Supplementary information for

Injectable biocompatible hydrogels with tunable strength based on crosslinked supramolecular polymer nanofibers

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Content

1. Synthesis

Synthetic routes

The synthesis rout for the BTU and BTP core units was done according to the before published procedures.[1]

Synthetic protocols

Crosslinker Synthesis

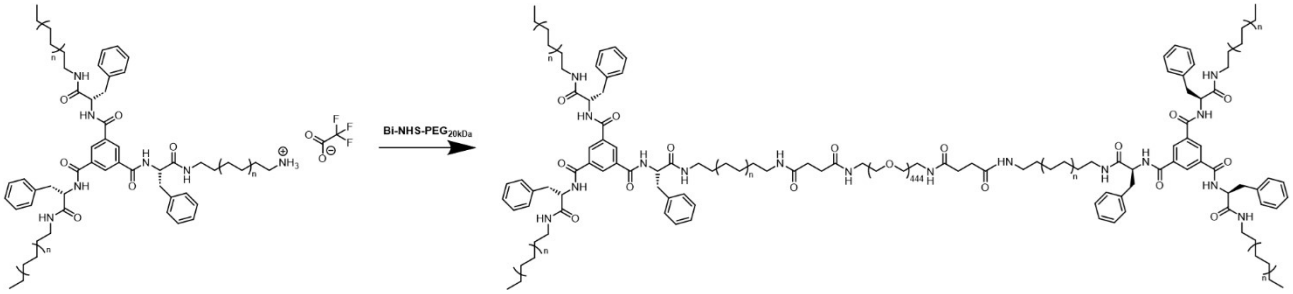

Figure S1: Synthesis route for the Crosslinker unites of BTP-C_6 (CL_6) and BTP-C_{12} (CL_{12}).

[[B][Phe]$_3$[C_6]$_3$]$_2$[PEO_{20kDa}] **CL_6**

The starting material BB_6 (25 mg, 0.024 mmol, 2.0 eq) and di-NHS-PEO_{20kDa} (243 mg, 0.012 µmol, 1 eq) were dissolved in 0.6 mL DMF. To this solution, 15 µL of triethylamine (0.104 mmol, 10 eq) were added and the mixture was stirred for 24 h at rt. After checking the conversion via SEC, the reaction mixture was diluted slowly with 20 mL water to obtain a rough concentration of 10 mg mL^{-1}. The obtained solution was dialysed (8 kDa cutoff) to remove traces of DMF and lyophilized.

Yield: 224.5 mg, 10.47 µmol (86%), white powder.

^{1}H-NMR (300 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.67 - 8.76 (m, 6 H, NH), 8.27 - 8.32 (m, 6 H, CH_{aromat}), 8.09 (br s, 6 H, NH), 7.86 (t, 2 H, NH), 7.76 (m, 2 H, NH), 7.15 - 7.35 (m, 28 H, CH_{aromat}), 4.66 - 4.78 (m, 6 H, CH), 3.70 - 3.81 (m, 15 H, CH_2), 3.39 - 3.67 (m, 2357 H, PEO), 2.97 - 3.20 (m, 32 H, CH_2) 2.28 (m, 9 H, CH_2) 1.36 (br s, 14 H, CH_2) 1.23 (br s, 34 H, CH_2) 0.84 (br t, *J*=6.51 Hz, 12 H, CH_3)

SEC (DMAc + 0.21 wt.% LiCl): M_n = 20.424 g mol^{-1}; M_w = 24.450 g mol^{-1}; Đ = 1.19

[[B][Phe]$_3$[C_{12}]$_3$]$_2$[PEO_{20kDa}] **CL_{12}**

CL_{12} was synthesised using according to the procedure of compound **CL_6** using **BB_{12}** as starting material.

Yield: 217.2 mg, 9.90 µmol (85%), white powder.

^{1}H-NMR (300 MHz, d_6-DMSO, 298 K): δ [ppm] = 8.70 (t, 6 H, NH) 8.27 (m, 6 H, CH_{aromat}) 8.08 (s, 6 H, NH) 7.86 (t, 2 H, NH) 7.74 (t, 2 H, NH) 7.29 - 7.15 (m, 30 H, CH_{aromat}), 4.73 (m, 6 H, CH) 3.69 - 3.78 (m, 10 H, CH_2) 3.51 (s, 2340 H, PEO) 3.18 - 2.92 (m, 24 H, CH_2) 2.27 (s, 11 H, CH_2) 1.36 (m, 16 H, CH_2) 1.22 (s, 114 H, CH_2) 0.75 - 0.91 (t, 12 H, CH_3)

SEC (DMAc + 0.21 wt.% LiCl): M_n = 24.832 g mol^{-1}; M_w = 31.275 g mol^{-1}; Đ = 1.26

2. Characterisation

2.1 Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) of polymers was performed on an Agilent system (series 1200) equipped with a PSS degasser, a G1310A pump, a G1362A refractive index detector and a PSS GRAM 30 and 1000 column with DMAc (+ 0.21*wt*% LiCl) as eluent at a flow rate of 1 mL min^{-1}. The column oven was set to 40 °C and polymethyl methacrylate (PMMA) standards were used for calibration.

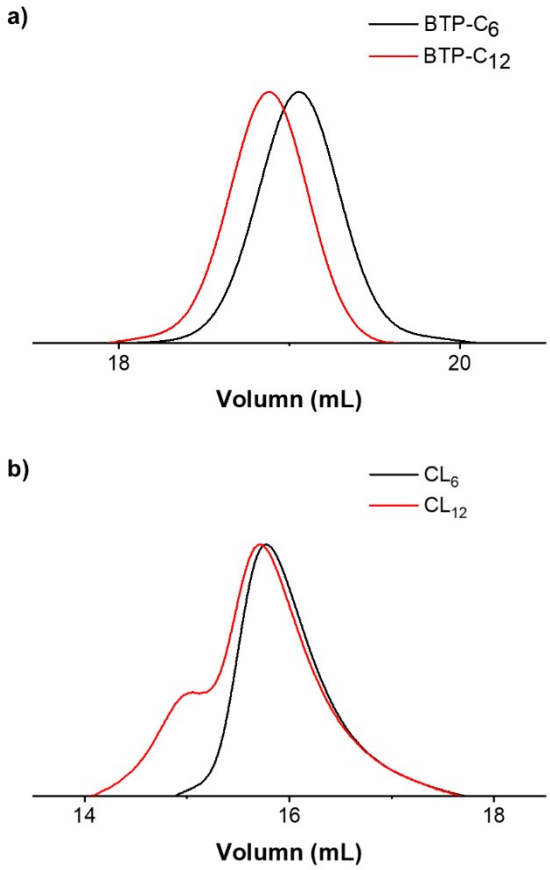

Figure S2: Overview of SEC measurements, showing elugrams of the BTP-Monomer (**a**) and BTP-Crosslinker units (**b**). Measured using DMAc (+ 0.21*wt*% LiCl) as eluent and a flow rate of 1 mL min^{-1}.

