Supporting Information

Tuning Polymer Hydrophilicity to Regulate Gel Mechanics and Encapsulated Cell Morphology

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Supporting Information

hMSC

Temperature Profile	Median Actin Area (μm²)	Avg. Actin Area (μm²)	Standard Deviation (μm², N = gels)
I	733.82	997.53	91.95
II	635.66	803.08	52.29
III	462.73	636.85	99.34

HUVEC

Temperature Profile	Median Actin Area (μm²)	Avg. Actin Area (μm²)	Standard Deviation (μm², N = gels)
I	271.09	525.52	33.06
II	236.04	387.33	61.05
III	243.05	361.90	74.56

hNPC

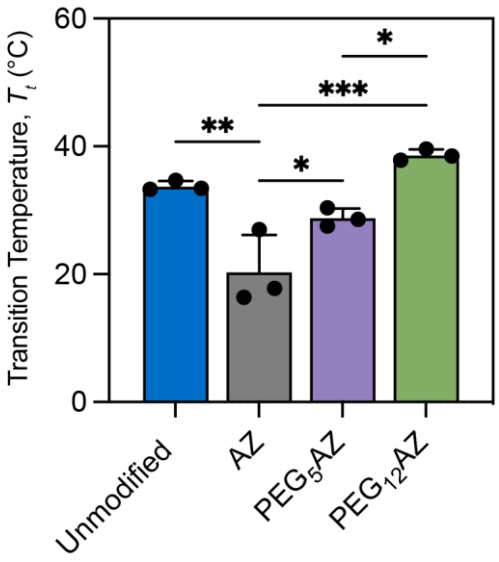
Temperature Profile	Median Actin Area (μm²)	Avg. Actin Area (μm²)	Standard Deviation (μm², N = gels)
I	52.11	70.94	2.04
II	54.43	76.74	5.40
III	57.90	87.73	8.48

Supplemental Table S1. Quantification and error measurements of actin area depicted in Figure 6. Standard deviation (*N* = gels) represents the variance between gel replicates. For hMSCs, *n* = 1,063, 823, and 899 (**I**, **II**, and **III**) cells examined over *N* = 3 independent gel samples. For HUVECs, *n* = 386, 448, and 503 (**I**, **II**, and **III**) cells examined over *N* = 3 independent gel samples. For hNPCs, *n* = 3,357, 3,013, and 3,067 (**I**, **II**, and **III**) cells examined over *N* = 3 independent gel samples.

