438	0	0.0000		0.0	7.31 ± 0.01	31.4 ± 0.7
	3			0.1 ± 0.1	7.06 ± 0.02	31.2 ± 0.8
	6			1.0 ± 0.9	7.05 ± 0.02	31.1 ± 0.7
	12			2.3 ± 0.9	6.99 ± 0.02	31.0 ± 0.7
	24	0.0029 ± 0.0007		5 ± 2	6.91 ± 0.02	30.9 ± 0.7
	48			7 ± 2	6.86 ± 0.02	30.9 ± 0.7

72	0.0023 ± 0.0008	6 ± 2	6.83 ± 0.02	30.7 ± 0.7
96		5 ± 1	6.77 ± 0.02	30.9 ± 0.7
120		4.6 ± 0.8	6.79 ± 0.01	30.8 ± 0.7
144	0.0026 ± 0.0006	5 ± 2	6.78 ± 0.02	30.7 ± 0.7

Polymer %(w/v)	Immersion time (h)	Gellan gum (%)	leached	Gelatin leached (%)	pH	Swelling ratio
4.125	0	0.0000		0.0	7.50 ± 0.01	26.1 ± 0.7
	3			0.1 ± 0.2	7.31 ± 0.02	25.9 ± 0.6
	6			0.6 ± 0.6	7.26 ± 0.02	25.8 ± 0.6
	12			1.0 ± 0.7	7.19 ± 0.02	25.7 ± 0.6
	24	0.0032 ± 0.0008		1.4 ± 0.8	7.15 ± 0.03	25.6 ± 0.6
	48			1.5 ± 0.6	7.12 ± 0.03	25.5 ± 0.6
	72	0.0032 ± 0.0009		1.5 ± 0.7	7.07 ± 0.03	25.6 ± 0.6
	96			1.5 ± 0.4	7.07 ± 0.02	25.5 ± 0.6
	120			1.5 ± 0.7	7.07 ± 0.02	25.5 ± 0.6
	144	0.004 ± 0.002		1.9 ± 0.8	7.12 ± 0.03	25.5 ± 0.6

Polymer %(w/v)	Immersion time (h)	Gellan gum (%)	leached	Gelatin leached (%)	pH	Swelling ratio
4.812	0	0.0000		0.0	7.44 ± 0.01	21.4 ± 0.7
	3			0.0	7.35 ± 0.02	21.2 ± 0.6
	6			0.1 ± 0.1	7.30 ± 0.02	21.2 ± 0.7
	12			0.4 ± 0.2	7.26 ± 0.03	21.1 ± 0.7

24	0.0033 ± 0.0007	0.9 ± 0.4	7.21 ± 0.02	21.1 ± 0.7
48		0.9 ± 0.4	7.16 ± 0.01	21.0 ± 0.7
72	0.0025 ± 0.0004	0.9 ± 0.5	7.15 ± 0.02	21.0 ± 0.7
96		0.7 ± 0.3	7.16 ± 0.02	21.0 ± 0.6
120		0.6 ± 0.2	7.20 ± 0.04	21.0 ± 0.6
144	0.0036 ± 0.0009	0.7 ± 0.2	7.31 ± 0.04	21.0 ± 0.7

Polymer %(w/v)	Immersion time (h)	Gellan gum leached (%)	Gelatin leached (%)	pH	Swelling ratio
5.500	0	0.0000	0.0	7.34 ± 0.01	19 ± 1
	3		0.4 ± 0.1	7.32 ± 0.01	19 ± 1
	6		1.4 ± 0.4	7.31 ± 0.02	19 ± 1
	12		2.4 ± 0.6	7.29 ± 0.03	19 ± 1
	24	0.0039 ± 0.0008	3 ± 1	7.13 ± 0.03	19 ± 1
	48		3 ± 1	7.14 ± 0.03	19 ± 1
	72	0.004 ± 0.001	3 ± 1	7.12 ± 0.04	19 ± 1
	96		2.3 ± 0.6	7.17 ± 0.04	19 ± 1
	120		2.0 ± 0.6	7.15 ± 0.04	19 ± 1
	144	0.0063 ± 0.0007	2.3 ± 0.7	7.17 ± 0.03	19 ± 1

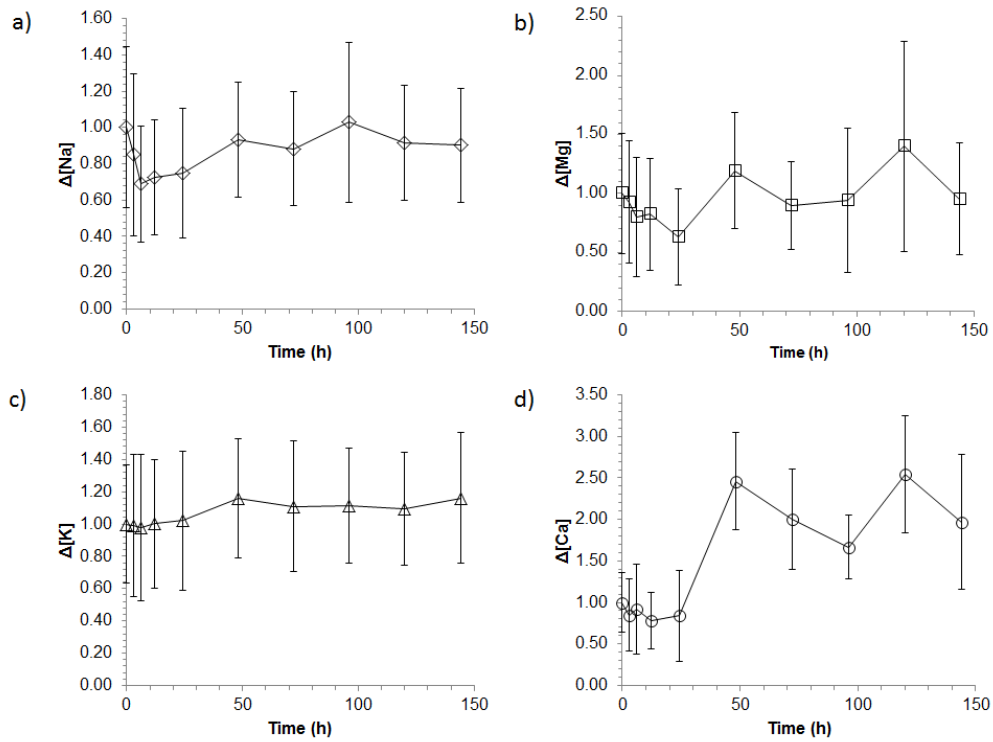


Figure S5. Change in concentration of sodium, magnesium, potassium and calcium ions in SBF with a 2.75% (w/w) polymer hydrogel immersed in them for different periods of time.