2.2 Rheologie Measurments

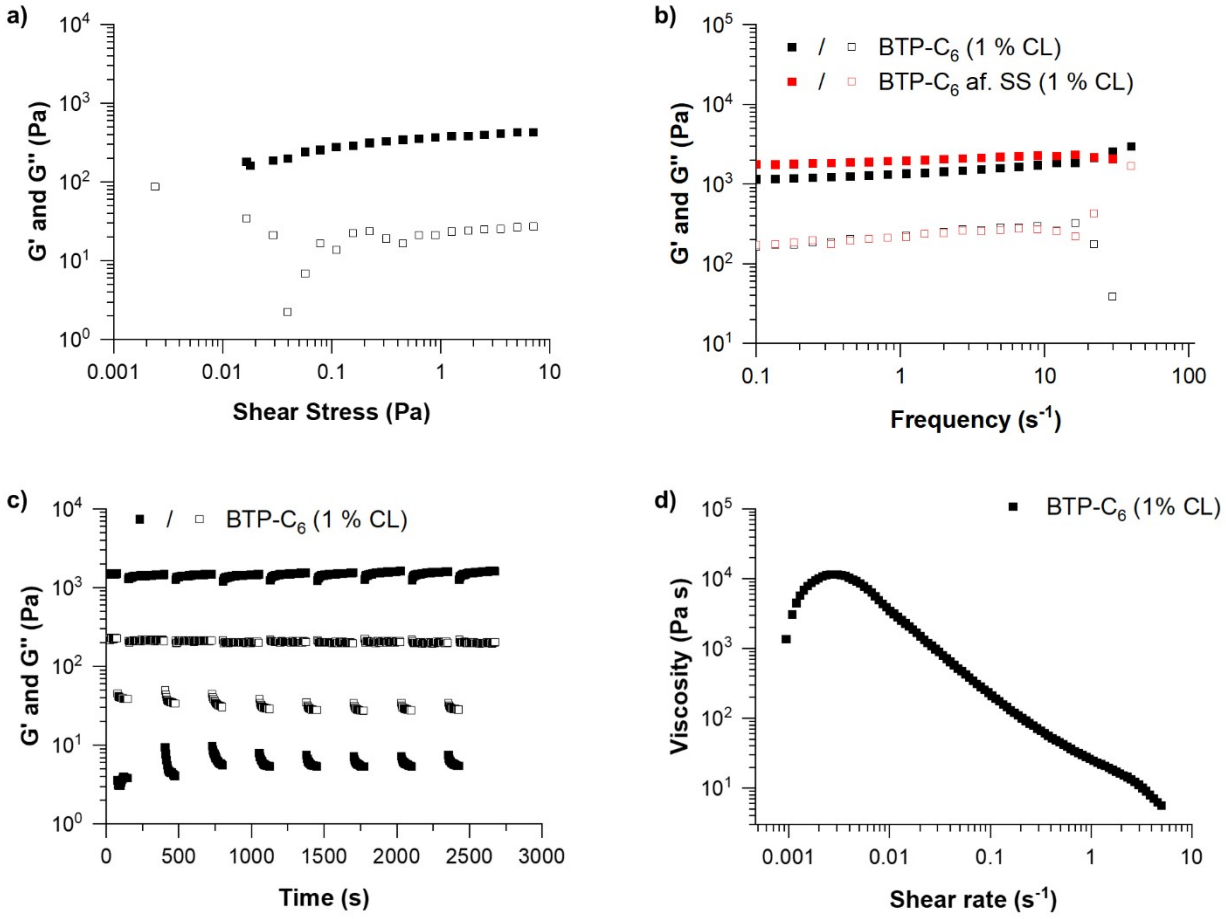

Figure S3: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurments (d) of BTP-C_6. The sample was prepaired using a solvent switch from THF and contained 2.5 w% BTP-C_6 with 1 mol% CL_6.

