Nonswelling thiol-yne crosslinked hydrogel materials as cytocompatible soft tissue scaffolds

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Contents

1. NMR and SEC analysis of alkyne and thiol PEG

The successful incorporation of the alkyne and thiol functionalities into the PEG-base polymers was confirmed by ^{1}H NMR spectroscopy and size exclusion chromatography (SEC) (Table S1).

Table S1. Additional characterisation data of alkyne and thiol-PEG precursors.

	End group conv. (%)	M_n NMR (kg /mol)	M_n SEC [c] (kg/mol)	$Đ_M$ [c]
$PEG_{1k}(C{\equiv}CH)_3$	97	1.2[a]	3.3	1.07
$PEG_{2k}(C{\equiv}CH)_4$	93	2.4[a]	5.9	1.08
$PEG_{1k}(SH)_3$	96	1.3[b]	1.2[d]	1.08[d]
$PEG_{2k}(SH)_4$	>99	1.9[b]	4.7	1.06
$PEG_{1k}(SH)_2$	97	1.2[b]	2.7	1.1
$PEG_{2k}(SH)_2$	>99	2.2[b]	2.7 [d]	1.26 [d]
$PEG_{3k}(SH)_2$	84	3.3[b]	8.7	1.1
$Pluronic_{3k}(SH)_2$	93	3.2[b]	5.8	1.2

[a]Determined by ^{1}H NMR spectroscopy in deuterated acetone. [b]Determined by ^{1}H NMR spectroscopy in deuterated chloroform. [c]Determined by SEC analysis in DMF against poly(methyl methacrylate). [d]Determined by SEC analysis in chloroform against polystyrene standards

a. ^{1}H NMR spectrum for alkyne and thiol PEG precursors

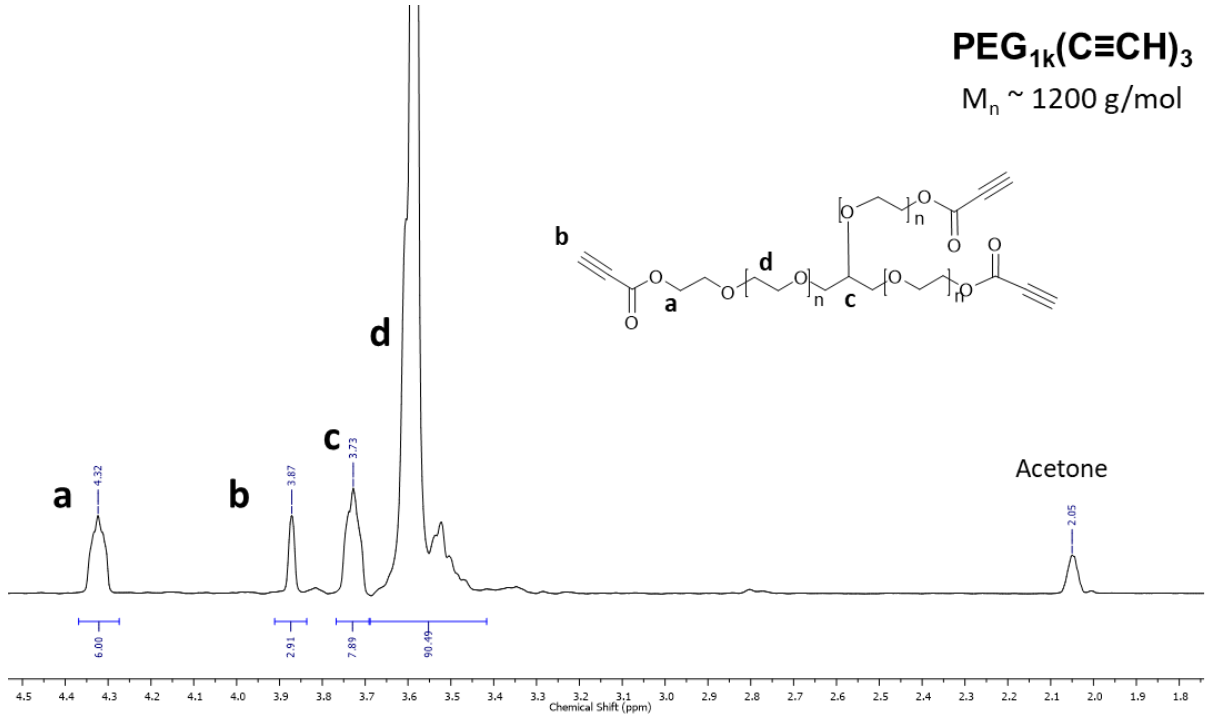

Figure S1. ^{1}H NMR spectrum of $PEG_{1k}(C{\equiv}CH)_3$ (1 kg/mol) in d_6-acetone (300 MHz, 298 K).

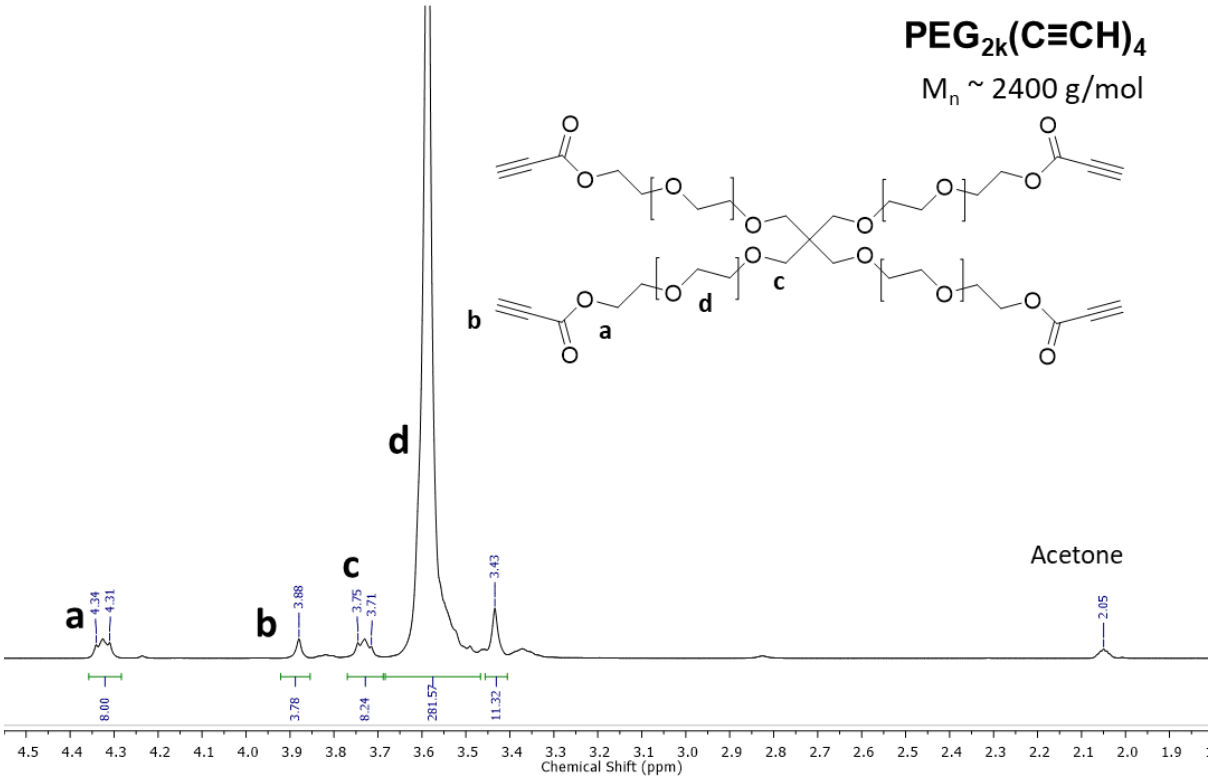

Figure S2. ^{1}H NMR spectrum of $PEG_{2k}(C{\equiv}CH)_4$ (2 kg/mol) in d_6-acetone (300 MHz, 298 K).

