6. Supporting Information

Ligand Presentation Controls Collective MSC Response to Matrix Stress Relaxation in Hybrid PEG-HA Hydrogels

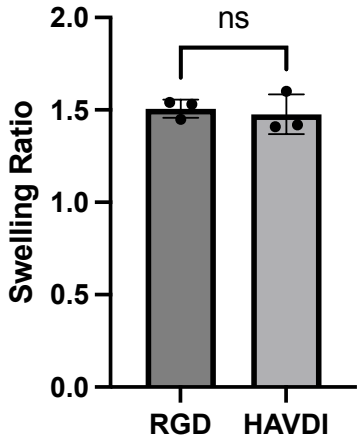


Figure S1. The swelling ratio for the 1 mM RGD and 1 mM HAVDI, 88% alkyl-hydrazone bonds conditions after 48 hours. The swelling ratio was found to be similar, ~150% the initial volume, regardless of the incorporated peptide within the hydrogel system.

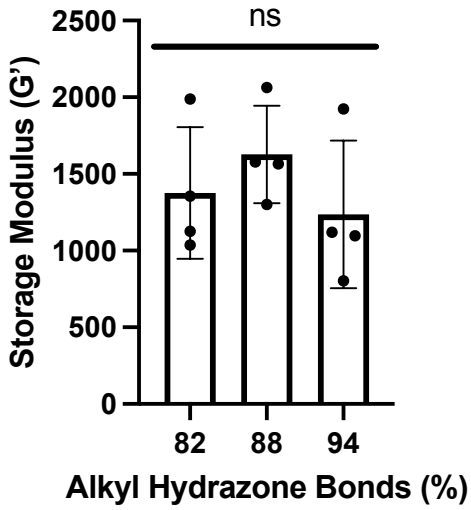


Figure S2. In-situ modulus for differing hydrogel compositions. The in-situ modulus was found to be similar across all alkyl-hydrazone bond conditions.

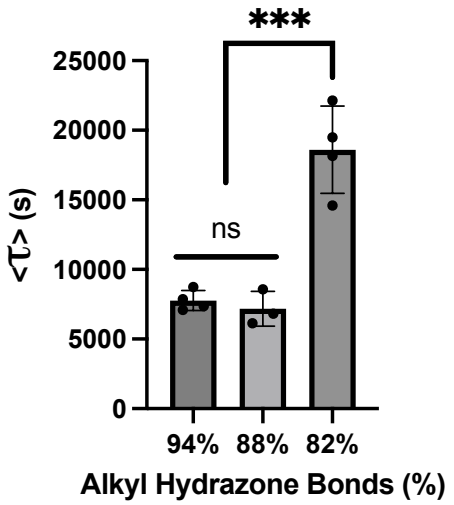


Figure S3. The average time constants for stress relaxation for the differing hydrogel compositions. The <τ> was found to be similar for the 94% and 88% alkyl hydrazone bonds conditions, with a constant of ~7000 seconds, which was significantly different from the 82% condition with a constant of ~19000 seconds.

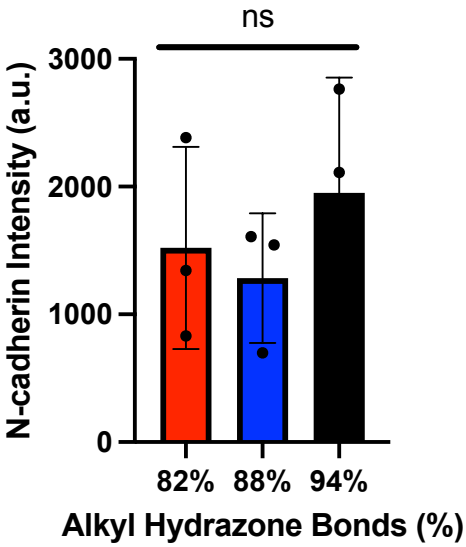


Figure S4. Relative expression of N-Cadherin for differing hydrogel compositions. The intensity expression of a cell-cell junction marker, N-Cadherin, indicating similar expression regardless of the extent of stress relaxation.