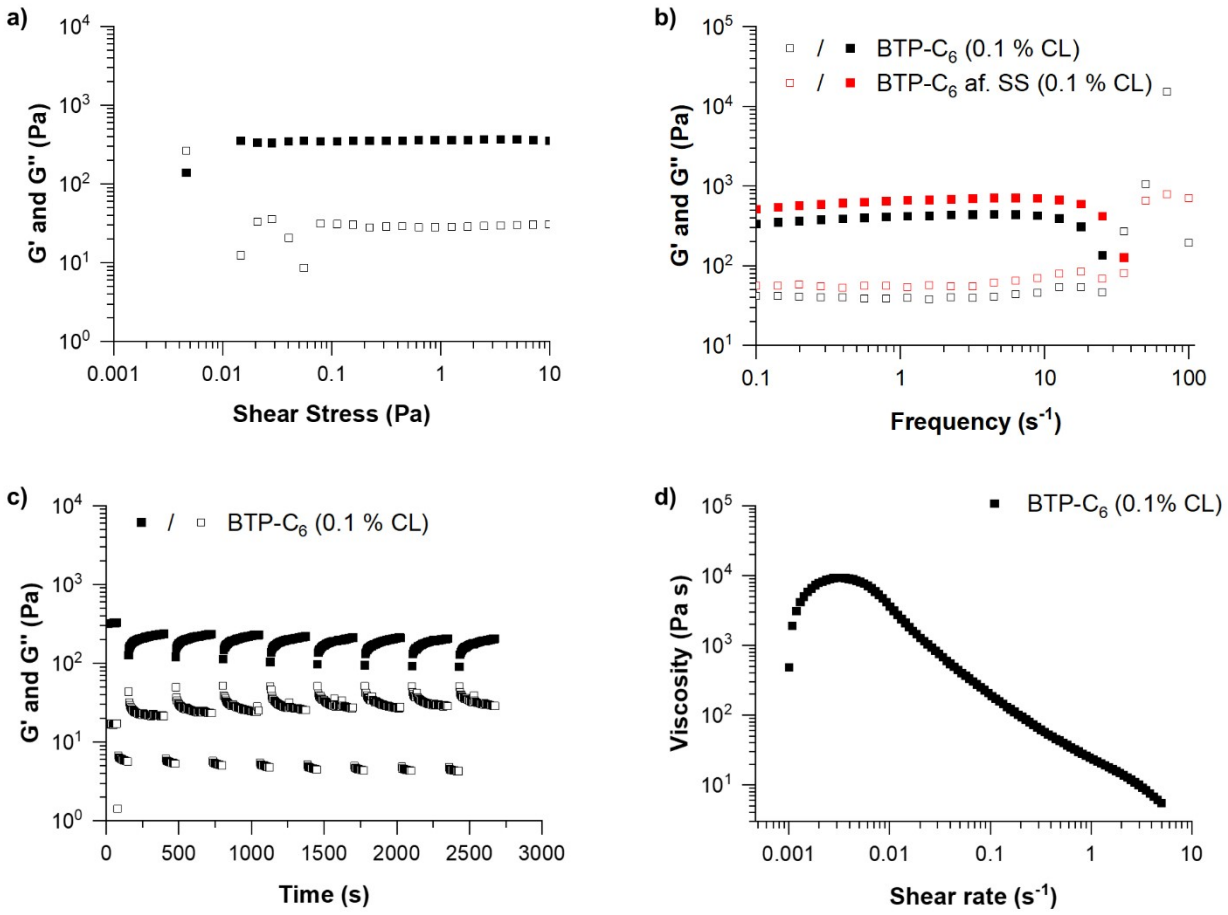

Figure S4: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurments (d) of BTP-C_6. The sample was prepaired using a solvent switch from THF and contained 2.5 w% BTP-C_6 with 0.1 mol% CL_6.

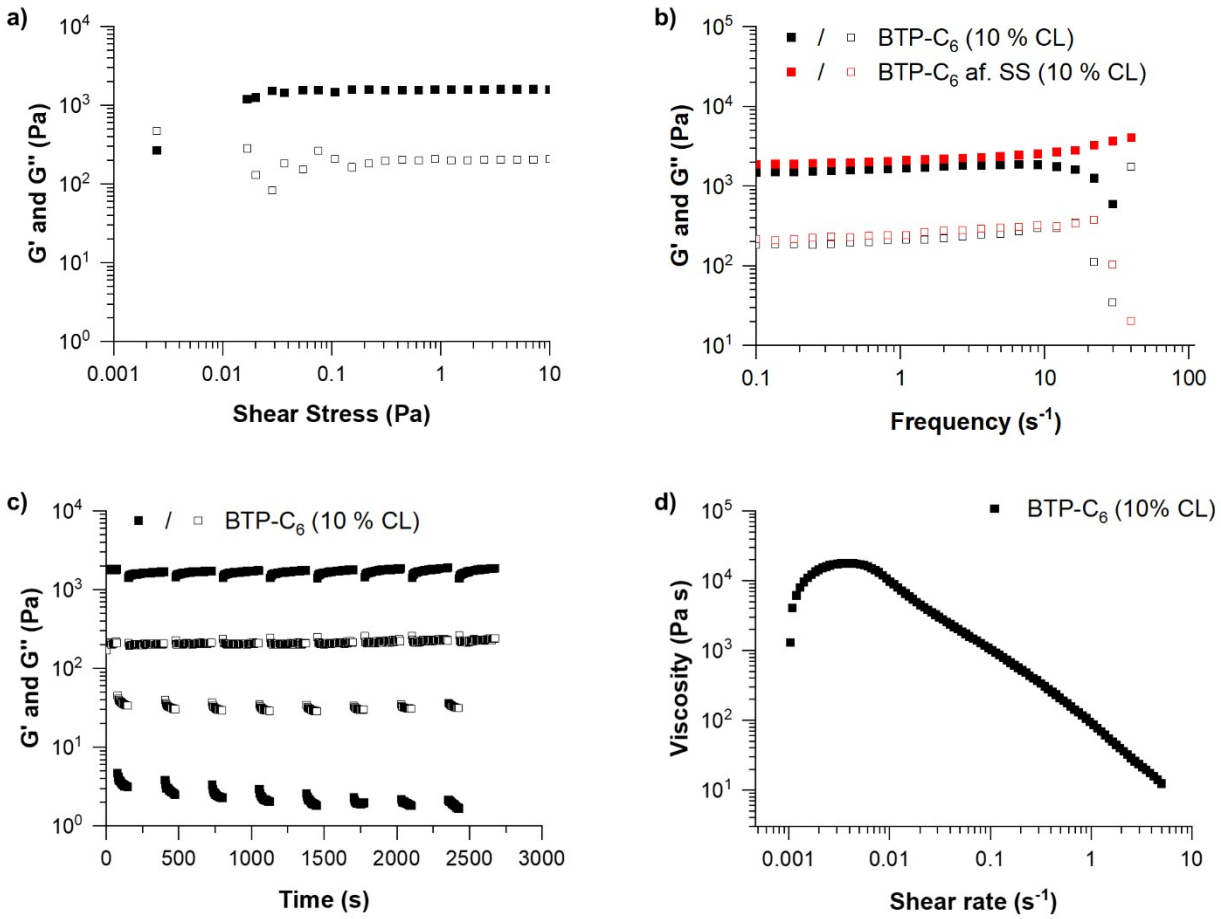

Figure S5: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurments (d) of BTP-C_6. The sample was prepaired using a solvent switch from THF and contained 2.5 w% BTP-C_6 with 10 mol% CL_6.