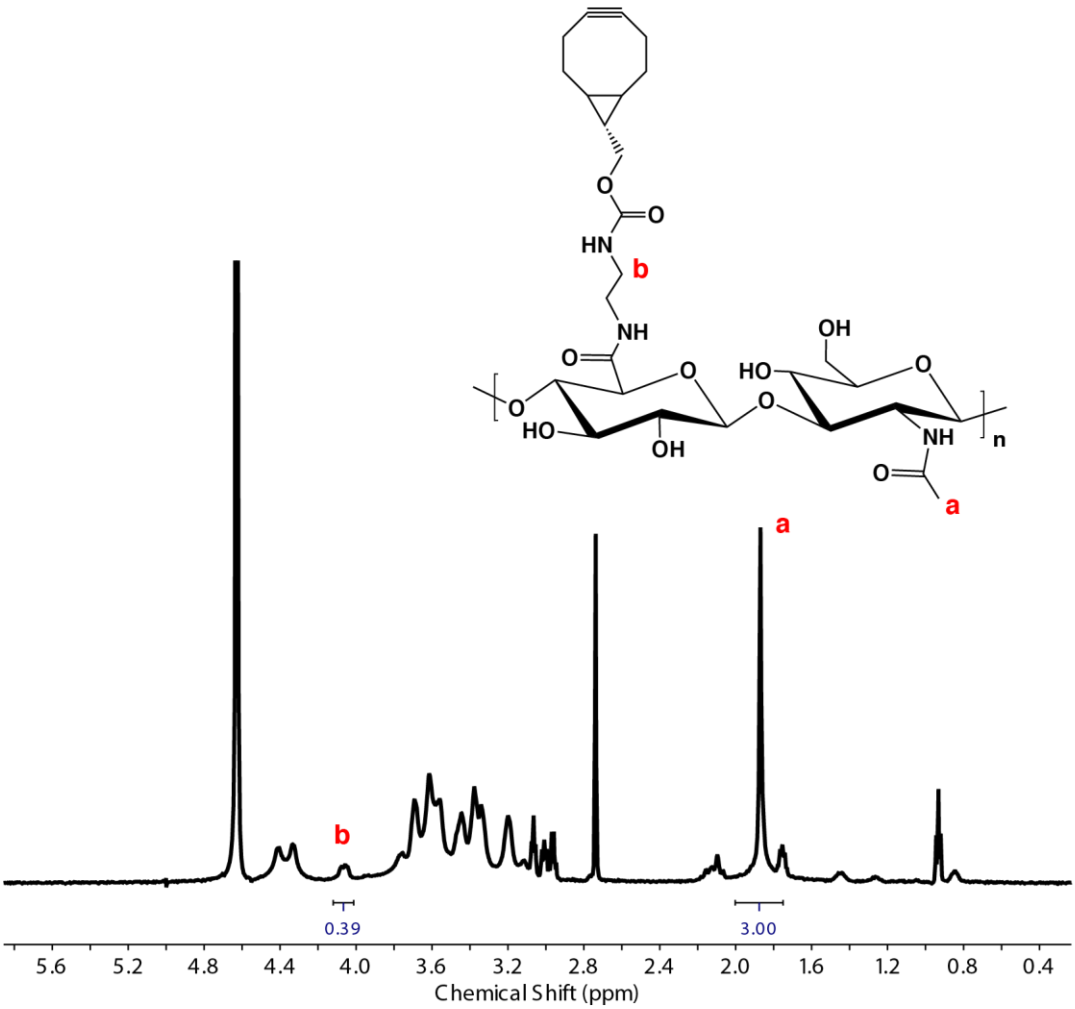
MASMTGGQQMGHHHHHHDDDDKLQ{LDASTVYAVTGRGDSPASSAASA[(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_3$VP}$_4$LE

Tags Cell-adhesive domain Elastin-like domain

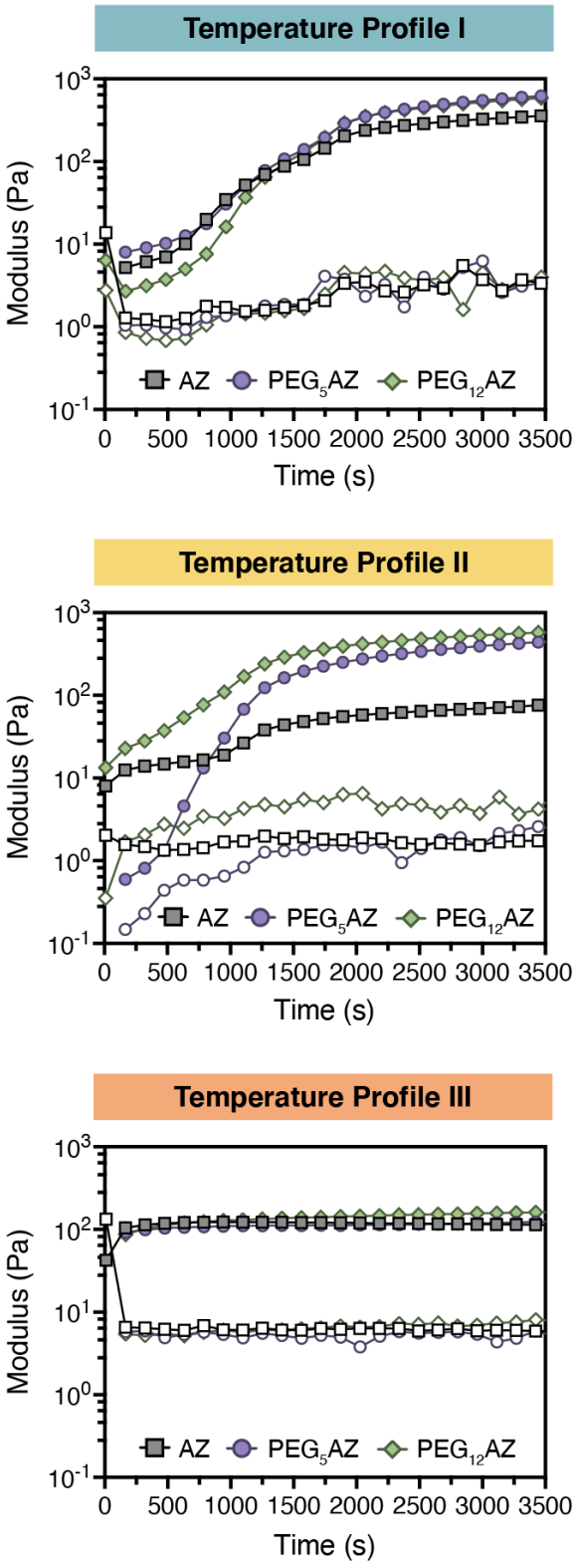
Supplemental Figure S1. Full amino acid sequence of cell-adhesive RGD-ELP with tag domains consisting of a T7 tag (MASMTGGQQMG), a histidine tag (HHHHHH), and an enterokinase cleavage site (DDDDK) to permit tag removal if desired. All ELP proteins were kept intact with no tag removal for these studies.



