ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Bio-Orthogonally Crosslinked, Engineered Protein Hydrogels with Tunable Mechanics and Biochemistry for Cell Encapsulation

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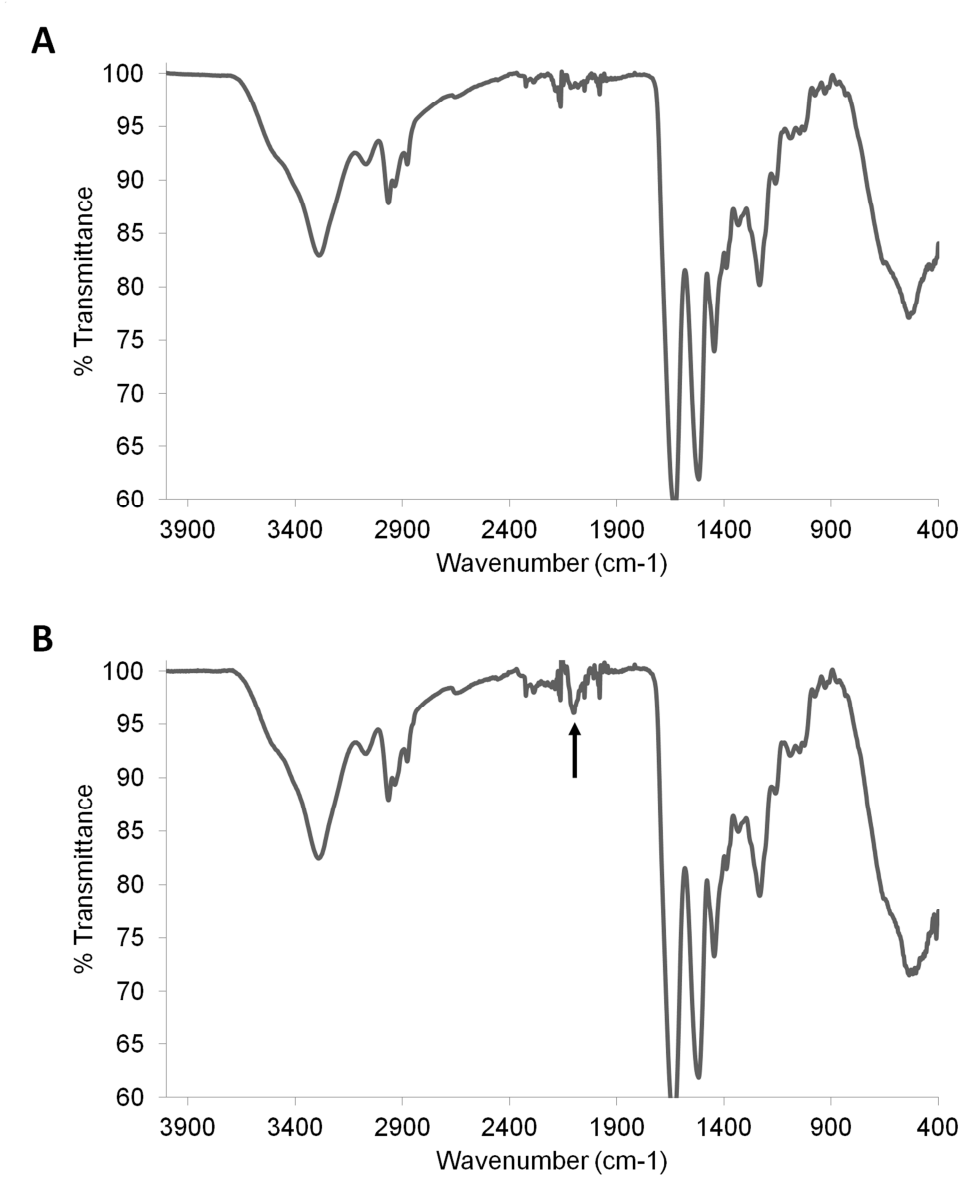

Figure S1. FT-IR Spectroscopy for Azide-Functionalized ELP. FT-IR spectra for **(A)** unmodified ELP and **(B)** azide-functionalized ELP. The appearance of a peak around 2100 cm^{-1} (denoted by a black arrow in B) is characteristic of the azide stretching frequency.