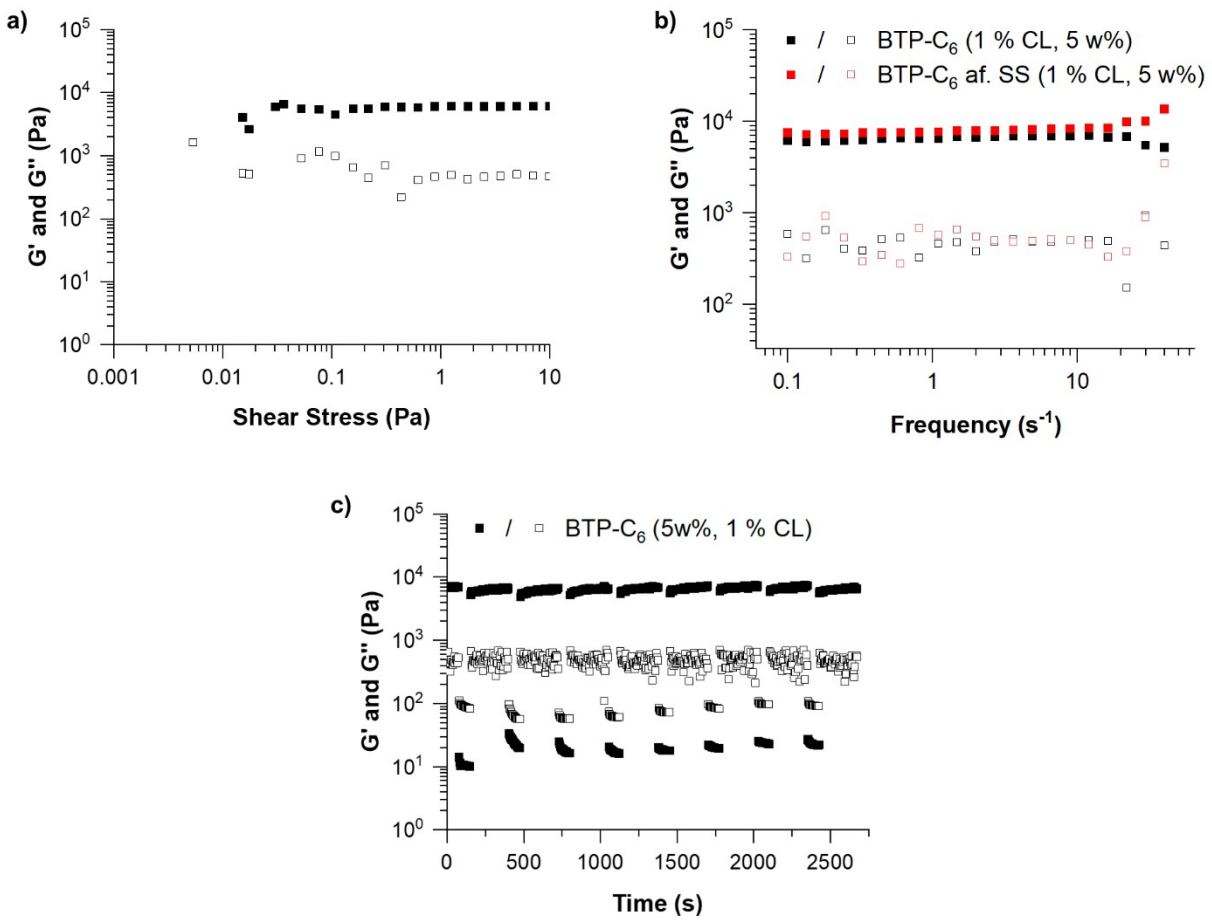

Figure S6: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) of BTP-C_6. The sample was prepared using a solvent switch from THF and contained 5 w% BTP-C_6 with 1 mol% CL_6.

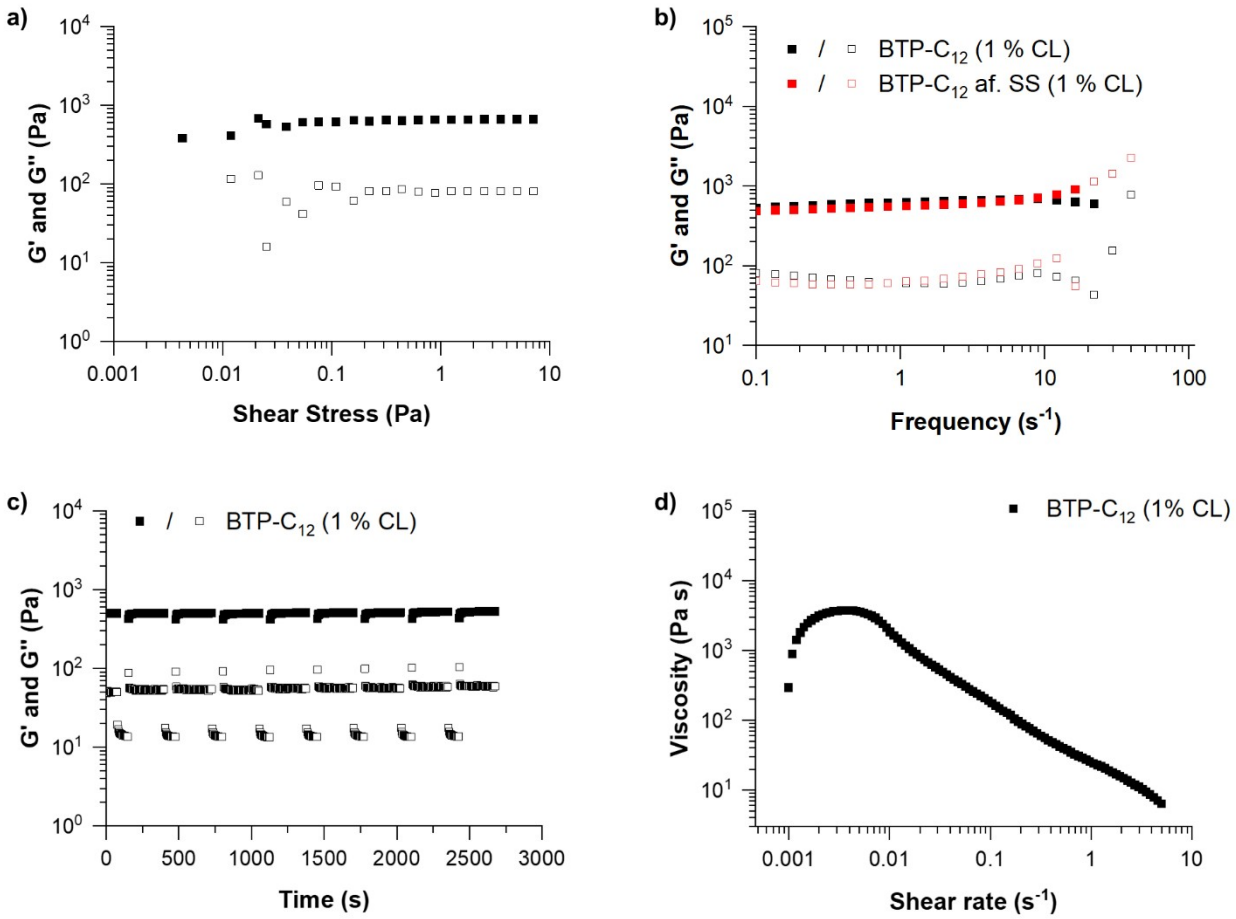

Figure S7: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurments (d) of BTP-C_{12}. The sample was prepaired using a solvent switch from THF and contained 2.5 w% BTP-C_{12} with 1 mol% CL_{12}.