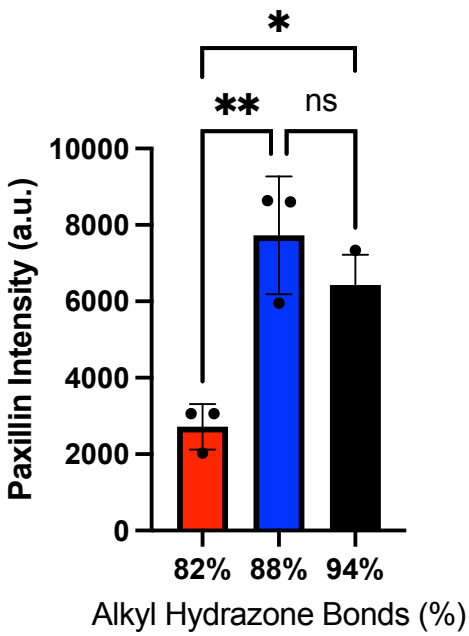
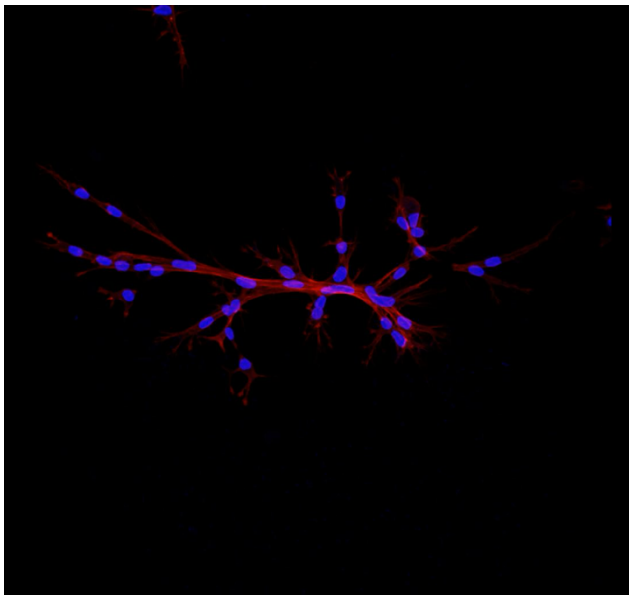
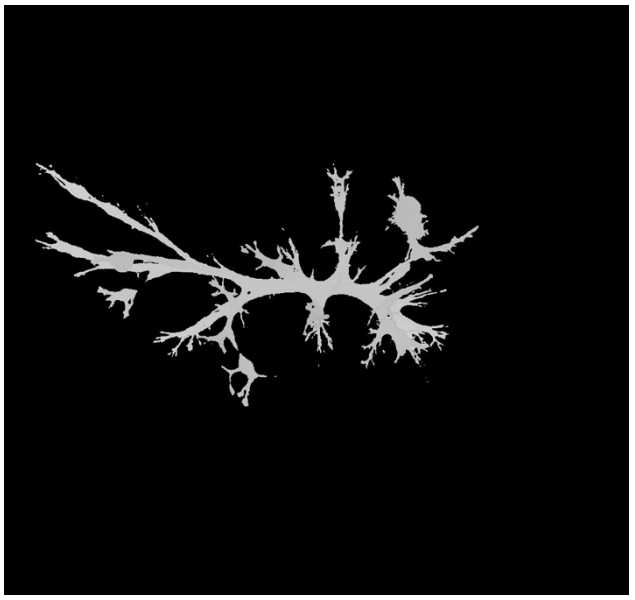


Figure S5. Relative expression of Paxillin for differing hydrogel compositions. The intensity expression of a mature focal adhesion marker, Paxillin, indicating increased expression with increasing extents of stress relaxation.

1. Obtain the visualized multi-cellular structure as imaged, in this case on a spinning disk confocal microscope, f-actin (red), DAPI (blue)

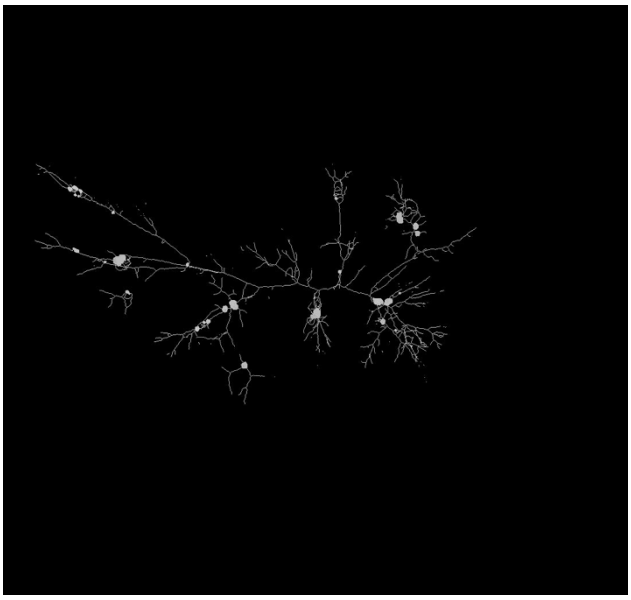


2. Binarize the multicellular structure volume based off of the f-actin (red) channel (Image -> Adjust ->



3. Repeat steps 1-2 for the DAPI (blue) channel, and subtract the DAPI threshold image from the f-actin threshold image (Process ->Image Calculator -> Image 1: F-actin Threshold -> Operator: Subtract -> Image 2: DAPI Threshold)