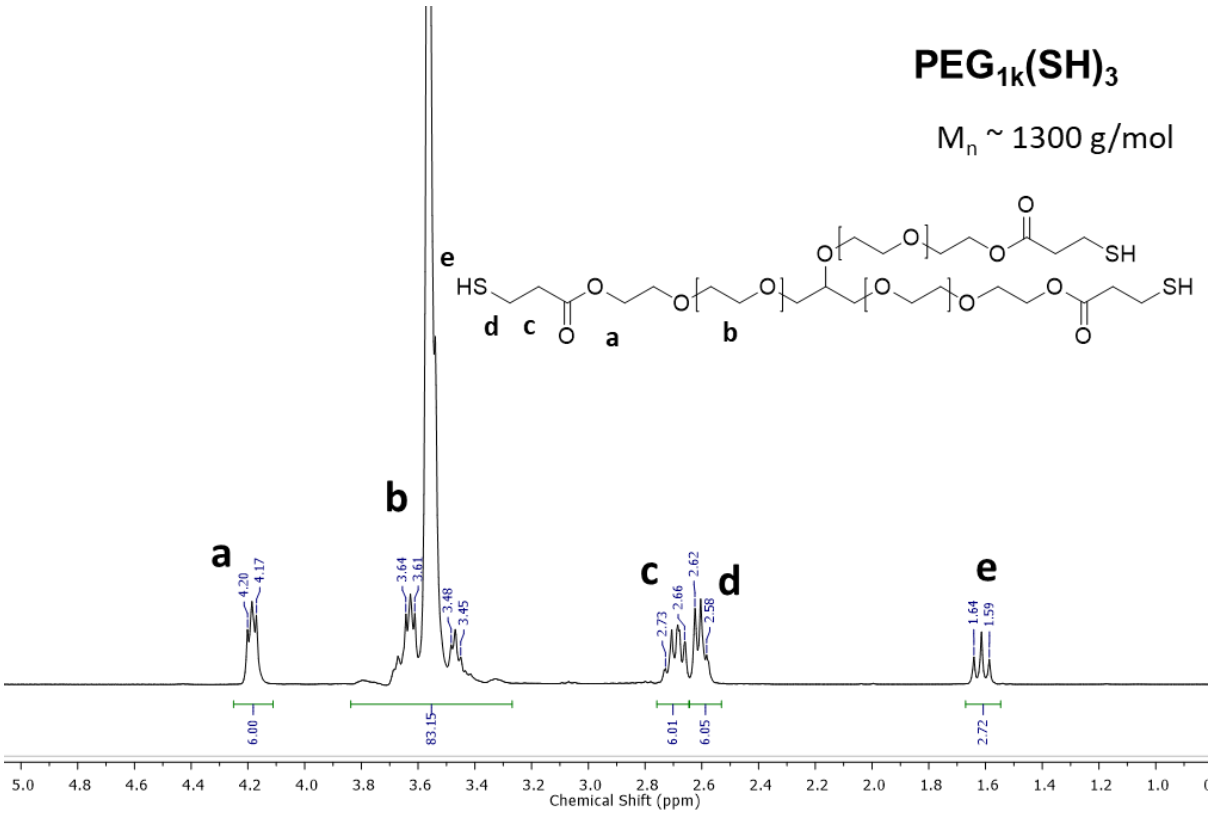

Figure S3. ^{1}H NMR spectrum of $PEG_{1k}(SH)_3$ (1 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

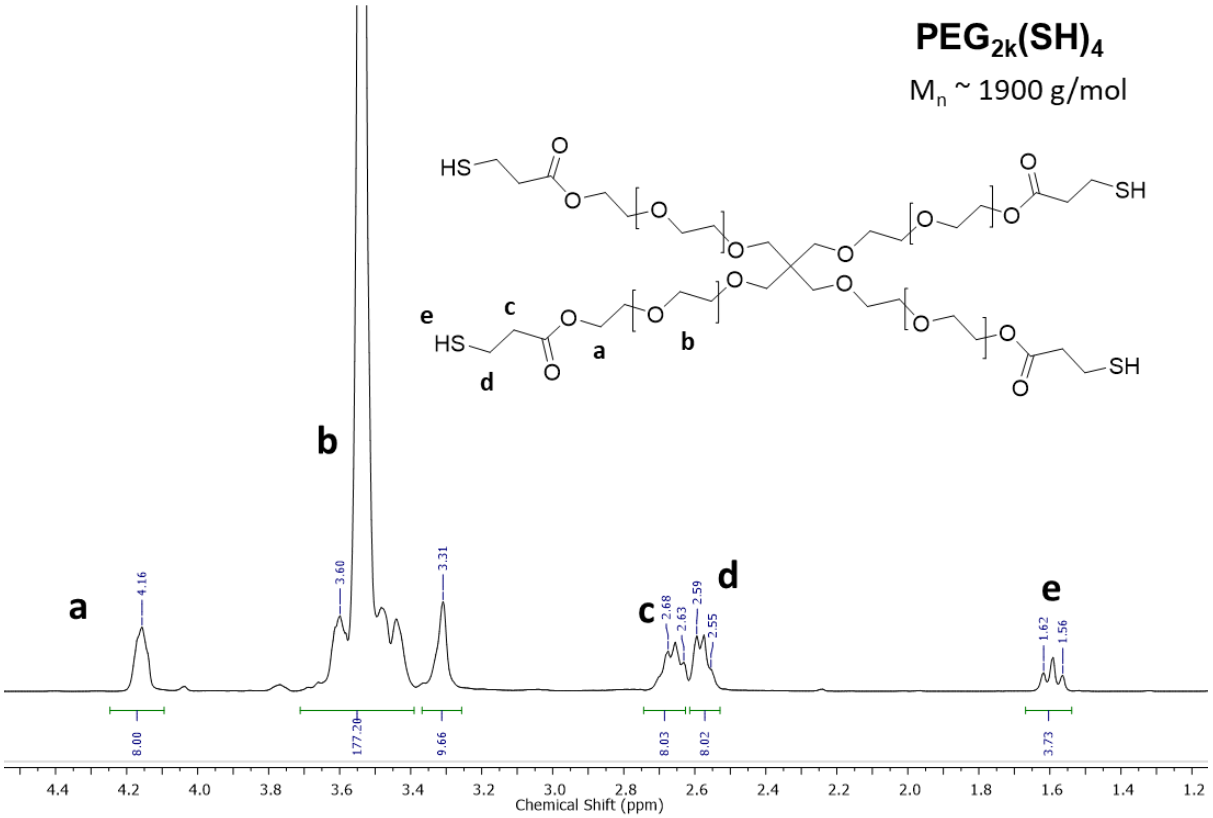

Figure S4. ^{1}H NMR spectrum of $PEG_{2k}(SH)_4$ (2 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

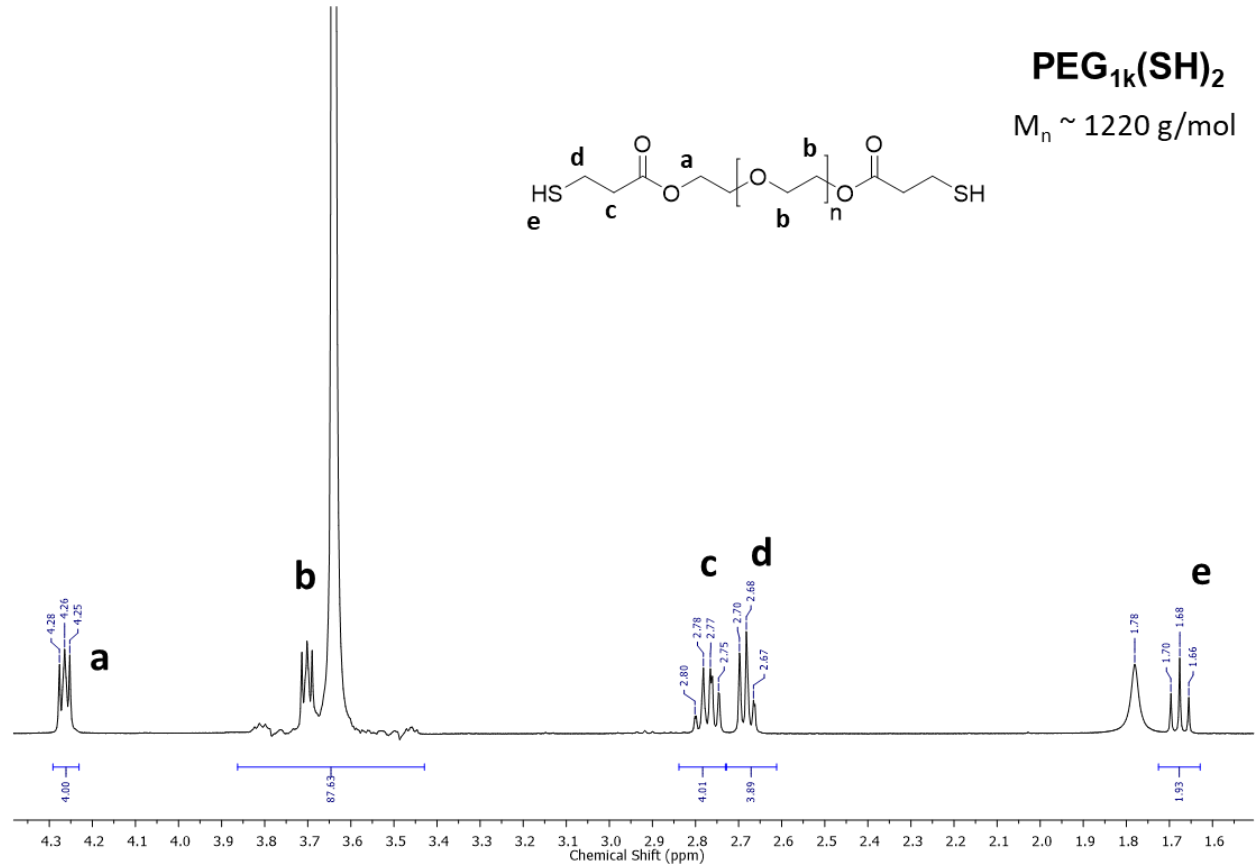

Figure S5. ^{1}H NMR spectrum of $PEG_{1k}(SH)_2$ (1 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