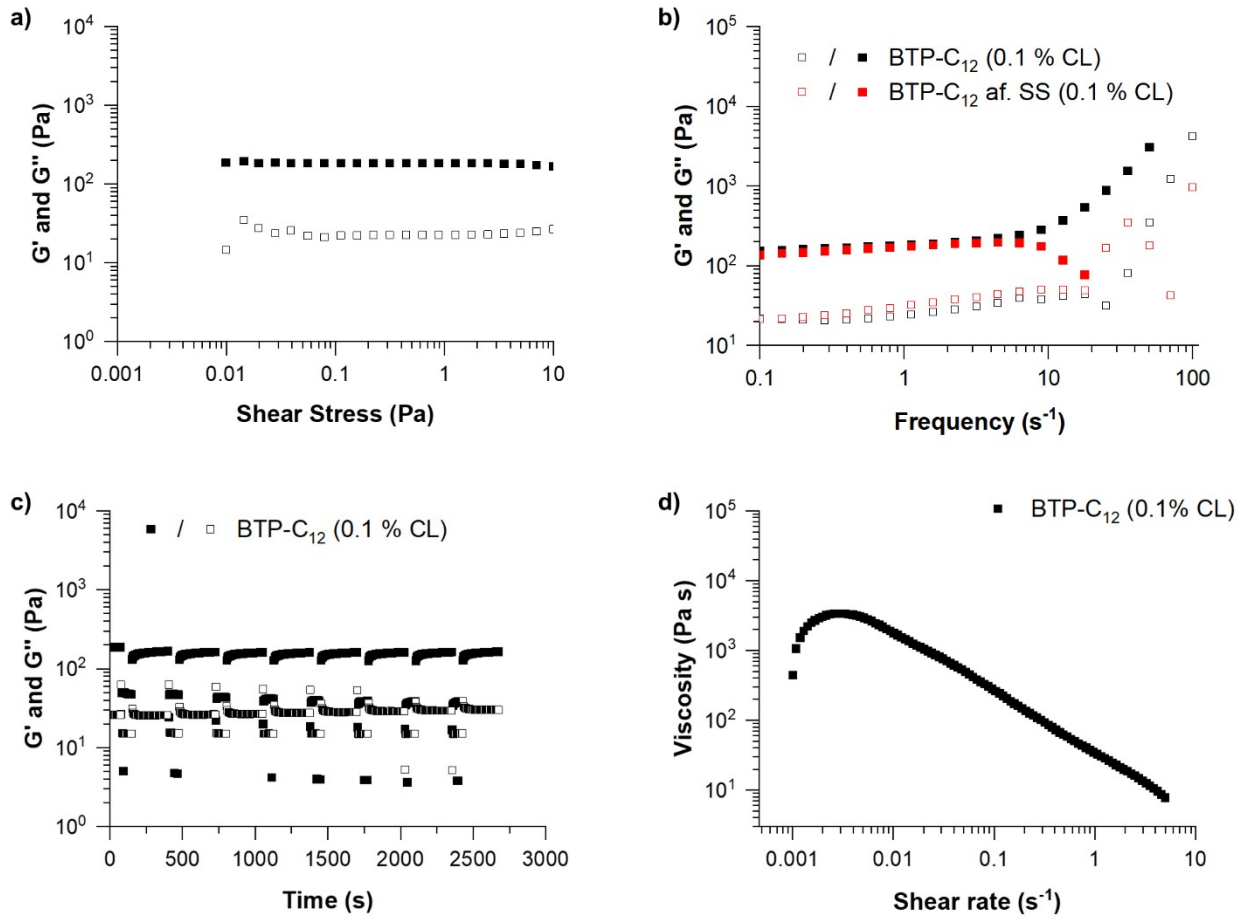

Figure S8: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurements (d) of BTP-C_{12}. The sample was prepared using a solvent switch from THF and contained 2.5 w% BTP-C_{12} with 0.1 mol% CL_{12}.