4. Skeletonized the multicellular structure image (Plugins -> skeleton -> skeletonize (2D/3D)



4. Obtain the XY-coordinates for the new skeletonized image (Analyze -> Tools -> Save XY Coordinates)

Figure S6. Processes for developing the skeletonized mask in FIJI. This yields the XY-coordinates, which can be run through a MATLAB script or other, to calculate the $\vec{R}_{cm}$,$<R_{g,\,structure}^2>^{1/2}$, and the α-value scaling analysis can be applied to.

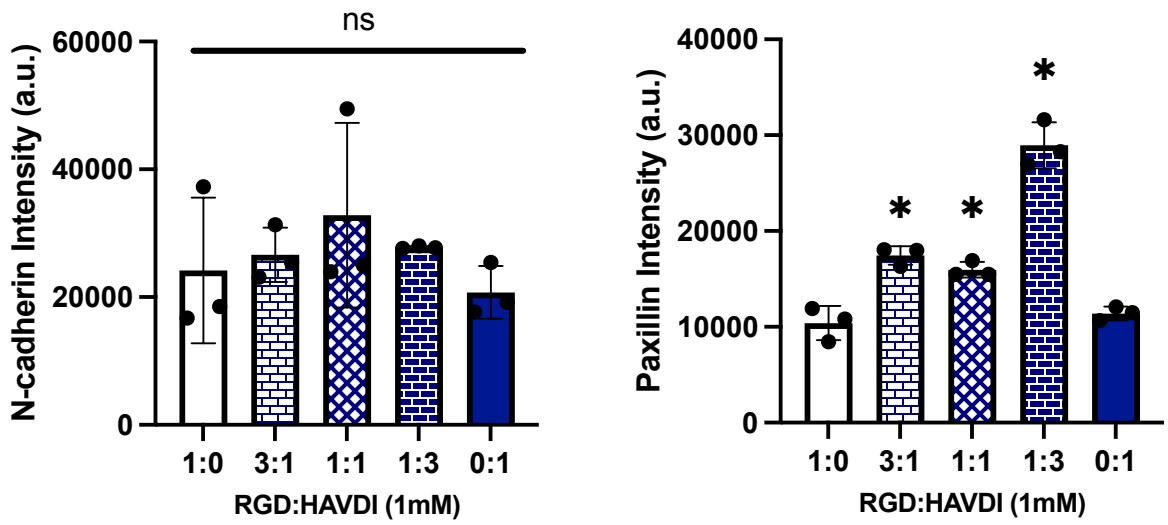


Figure S7. The N-Cadherin and Paxillin intensities for the multicellular structures. The N-Cadherin and Paxillin intensities were calculated for the 1:0, 3:1, 1:1, 1:3, and 0:1 [RGD] to [HAVDI] conditions within the 88% alkyl hydrazone bonds condition.

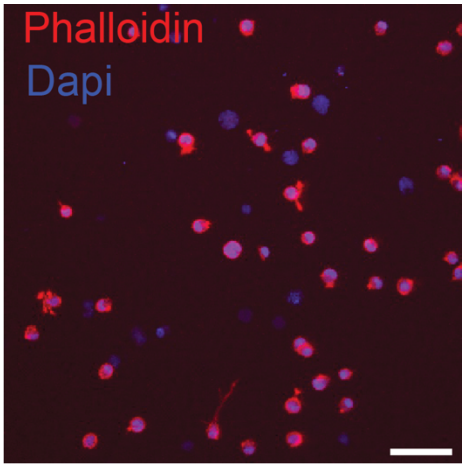


Figure S8. No peptide control. The 88% alkyl hydrazone bond condition containing no RGD of HAVDI. Scale bar = 50 μm.