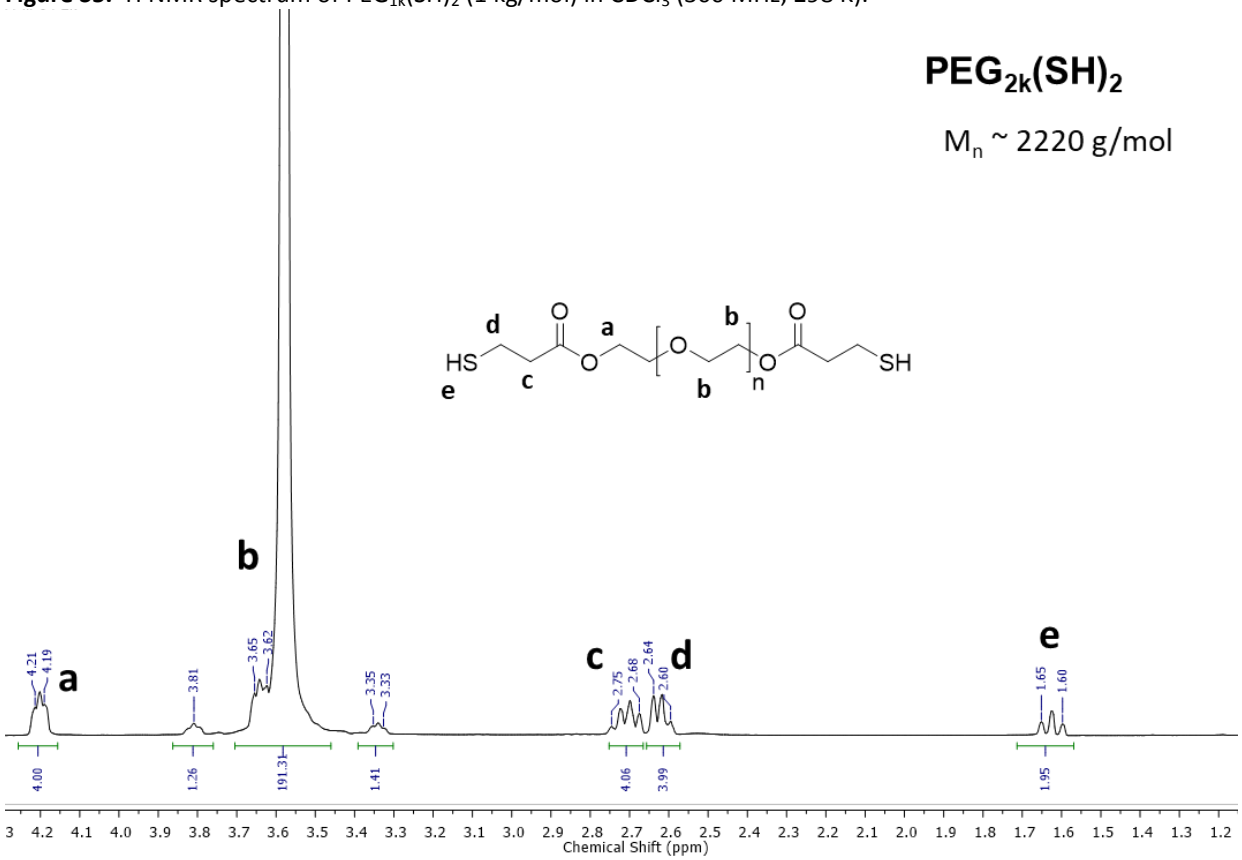

Figure S6. ^{1}H NMR spectrum of $PEG_{2k}(SH)_2$ (2 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

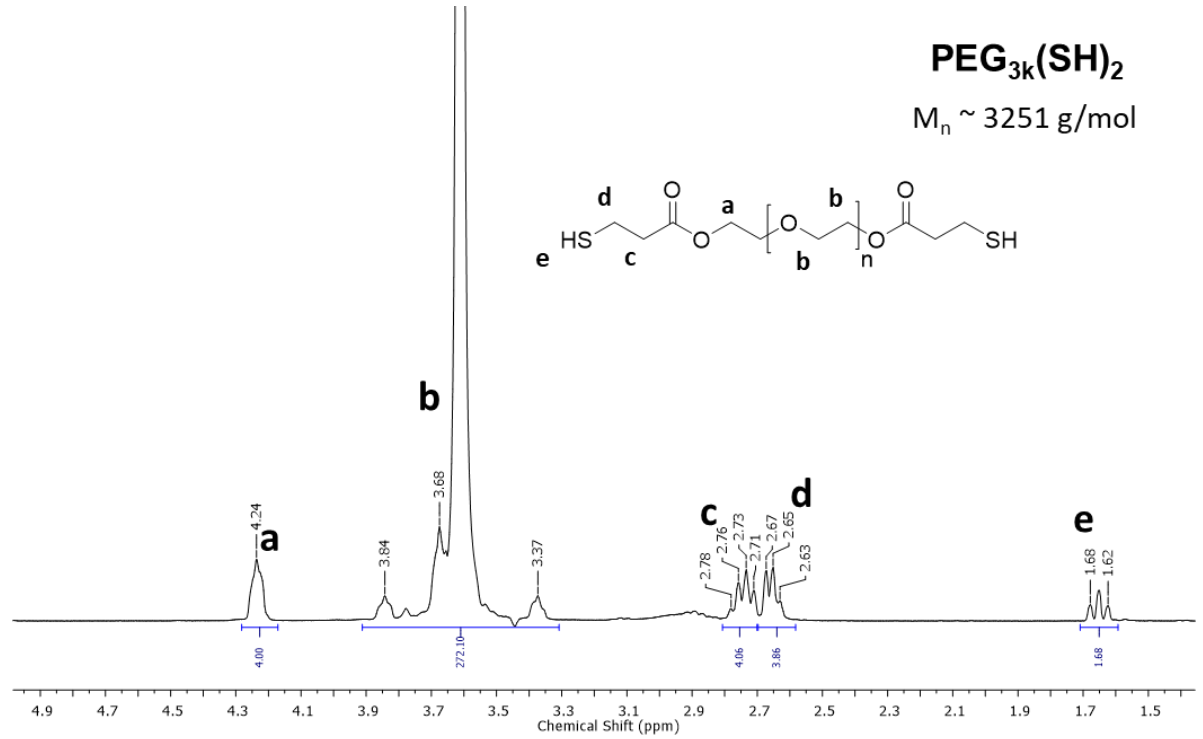

Figure S7. ^{1}H NMR spectrum of $PEG_{3k}(SH)_2$ (3 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