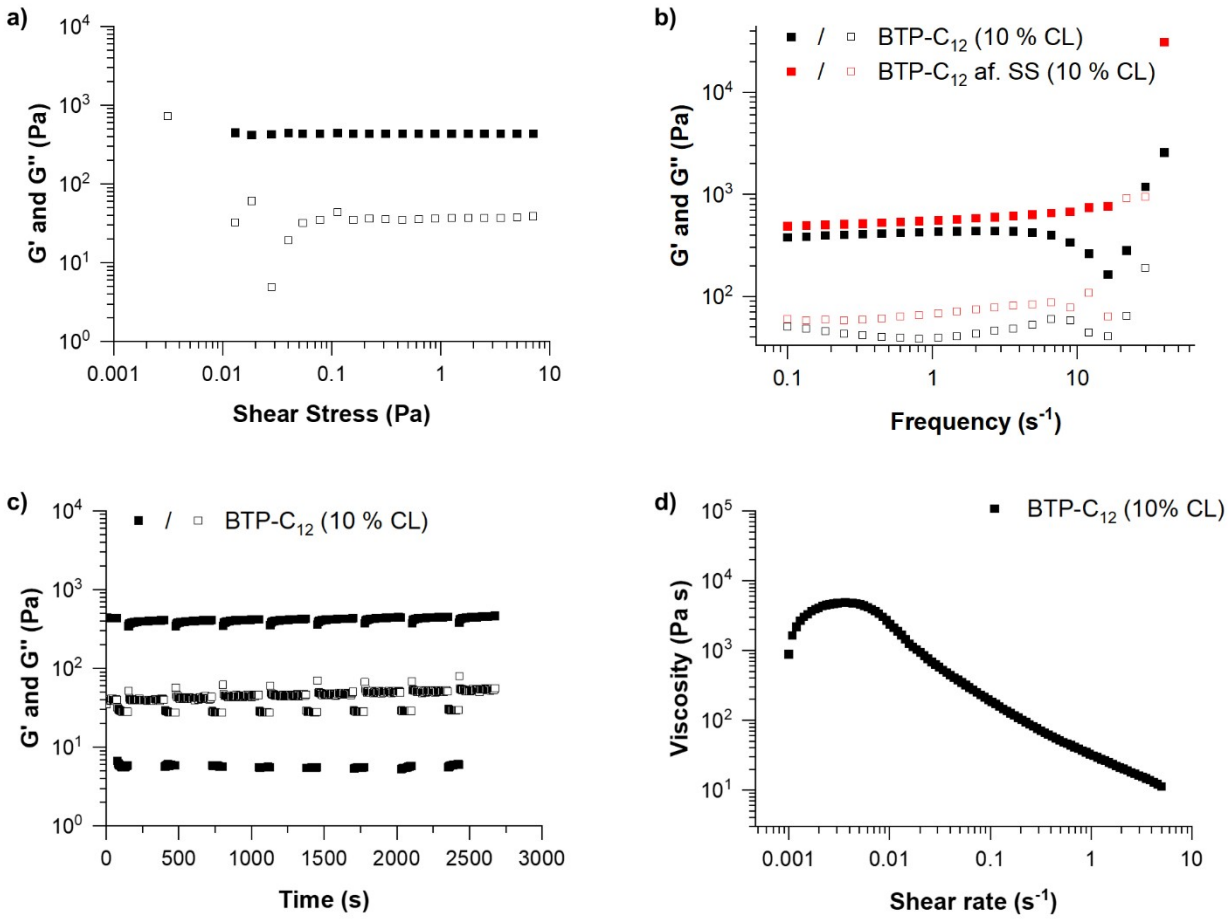

Figure S9: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) and sheer-viscosity measurements (d) of BTP-C_{12}. The sample was prepared using a solvent switch from THF and contained 2.5 w% BTP-C_{12} with 10 mol% CL_{12}.

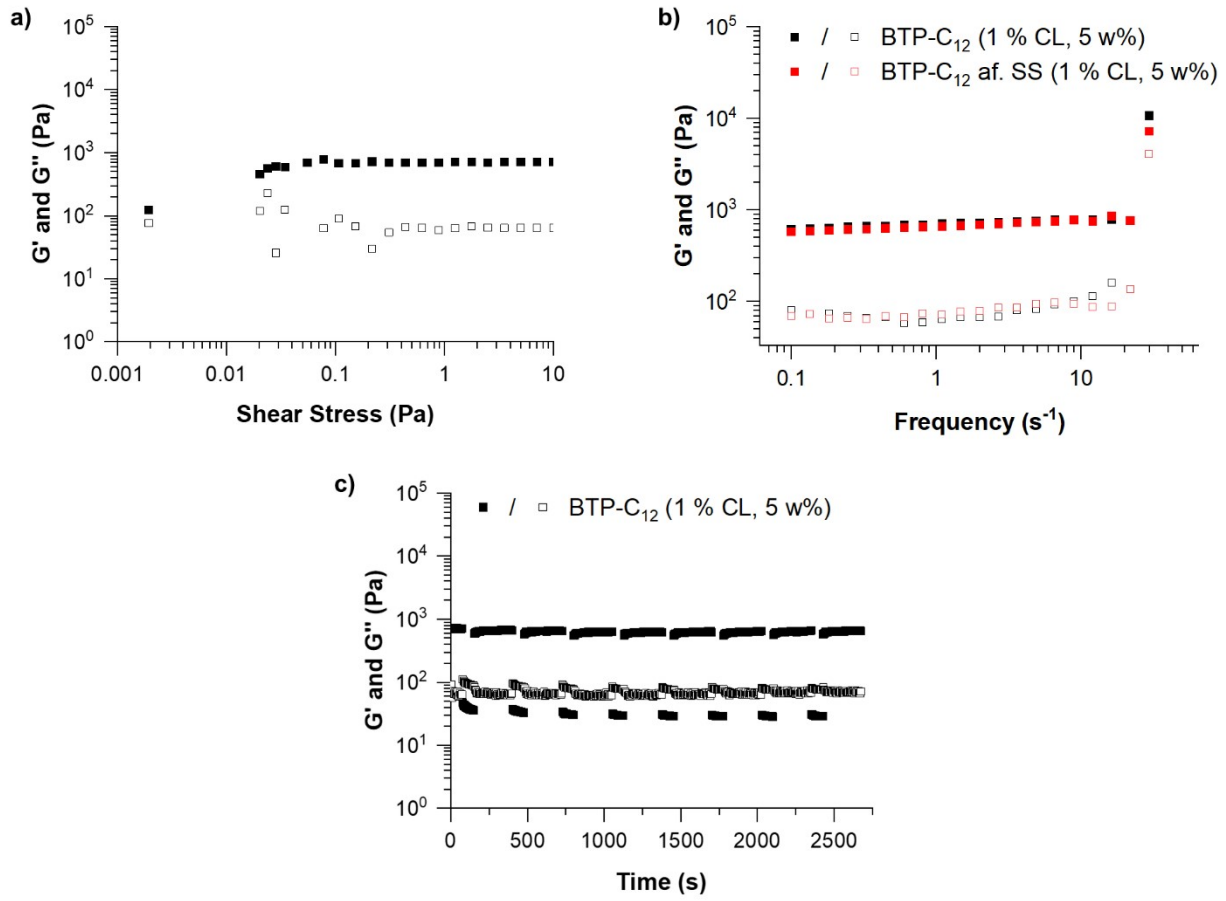

Figure S10: Stress sweep (a), Frequency sweep (b) before (black) and after (red) step strain measurements (c) of BTP-C12. The sample was prepared using a solvent switch from THF and contained 5 w% BTP-C_{12} with 1 mol% CL_{12}.