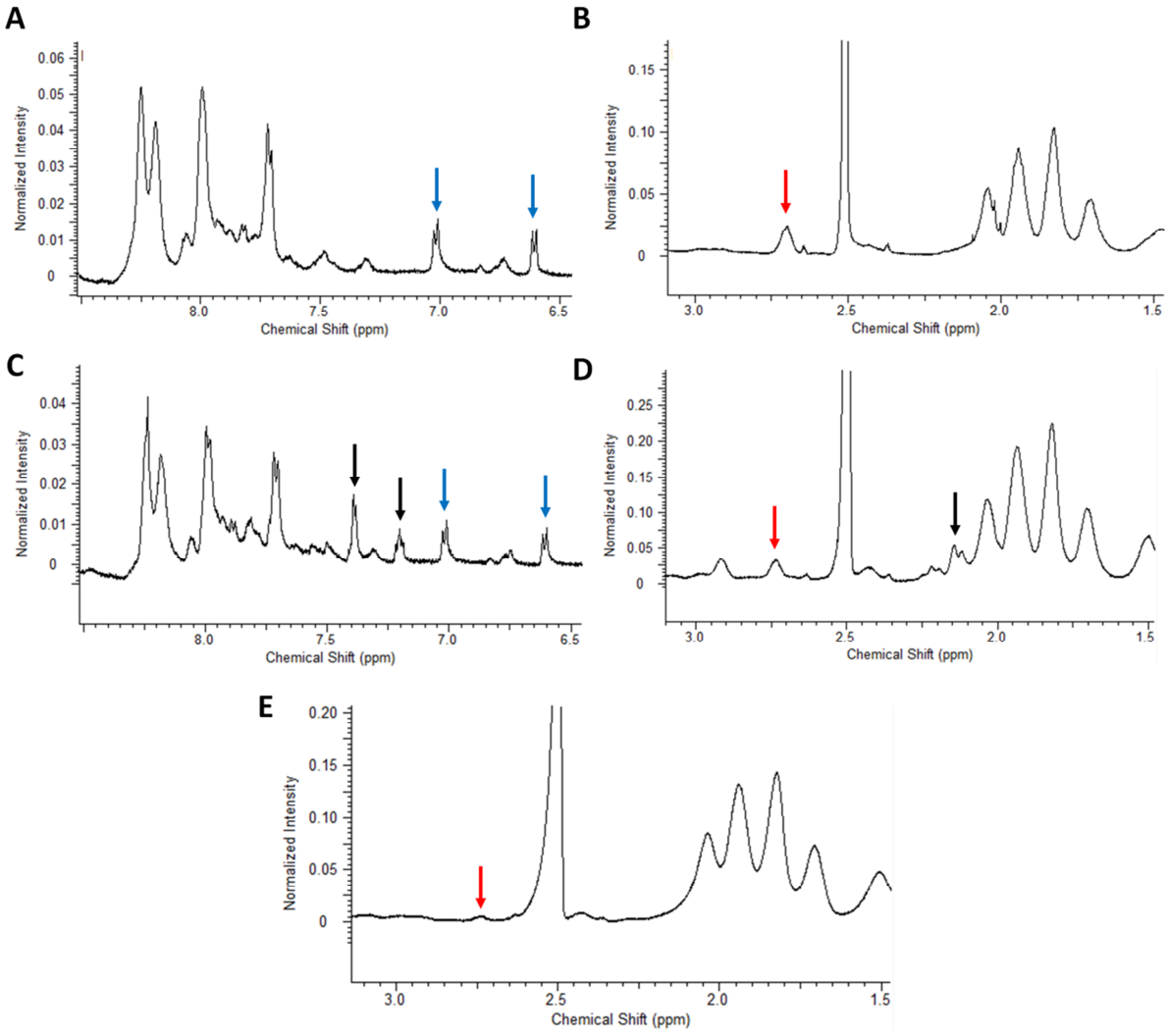

Figure S2. ^{1}H-NMR Spectroscopy for Triarylphosphine- and BCN-Functionalized ELP. **(A,B)** Relevant regions of the ^{1}H-NMR spectrum for unmodified ELP. **(C)** ^{1}H-NMR spectrum of triarylposphine-functionalized ELP. Black arrows denote appearance of aromatic peaks characteristic of the triarylphosphine reagent. **(D)** ^{1}H-NMR spectrum of BCN-functionalized ELP. Black arrow denotes appearance of peaks characteristic of the BCN group. **(E)** ^{1}H-NMR spectrum of azide-functionalized ELP. In A and C, blue arrows denote peaks corresponding to tyrosine residues used as references for peak integration. In A, D, and E, red arrows denote the peak corresponding to protons on the carbon adjacent to the primary amine of the lysine side chain. Decrease in the intensity of this peak was used to estimate the fraction of reacted amines.