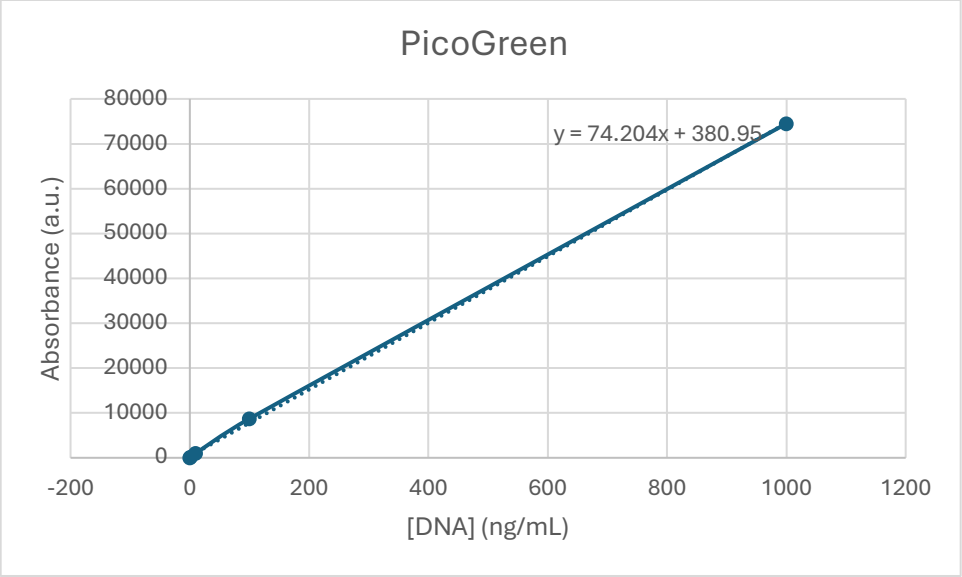


Figure S9. Characterization of the [DNA] (μg/mL) for the cytokine array hydrogel conditions. The PicoGreen standards, enabling the calculation of the [DNA] for each condition, and the ensuing normalization of the secreted cytokines.

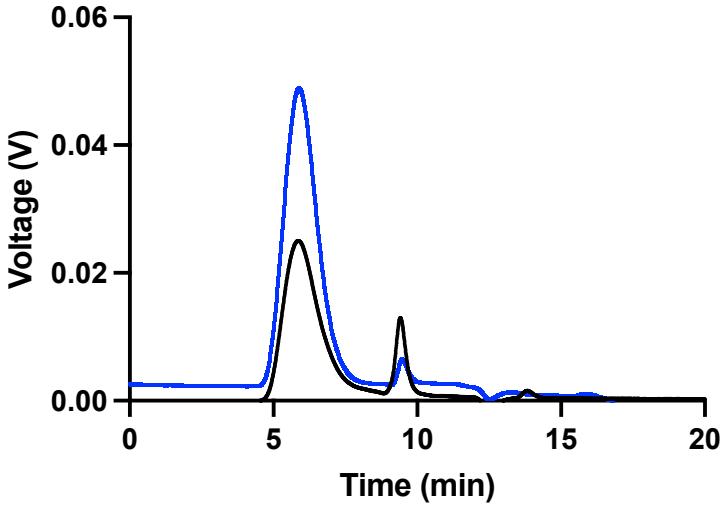


Figure S10. GPC Characterization of HA-Ald and HA-Hyd. GPC traces of HA-Ald (black) and HA-Hyd (blue), determined to be ~96 kDa and ~85 kDa, respectively.

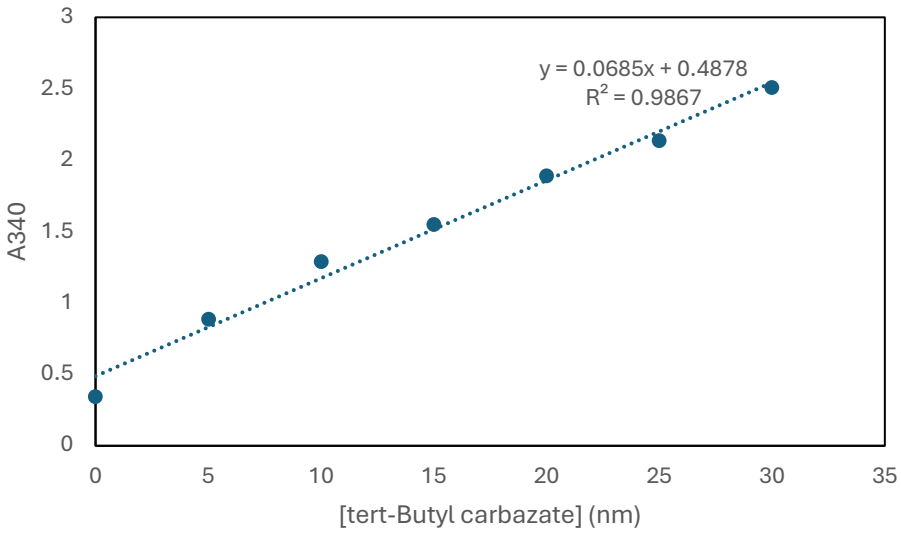


Figure S11. Characterization of HA-Ald. The HA-Ald modification was quantified via a TNBS assay, where tert-Butyl carbazate was reacted with the HA-Ald and quantified with TNBS. HA-Ald was found to have ~37% modification of the disaccharide repeat units.