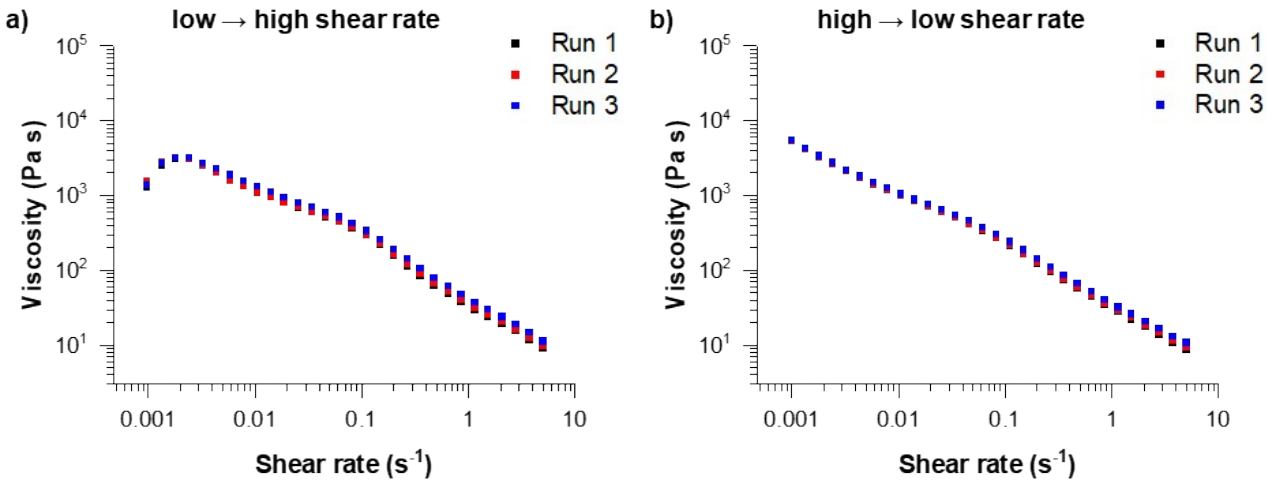

Figure S11: Multiple successive viscosity measurements in of BTP-C_{12} (1 mol% CL) with a shear rate increase (a) and decrease (b) for each run.

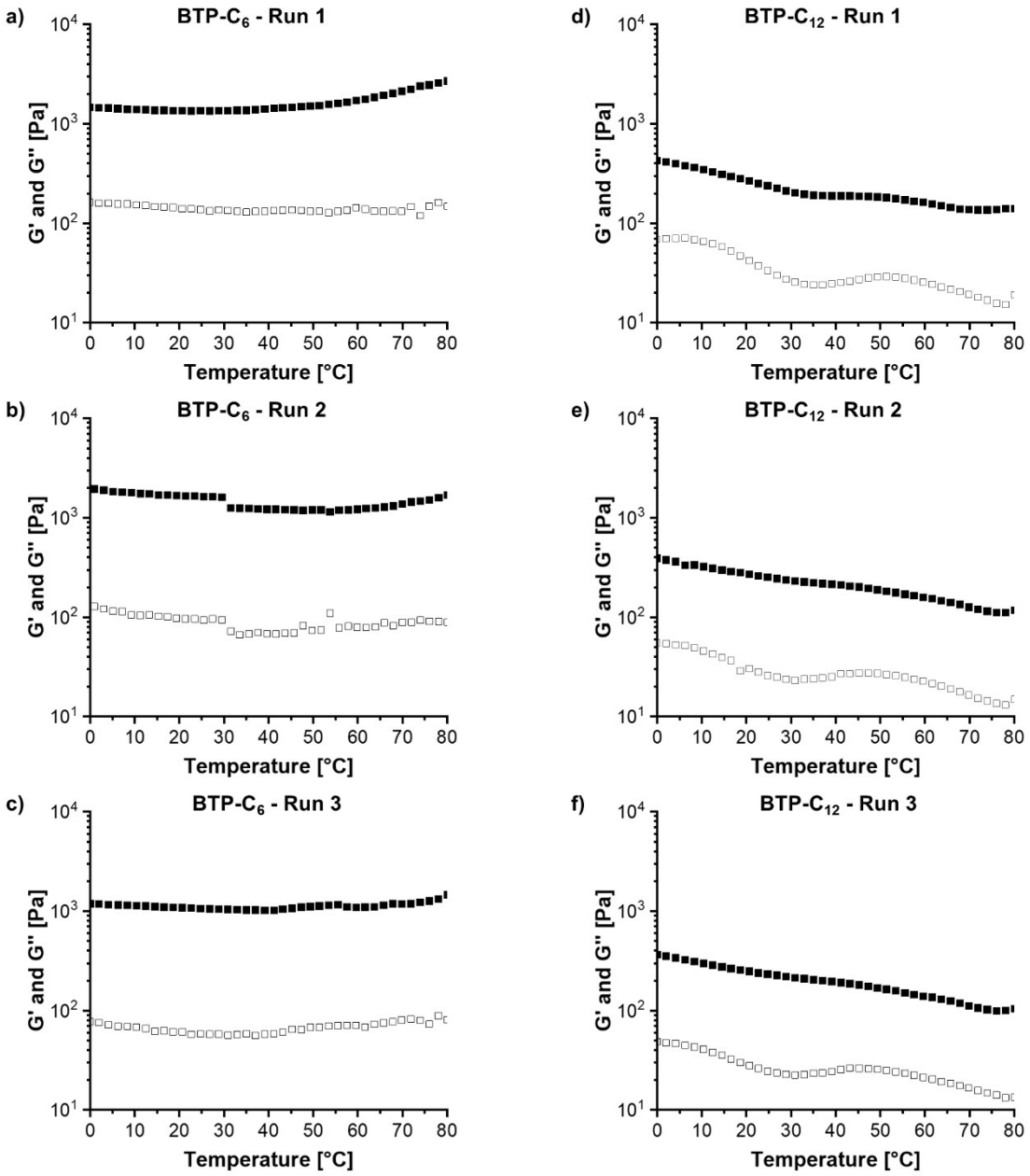

Figure S12: Repeated temperature sweep measurements performed on the same BTP-C_6 (a-c) and BTP-C_{12} (d-f). The sample was prepared using a solvent switch from THF and contained 2.5 w% of the corresponding BTP with 1 mol% CL.

2.3 Biocompatibility tests

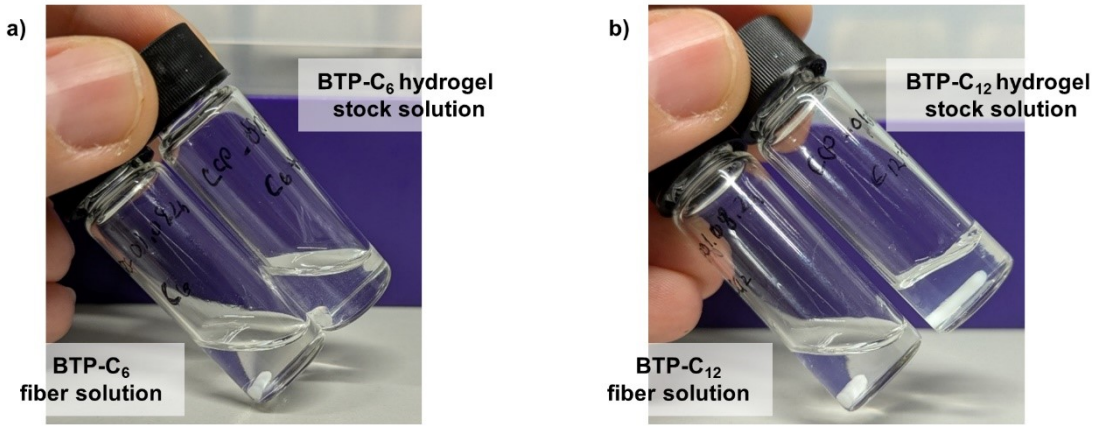

Figure S13: Following solvent switch, constitutional difference in BTP-C_6 (a) and BTP-C_{12} (b) fiber and hydrogel stock solutions.