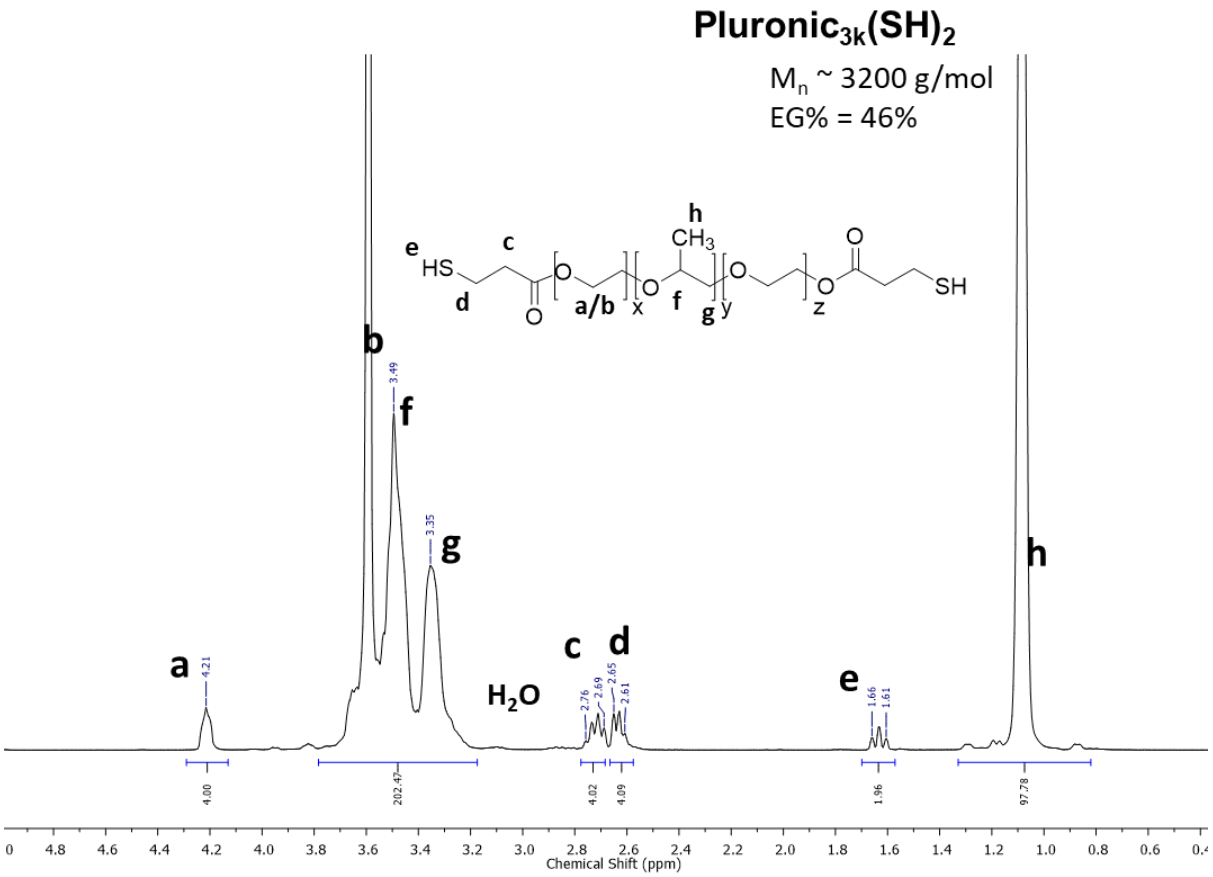

Figure S8. ^{1}H NMR spectrum of $Pluronic_{3k}(SH)_2$ (3 kg/mol) in $CDCl_3$ (300 MHz, 298 K).

b. SEC data for alkyne and thiol PEG precursors

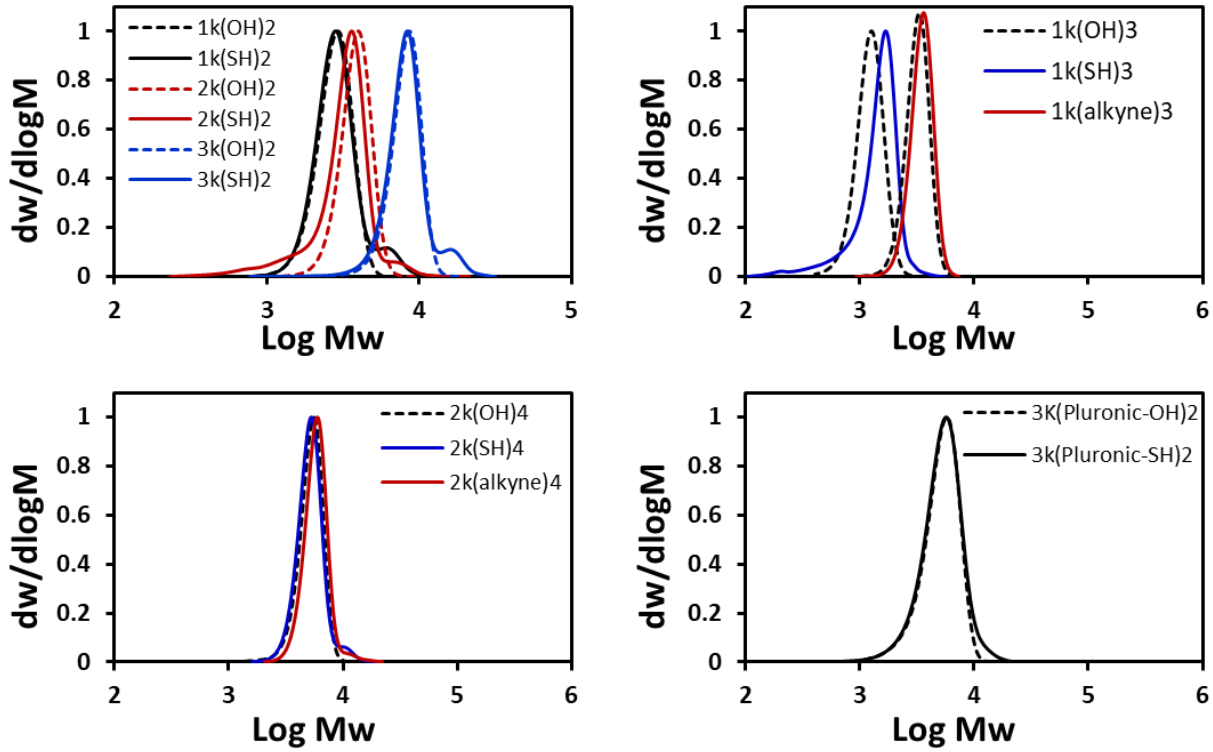

Figure S9. SEC chromatograms of alkyne and thiol PEG precursors and starting PEG materials. Molecular weight determined against poly(methyl methacrylate) standards using DMF (5 mM NH_4BF_4) as eluent or against polystyrene standards using chloroform (0.5%NEt_3), (**$PEG_{1k}(SH)_3$** and **$PEG_{2k}(SH)_2$**).

2. LCST for Pluronic$_{3K}$(SH)$_2$

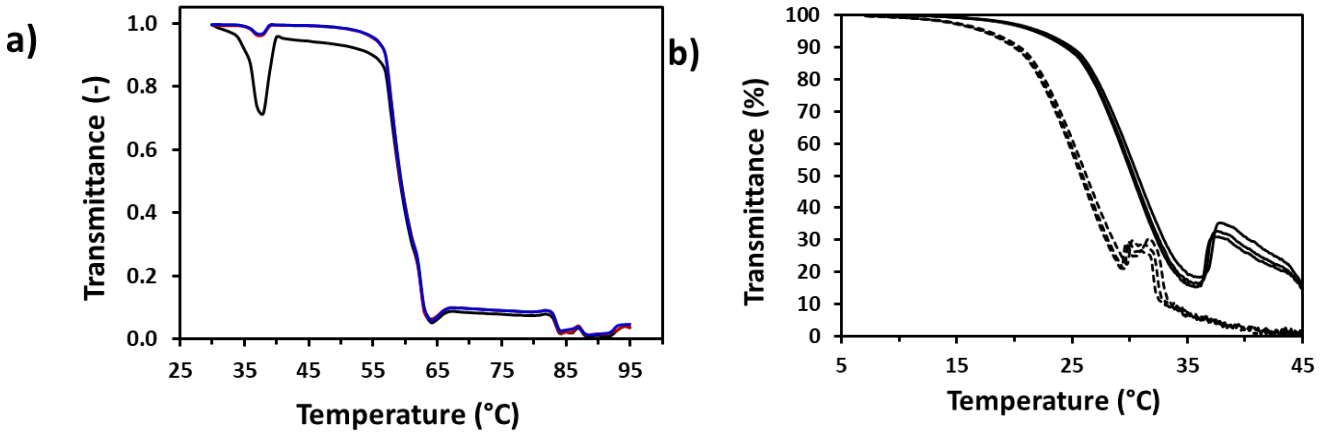

Figure S10. LCST response of Pluronic® (a) before and (b) after modification to obtain Pluronic$_{3k}$(SH)$_{2.}$

3. Additional hydrogel characterisation data

Table S2. Additional mechanical data of gels at 10 w/v% in PBS (1:1 molar ratio of alkyne to thiol end groups): stress and strain values before immersion in PBS /after a certain interval of time at 37 °C.