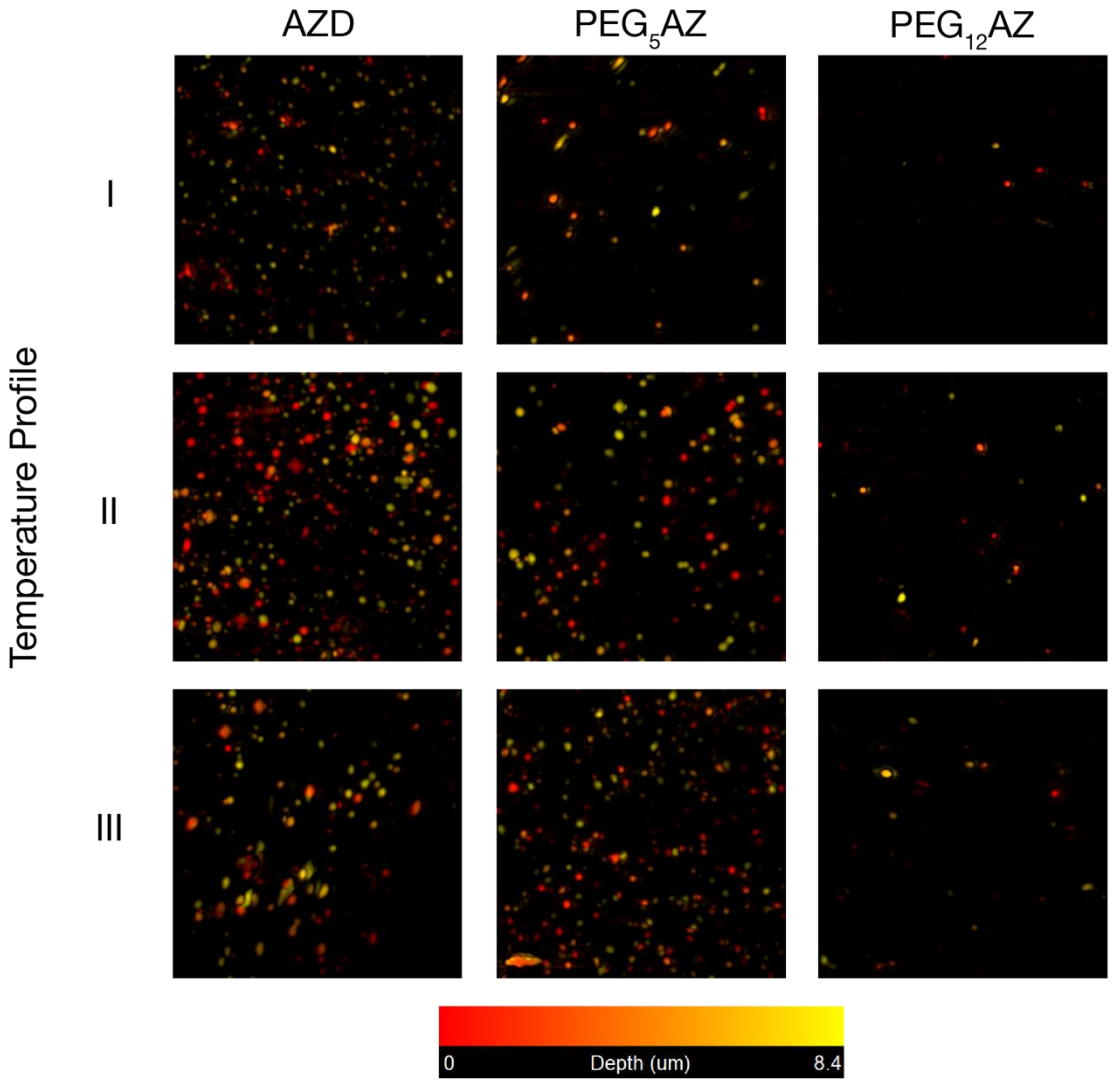
Supplemental Figure S2. Quantification of ELP transition temperatures before and after coupling reaction (n = 3, data are averages ± standard deviation, *p < 0.1, **p < 0.01, ***p < 0.001, one-way ANOVA with Tukey *post hoc* test).