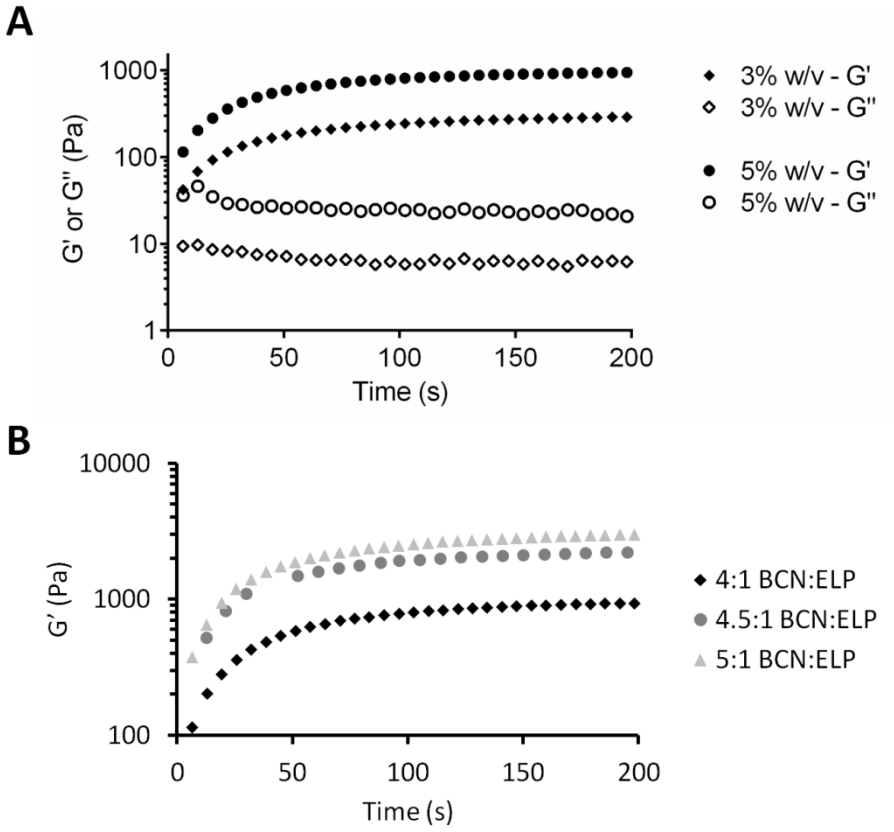

Figure S3. Time Sweep Data for SPAAC-Crosslinked Gels. (A) Time sweep showing storage (G') and loss (G'') moduli during SPAAC-based crosslinking of ELP gels with a BCN:ELP molar ratio of 4:1 at 3% (w/v) and 5% (w/v) total protein content. **(B)** Time sweep showing storage (G') moduli during SPAAC-based crosslinking of ELP gels with a varying BCN:ELP molar ratio at 5% (w/v) total protein content. The plateau moduli for these time sweeps are ~1000 Pa, 2300 Pa, and 3300 Pa for 4:1, 4.5:1, and 5:1 BCN:ELP, respectively.