	Stress at break (kPa)	**Strain at break (%)**
$\mathbf{3_{1A}2_{1S}}$ [a]	1944 ± 20 / 1663 ± 80	96.3% ± 0.1% / 96.7% ± 0.3%
$\mathbf{3_{1A}2_{2S}}$ [a]	2228 ± 224 / 953 ± 104	95.5% ± 0.5% / 97.1% ± 0.3%
$\mathbf{3_{1A}2_{3S}}$ [a]	2357 ± 250 / 1045 ± 52	95.4% ± 0.6% / 96.7% ± 0.3%
$\mathbf{3_{1A}2P_{3S}}$ [b]	2469 ± 202 / 4628 ± 252	95.3% ± 0.2% / 93.1% ± 0.2%
$\mathbf{3_{1A}2_{3S\text{-}6040}}$ [c]	2512 ± 55 / 638 ± 249	94.8% ± 0.2% / 97.2% ± 0.5%
$\mathbf{3_{1A}3_{1S}}$	93 ± 8.8 / 275 ± 37 (3 days)	66.4 ± 2.2 / 68.3 ± 3.6
$\mathbf{3_{1A}4_{2S}}$	116 ± 8.7 / 317 ± 77.5 (24hrs)	61.2 ± 1.89 / 66.4 ± 3.7
$\mathbf{4_{2A}3_{1S}}$	131 ± 10.5 / 315 ± 63 (24 hrs)	58.0 ± 2.5 / 57.8 ± 4.0
$\mathbf{4_{2A}4_{2s}}$	120 ± 13 / 459 ± 73.5 (5 days)	48.2 ± 3.7 / 66.7 ± 3.6

[a)] After 48h in PBS; [b)] after 7 days in PBS; [c)] after 14 days in PBS; and [d)] maximum stress in PBS at 37 °C.

Table S3. Compressive Young's Modulus of gels at 10 w/v% in PBS (1:1 molar ratio of alkyne to thiol end groups) before/after being immersed in PBS at 37 for certain intervals of time. Storage (G') and loss (G'') modulus data obtained from the frequency sweep measurement.

	Modulus (kPa)		**G' (kPa)**	**G'' (kPa)**
	As prepared	**24 hours**		
$\mathbf{3_{1A}2_{1S}}$	25 ± 2.5	11 ± 2.2	2154 ± 578	40 ± 28
$\mathbf{3_{1A}2_{2S}}$	29 ± 1.9	4.6 ± 1.0	4064 ± 203	11 ± 7.3
$\mathbf{3_{1A}2_{3S}}$	34 ± 2.6	12.4 ± 1.1	2304 ± 428	42 ± 19
$\mathbf{3_{1A}2P_{3S}}$	18 ± 2.2	83.7 ± 11.3	3356 ± 421	340 ± 78
$\mathbf{3_{1A}2_{3S\text{-}6040}}$	24 ± 1.9	26.3 ± 1.6	3904 ± 570	205 ± 50
$\mathbf{3_{1A}3_{1S}}$	0.25 ± 0.08	0.36 ± 0.14	6688 ± 78	7.7 ± 5.2
$\mathbf{3_{1A}4_{2S}}$	0.44 ± 0.1	0.96 ± 0.31	124 ± 7.1	7.9 ± 3.6
$\mathbf{4_{2A}3_{1S}}$	0.67 ± 0.24	1.25 ± 0.2	1289 ± 127	44 ± 12
$\mathbf{4_{2A}4_{2s}}$	0.75 ± 0.3	2.18 ± 0.47	20458 ± 830	17.5 ± 4.5

a. Swellable hydrogels compression data

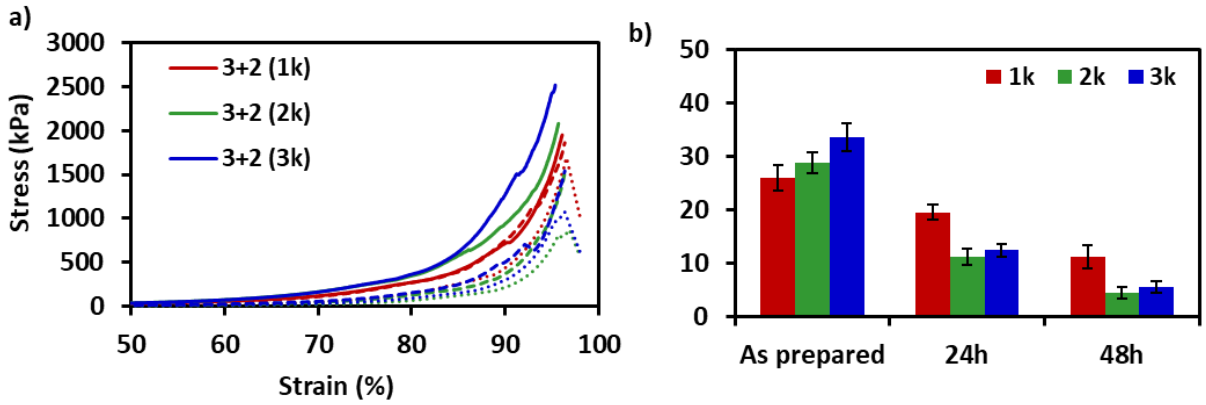

Figure S11. (a) Stress/Strain curves for the swellable hydrogels b) Young's Modulus for the swellable hydrogels at 3 time points.

b. Thermoresponsive blended hydrogels

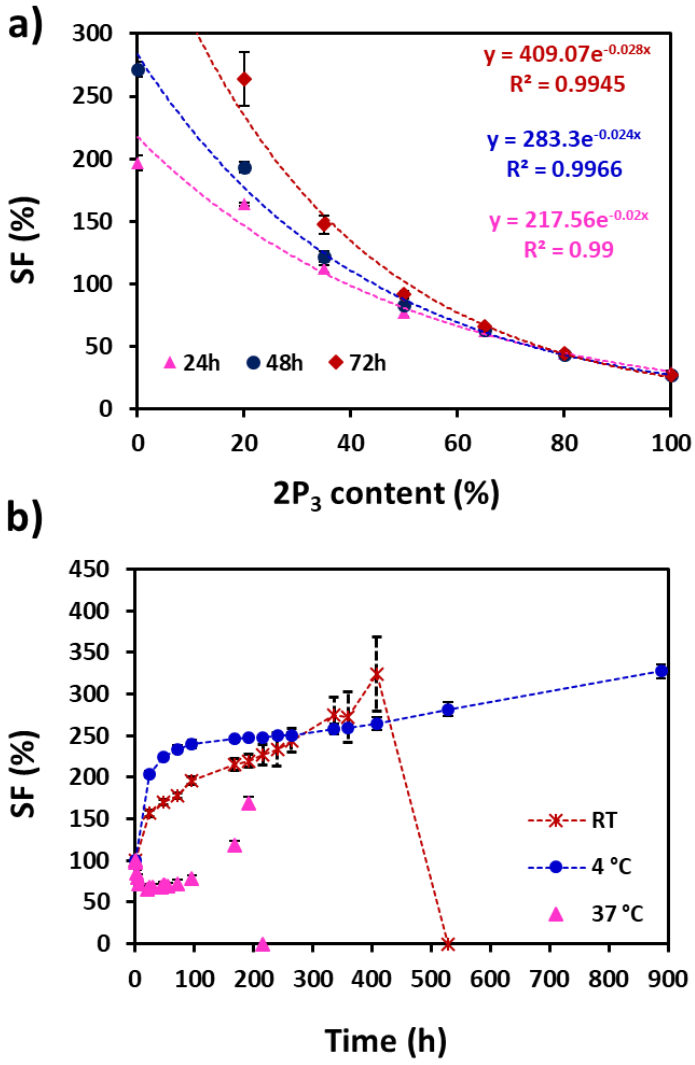

Figure S12. Swelling factor (SF) (%) of thermoresponsive blended hydrogels as a function of $Pluronic_{3k}(SH)_2$ precursor. b) Swelling response of $3_{1A}2_{3S\text{-}6040}$ hydrogels in PBS at different temperatures.

c. Additional Cryo-SEM images

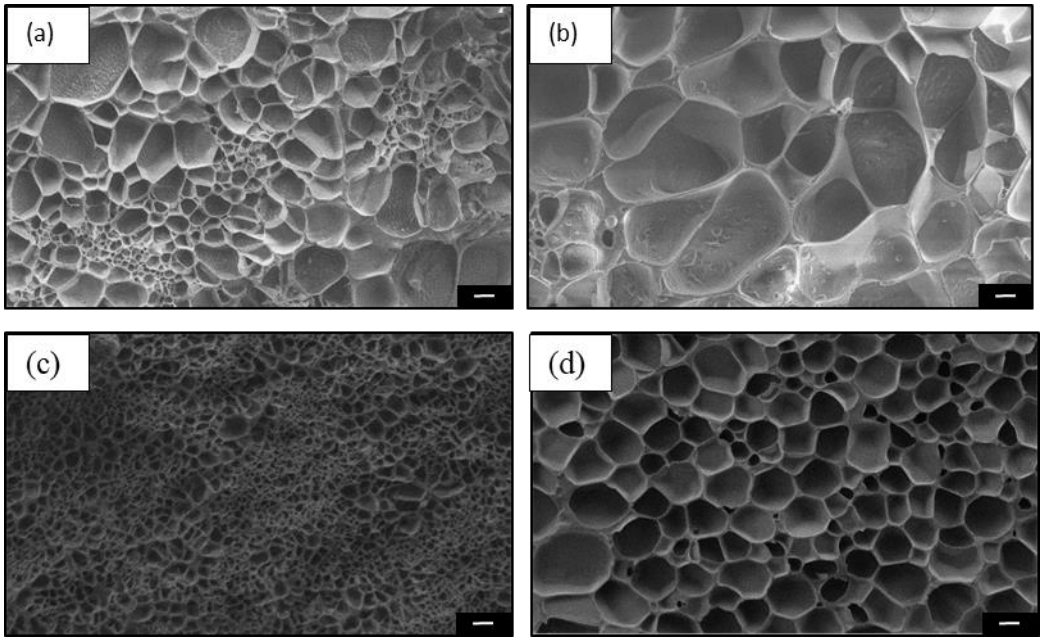

Figure S13. Additional cryo-SEM images of nonswellable thiol-yne PEG hydrogels, (Scale bar= 2 µM: (a) PEG $4_{2A}4_{2S}$, (b) PEG $4_{2A}3_{1S}$, (c) $PEG3_{1A}4_{2S}$, (d) $PEG3_{1A}3_{1S}$