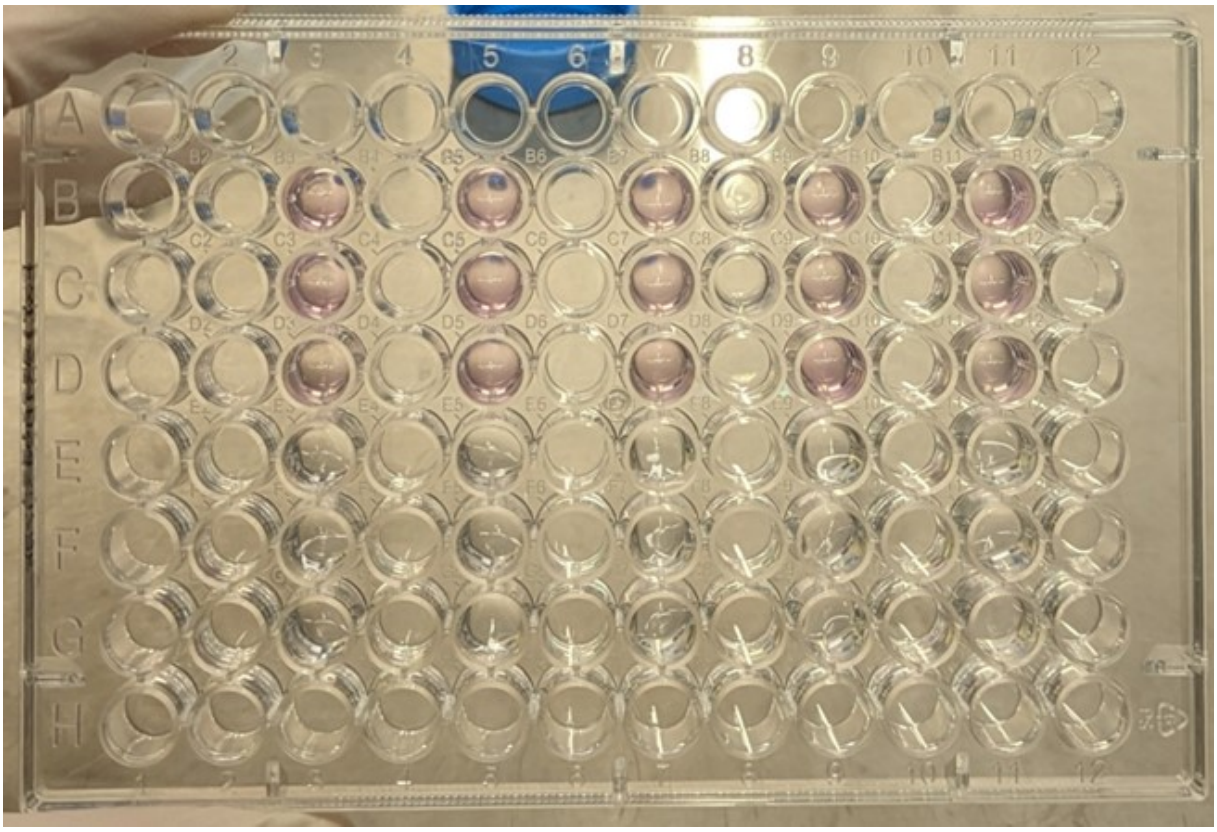

Figure S14: The overview of 96-well plate seeded with BTP-C_6 hydrogels (2.5 wt% and 1 mol% CL) after THF evaporation. Upper triplicates represent the gels treated D10 and the bottom triplicates depict the gels treated with PBS (1x).

Table S1. Mean and standard deviation values for relative metabolic activity (%)

Sample	Mean	Std. Deviation
Control P1	100	3.9
Control P2	100	2.8
Control P3	100	4.9
Control P4	100	4.2
Gel P1	92.6	7.8
Gel P2	87.7	11.7
Gel P3	92.0	6.4
Gel P4	88.2	4.8

3. Injection parameter calculation

Using the following equation one can calculate the pressure (*P*) needed to inject the sample through a syringe.[2]

$$P = \left(\frac{3n+1}{n}\right)^n K \left(\frac{Q}{\pi}\right)^n \frac{2l}{R^{3n+1}}$$

n = Fitted shear thinning parameter

l = needle length

K = consistency index

R = needle radius

Q = flow rate

To the force (*F*) needed for extrusion was calculated using the following equation.

$$F = P\pi R_{syringe}{}^2$$

Table S2. Calculated values of the force needed to extrude the hydrogels (25 mg mL^{-1}, 1 mol% CL) using different neeldes and a 1 mL syringe.

Gauge	Sample	n	K	Q	l	R	$R_{syringe}$	F

			[Pa s^{-1}]	[mL s^{-1}]	[mm]	[mm]	[mm]	[N]
21	BTP-C_6	0.14	25.12	0.1	50	0.29	2.5	0.62
	BTP-C_{12}	0.16	25.48					0.76
23	BTP-C_6	0.14	25.12		12	0.17		0.33
	BTP-C_{12}	0.16	25.48					0.42
23	BTP-C_6	0.14	25.12		80	0.17		2.13
	BTP-C_{12}	0.16	25.48					2.72
27	BTP-C_6	0.14	25.12		12	0.11		0.63
	BTP-C_{12}	0.16	25.48					0.84

4. References

[1] T. Klein, H. F. Ulrich, F. V. Gruschwitz, M. T. Kuchenbrod, R. Takahashi, S. Fujii, S. Hoeppener, I. Nischang, K. Sakurai, J. C. Brendel, *Impact of amino acids on the aqueous self-assembly of benzenetrispeptides into supramolecular polymer bottlebrushes. Polym. Chem.* **2020**, *11*, 6763-6771.

[2] S. Correa, A. K. Grosskopf, H. Lopez Hernandez, D. Chan, A. C. Yu, L. M. Stapleton, E. A. Appel, *Translational Applications of Hydrogels. Chem. Rev.* **2021**, *121*, 11385-11457.