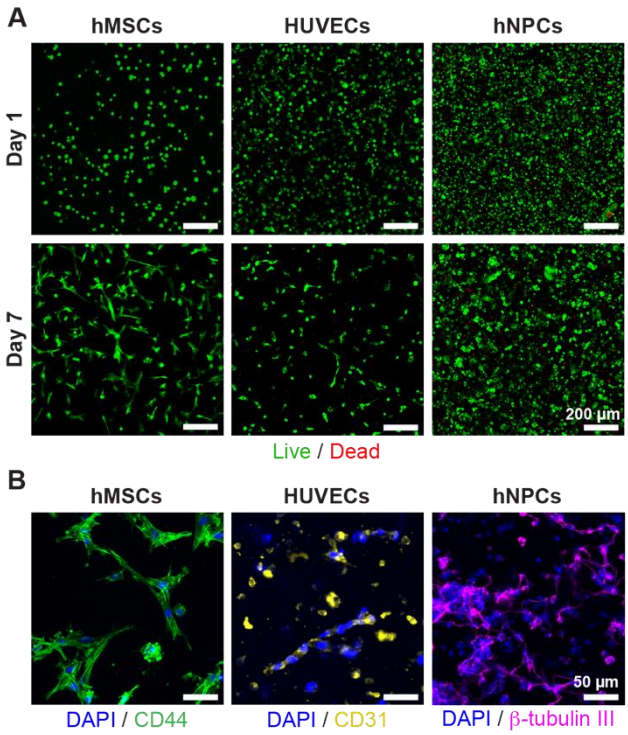
Supplemental Figure S3. ^{1}H NMR 600 MHz spectrum of hyaluronic acid conjugated with bicyclononyne.



Supplemental Figure S4. Representative gelation time sweep data showing storage moduli (*G'*, filled symbols) and loss moduli (*G"*, open symbols) during SPAAC-crosslinking of HELP-SPAAC hydrogels for the three temperature profiles and each of the HELP-SPAAC gel formulations.



Supplemental Figure S5. Representative maximum projections of CARS images of HELP-SPAAC gels crosslinked with all three temperature profiles. Regions of bright intensity are protein-rich ELP aggregates.



Supplemental Figure S6. HELP-SPAAC hydrogels support 3D cell culture. A) Live/Dead staining of hMSCs, HUVECs, and hNPCs cultured within HELP-SPAAC hydrogels (formulated with PEG_5AZ and temperature profile **I**) indicated high viability for all cell types 1 and 7 days post-encapsulation. B) Representative confocal images of immunostained hMSCs, HUVECs, and hNPCs cultured within HELP-SPAAC hydrogels (formulated with PEG_5AZ and temperature profile **I**). After 7 days in culture, hMSCs stain positive for the MSC surface marker CD44 (green), HUVECs stain positive for the endothelial marker CD31 (yellow), and hNPCs stain positive for the neuronal marker β-tubulin III (pink). Nuclei are counter-stained with DAPI (blue).