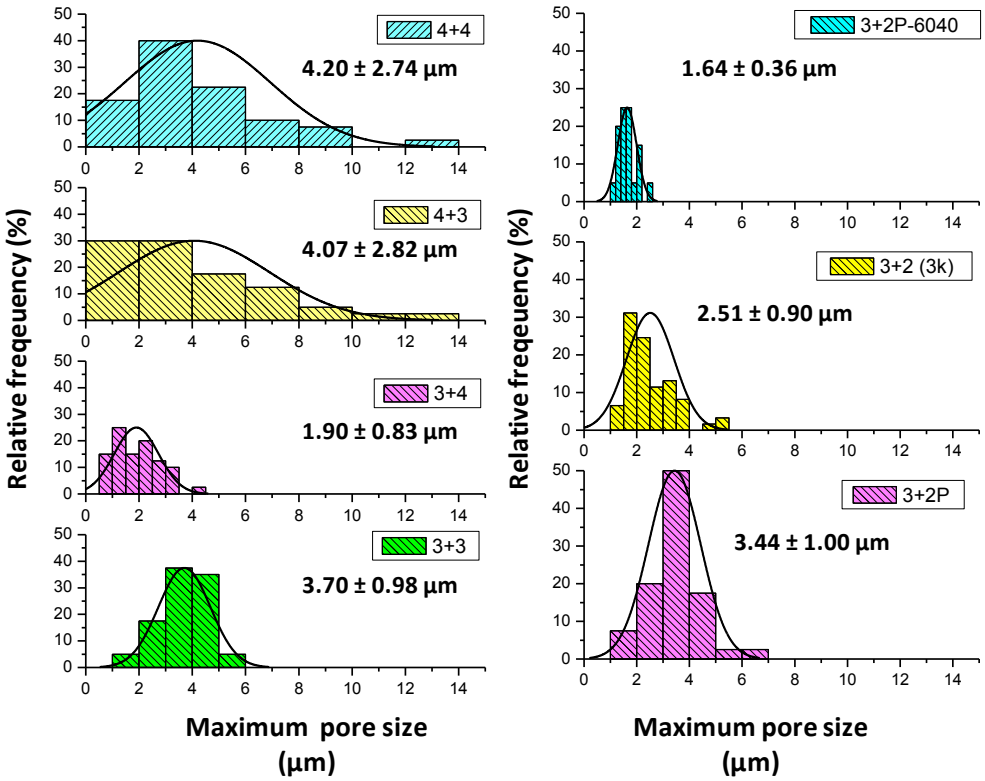

Figure S14. Maximum pore size histograms from cryo-SEM images of nonswellable thiol-yne PEG hydrogels. Indicating the maximum size the ice crystals can grow in the hydrogel during the freezing process.

d. Multiple arm compression stress/strain charts

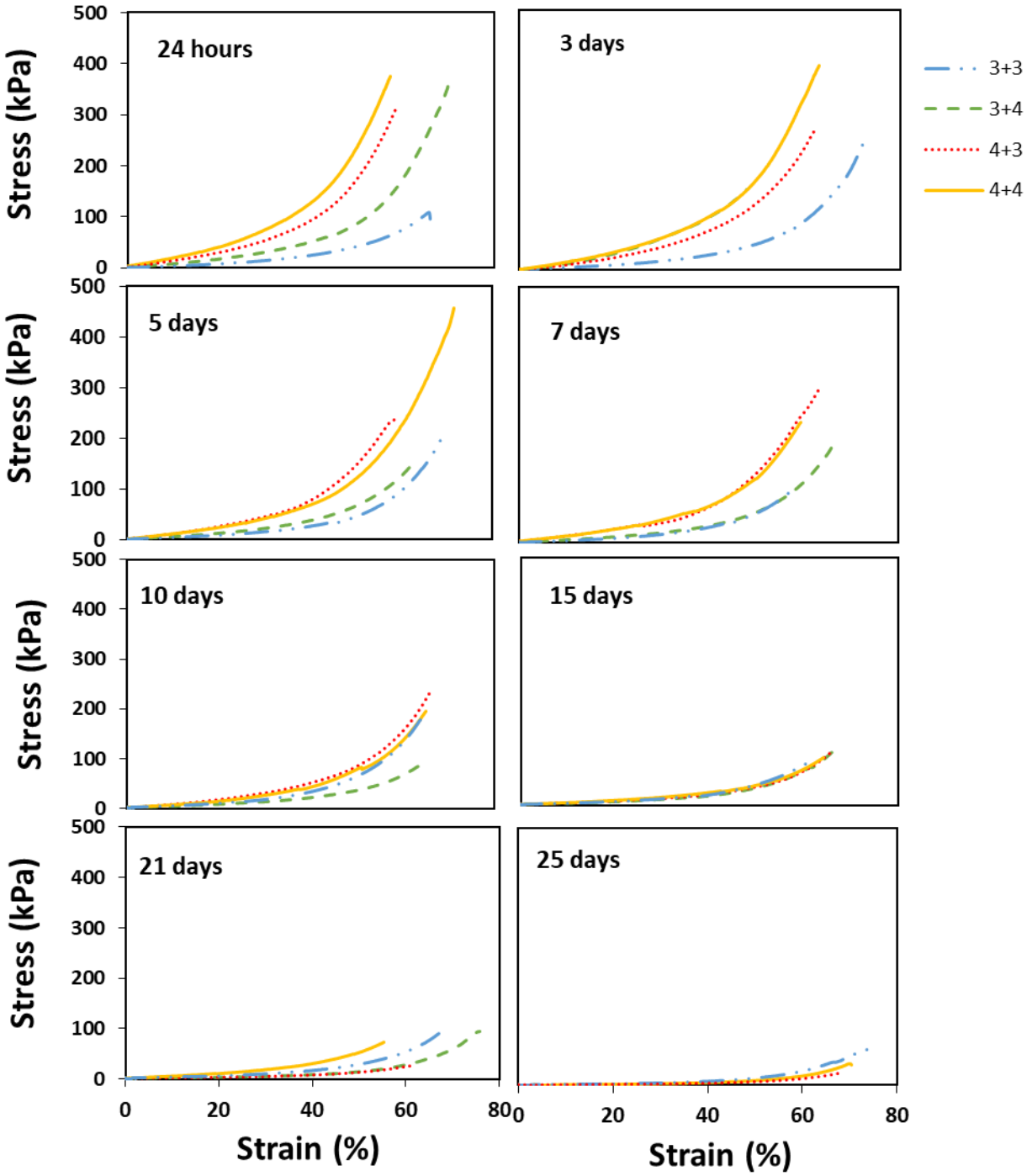

Figure S15. Stress/ strain curves for the hydrogels prepared with multi-arm PEG precursors after being immersed in PBS at 37 °C for different intervals of time.