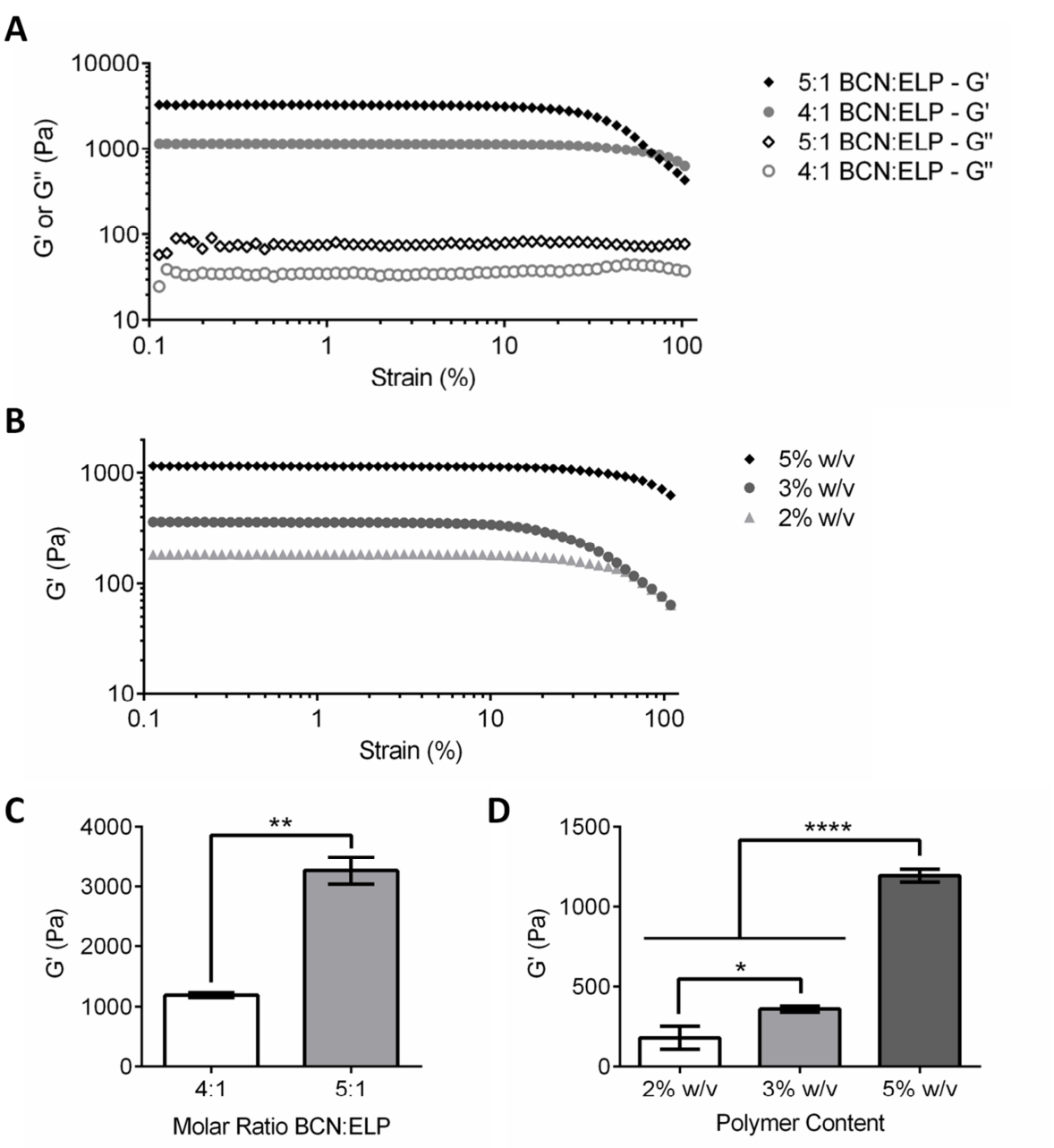

Figure S4. Rheological Characterization of SPACC-Crosslinked ELP Hydrogels.

(A) Average storage (G') and loss (G'') moduli of SPAAC-crosslinked ELP gels during a strain sweep at a fixed oscillatory frequency of 1 Hz, varying the stoichiometry of BCN groups per ELP polymer at 5% (w/v) polymer content. **(B)** Average storage moduli during a strain sweep at a fixed oscillatory frequency of 1 Hz, varying the polymer content of the hydrogels with a fixed BCN:ELP ratio of 4:1. **(C)** Average storage moduli of 5% (w/v) SPAAC-crosslinked ELP gels at 1% strain and 1 Hz oscillatory frequency, varying the

stoichiometry of BCN groups per ELP polymer. **(D)** Average storage moduli of gels with a fixed molar ratio of BCN:ELP of 4:1 at 1% strain and 1 Hz oscillatory frequency, varying the polymer content of the hydrogels. Error bars are ± *SD*. *p<0.05, **p<0.01, ****p<0.0001.