e. Additional $3_{1A}2P_{3S}$ compression stress/strain charts

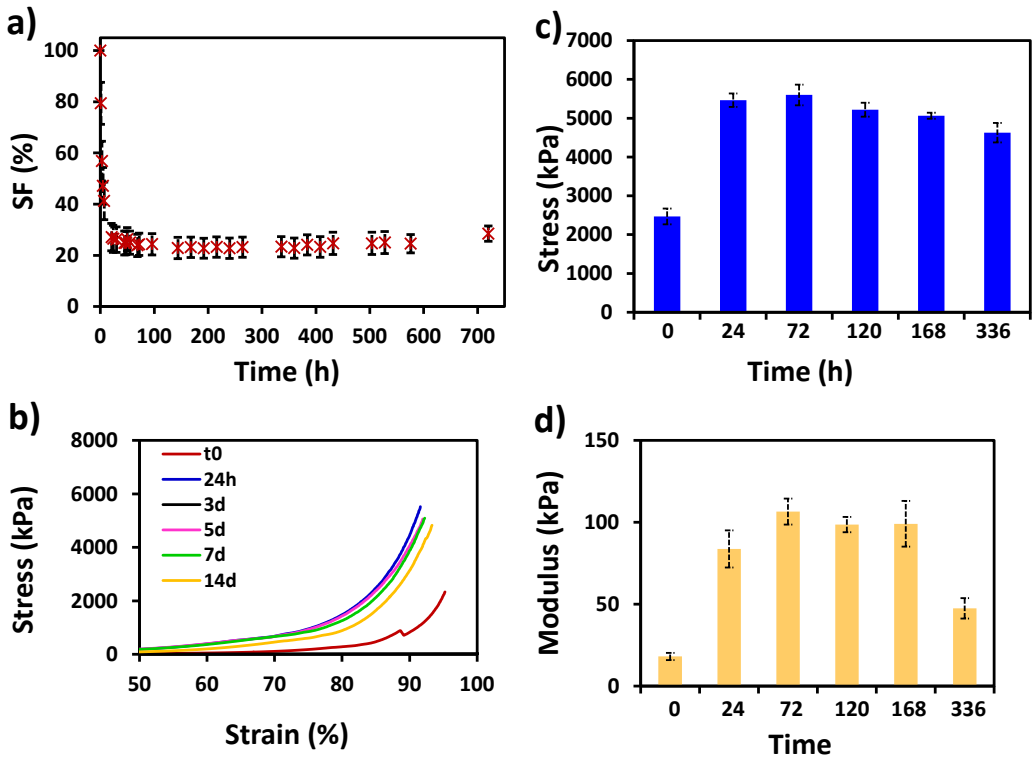

Figure S16. Additional mechanical data for $3_{1A}2P_{3S}$ thermoresponsive nonswellable hydrogel: a) SF (%) as a function of time in PBS at 37 °C; b) compressive stress-strain curves for different intervals of time in PBS at 37 °C; and evolution of compressive strength (c) and Young's Modulus values (d) with immersion time.

f. Additional Mechanical data for nonswellable hydrogels

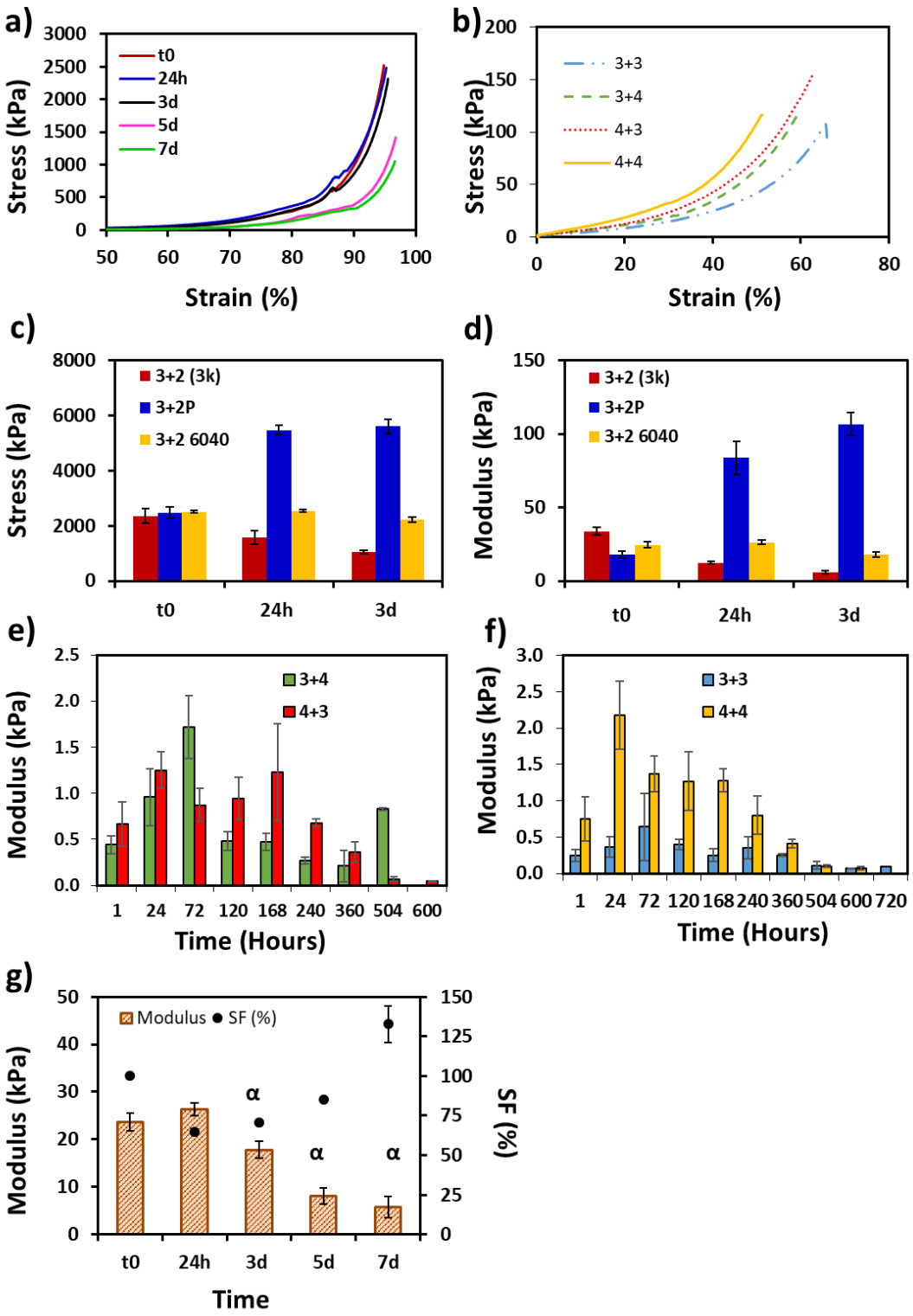

Figure S17. Additional mechanical data for nonswellable hydrogels: representative compressive stress-strain curves for a) $3_1 2_{3S\text{-}6040}$ hydrogels and b) the multi-arm hydrogels after 1 hour; c) Stress at break and d) Young's Modulus with swelling time for $3_{1A}2_{3S}$, $3_{1A}2P_{3S}$ and $3_{1A}2_{3S\text{-}6040}$ hydrogels; and Young's Modulus with swelling time for e) $4_{2A}3_{1S}$ and $3_{1A}4_{2S}$; f) $4_{2A}4_{2A}$ and $3_{1A}3_{1S}$; and g) $3_{1A}2_{3S\text{-}6040}$ hydrogels.

g. Additional biocompatible images of the thiol-yne PEG hydrogels

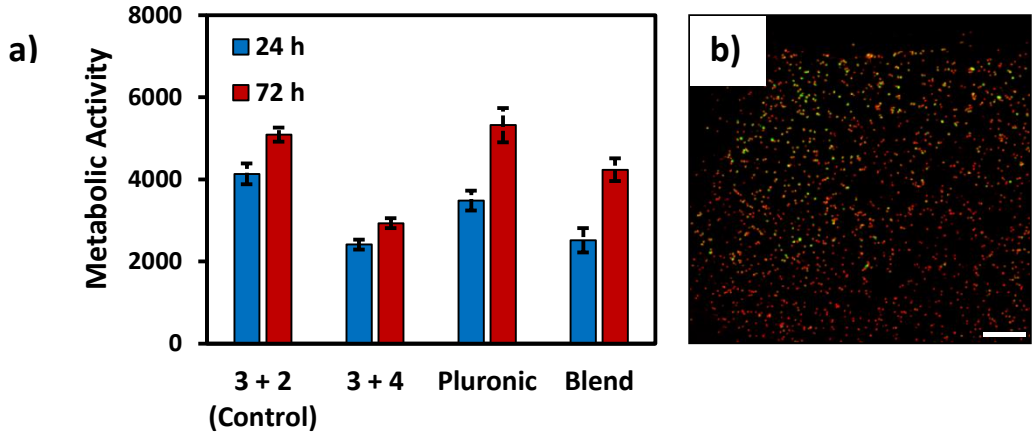

Figure S18. a) Metabolic activity of cells seeded on the top of 3+4, Pluronic and Blend hydrogels after 24 and 72 h. b) Fluorescence image of cells stained with live/dead dyes after 72hour encapsulation in Pluronic gels. Scale bar is 200 µm.

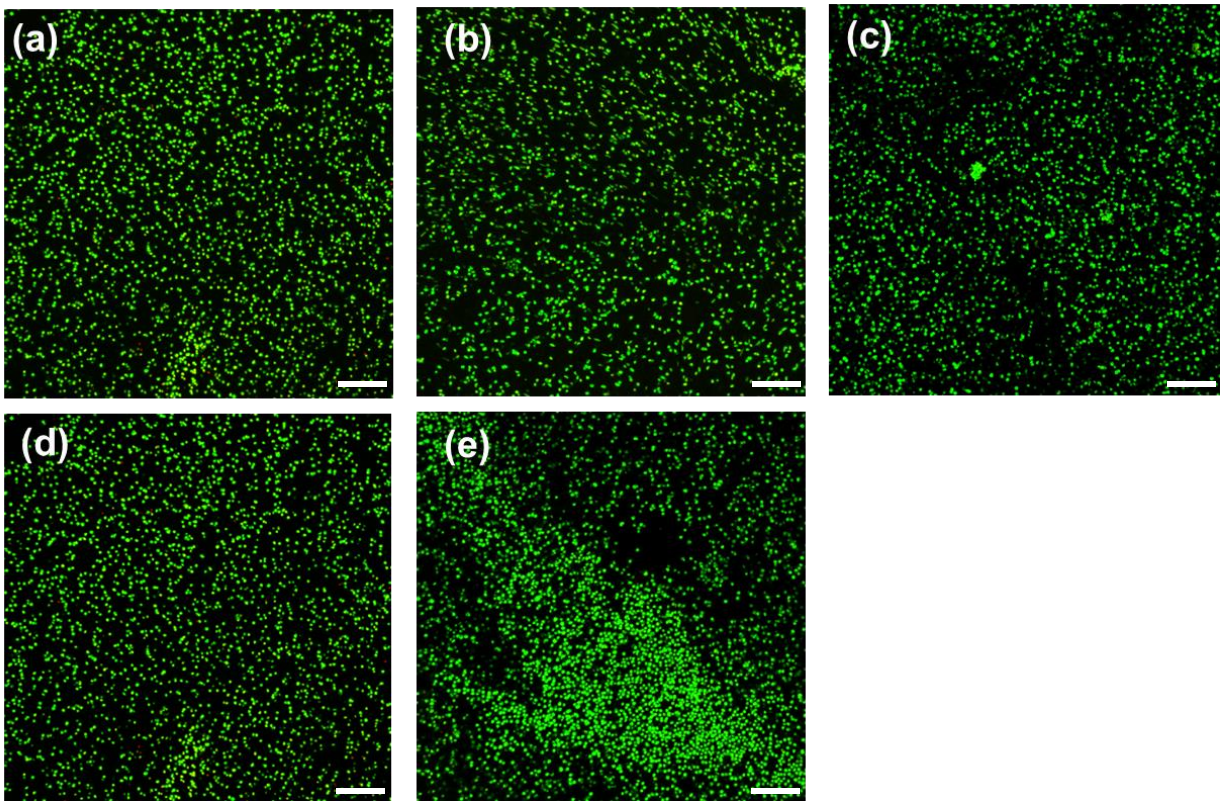

Figure S19. 3D encapsulation after 24 hours (a) $\mathbf{3_{1A}2_{3S}}$ at room temperature (b) $\mathbf{3_{1A}2_{3S}}$ prepared in ice (c) $\mathbf{3_{1A}2_{3S\text{-}6040}}$ (d) $\mathbf{3_{1A}2P_{3S}}$ (e) $\mathbf{3_{1A}4_{2S}}$. Scale bar is 200 µm.

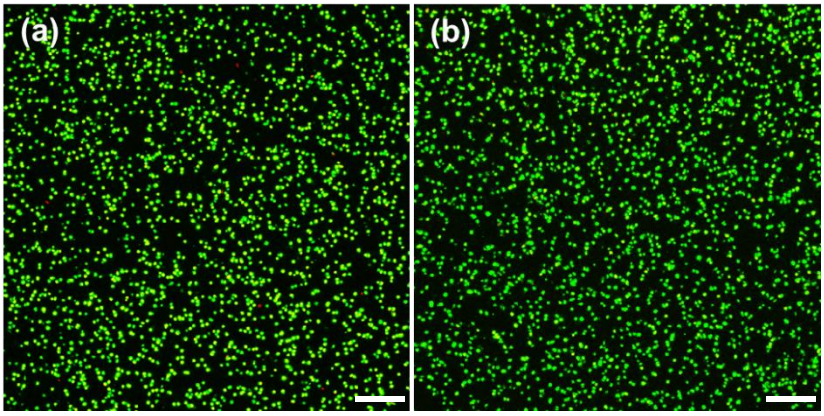

Figure S20. 3D encapsulation after 72 hours (a) $\mathbf{3}_{1A}\mathbf{2}_{3S}$ at room temperature (b) $\mathbf{3}_{1A}\mathbf{2}_{3S}$ prepared in ice. Scale bar is 200 µm.

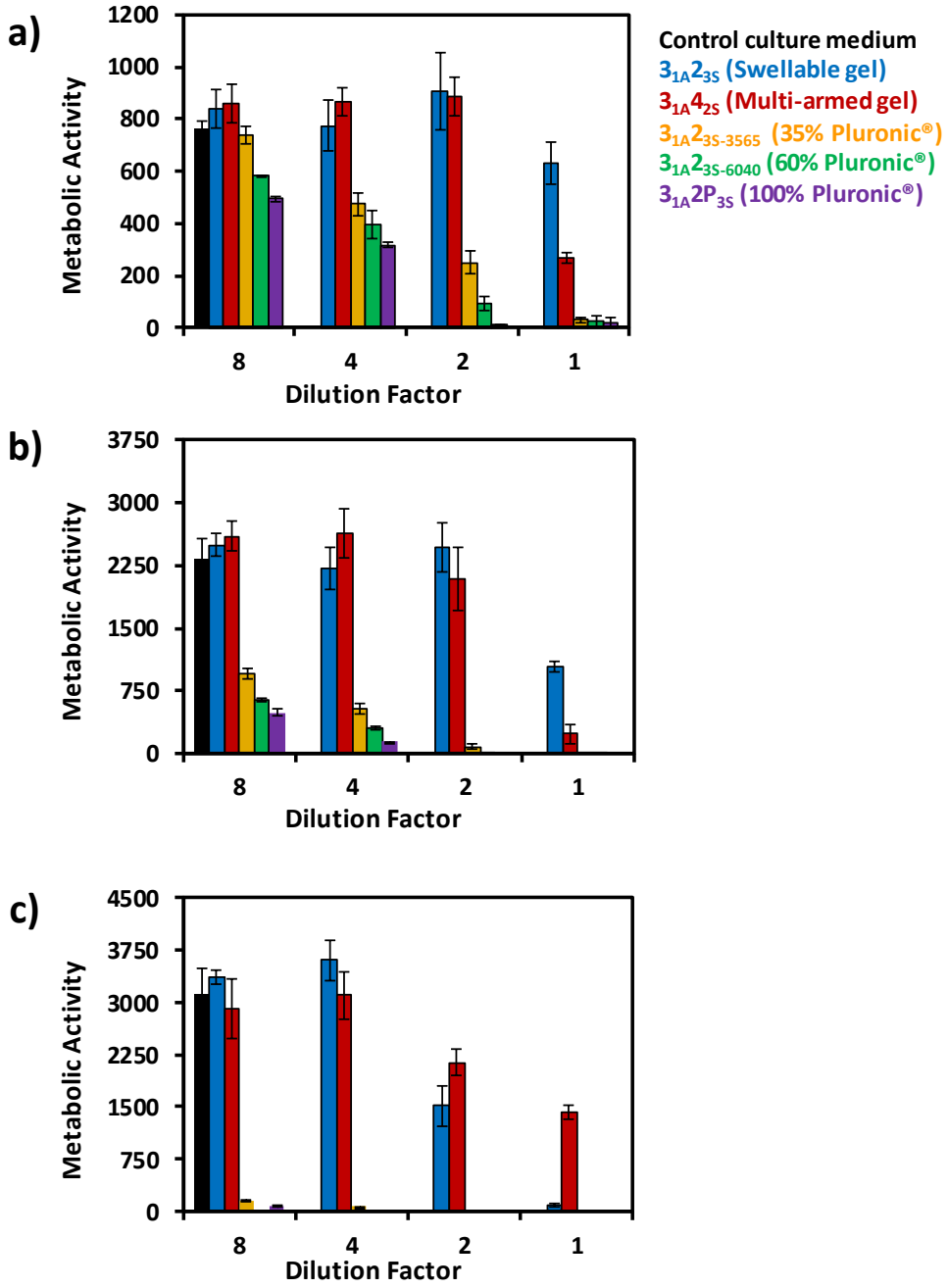

Figure S21. Metabolic activity at different time points of cells incubated in the presence of culture medium that had been in contact with gels (DF 1) for 6 days at 37 °C as well as three diluted solutions (DF 2, 4, and 8): (a) 24 h, (b) 72 h, and (c) 7 days. Unaltered culture medium was also used as a control without dilution (black bar at DF 8).