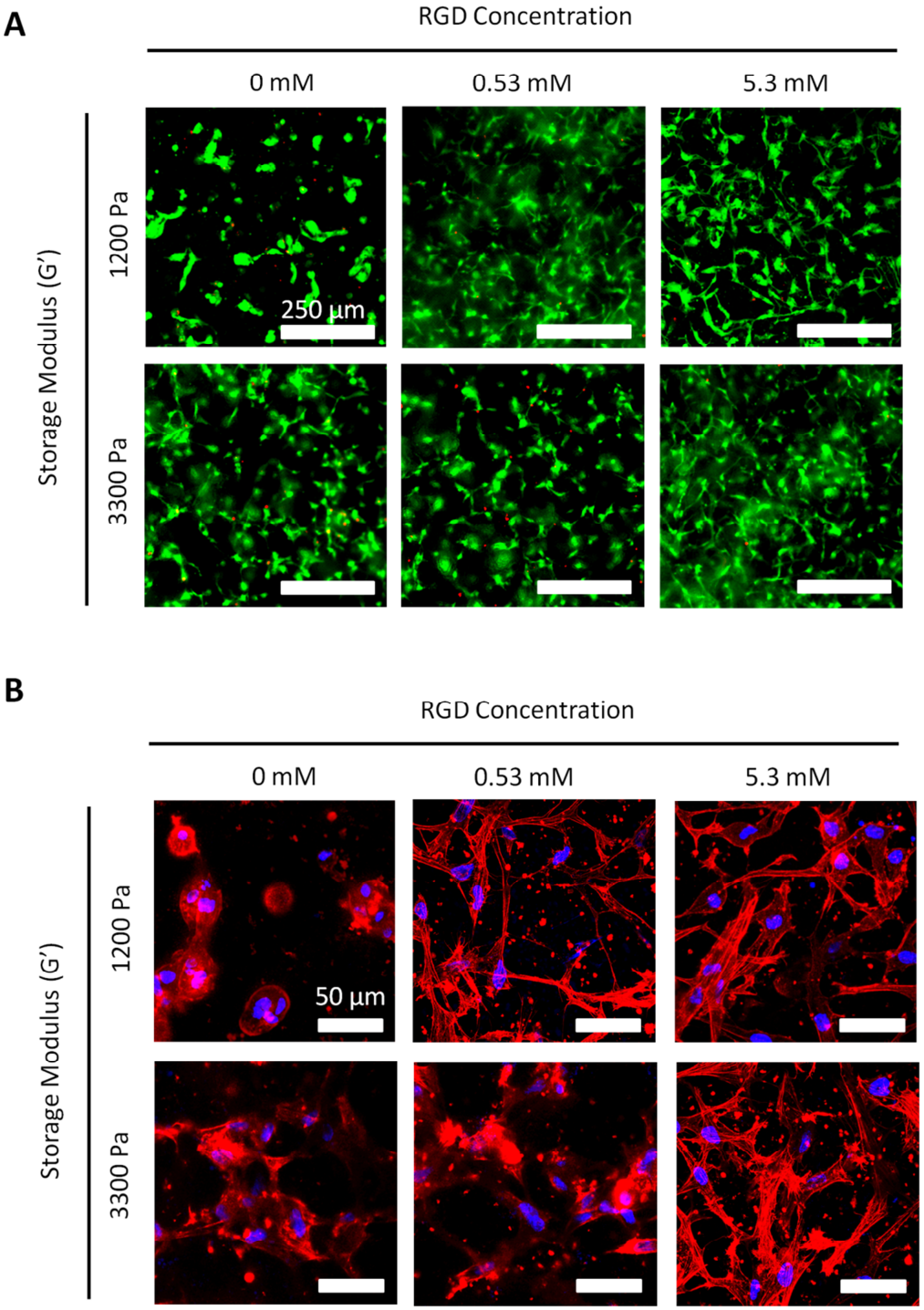

Figure S5. Fluorescence Microscopy Images of Human MSCs Cultured in SPAAC-ELP Gels with Varying RGD Concentration and Stiffness. (A) Representative Live/Dead images of hMSCs cultured for 2 days. Green: live (calcein-AM), Red: dead (ethidium homodimer). **(B)** Representative phalloidin-stained images of hMSCs cultured for 2 days. Blue: nuclei (DAPI), Red: F-actin (phalloidin).