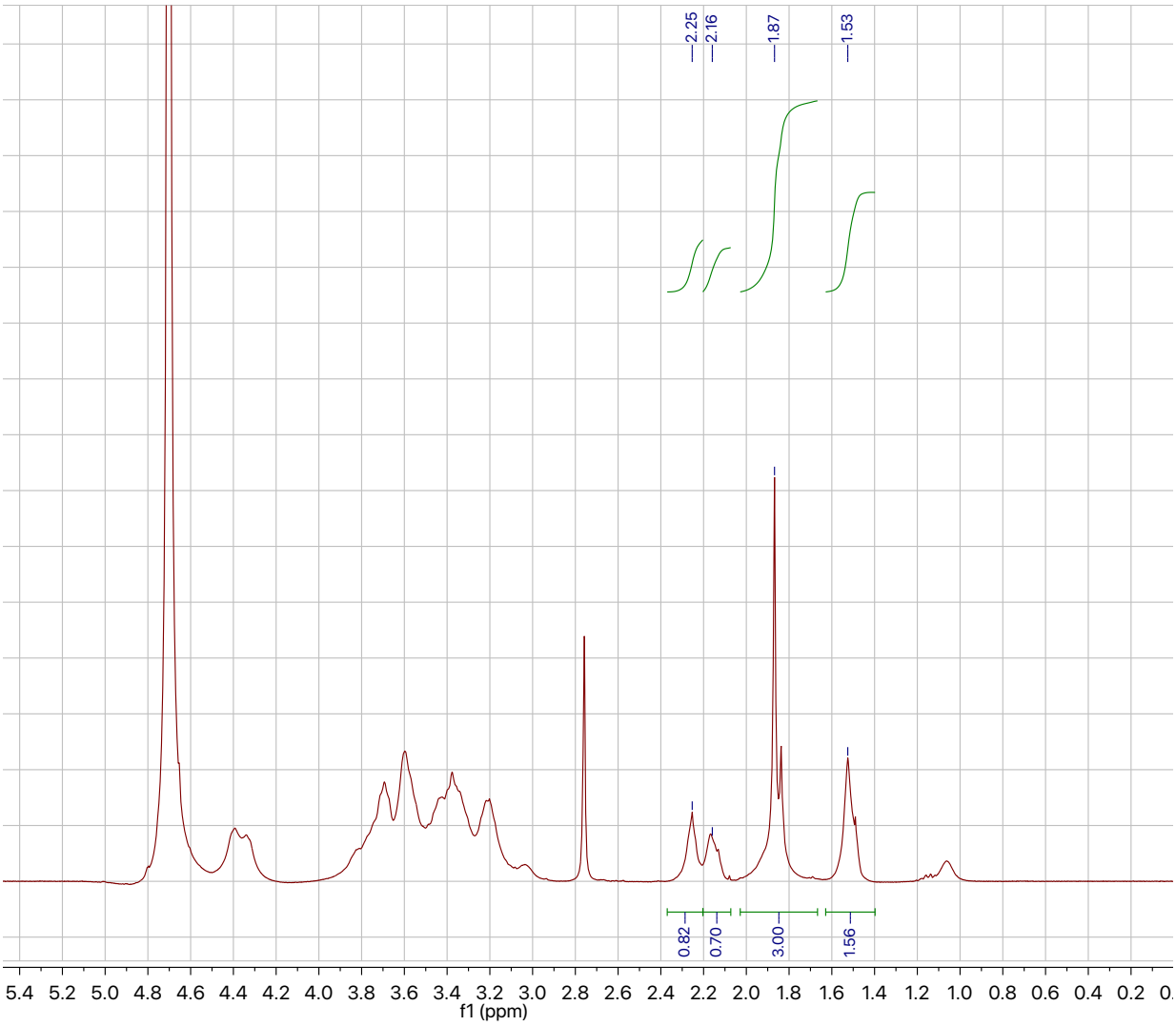


Figure S12. ^{1}H NMR characterization of HA-Hyd. The HA-Hyd modification was determined using ^{1}H NMR (400 MHZ, deuterium oxide) and integrating the butyl linker (8H, 2.1-2.4 ppm, 1.4-1.6 ppm) relative to the methyl group of HA (3H, 1.7-2.0 ppm). Functionalization was found to be ~39% of disaccharide repeat units.

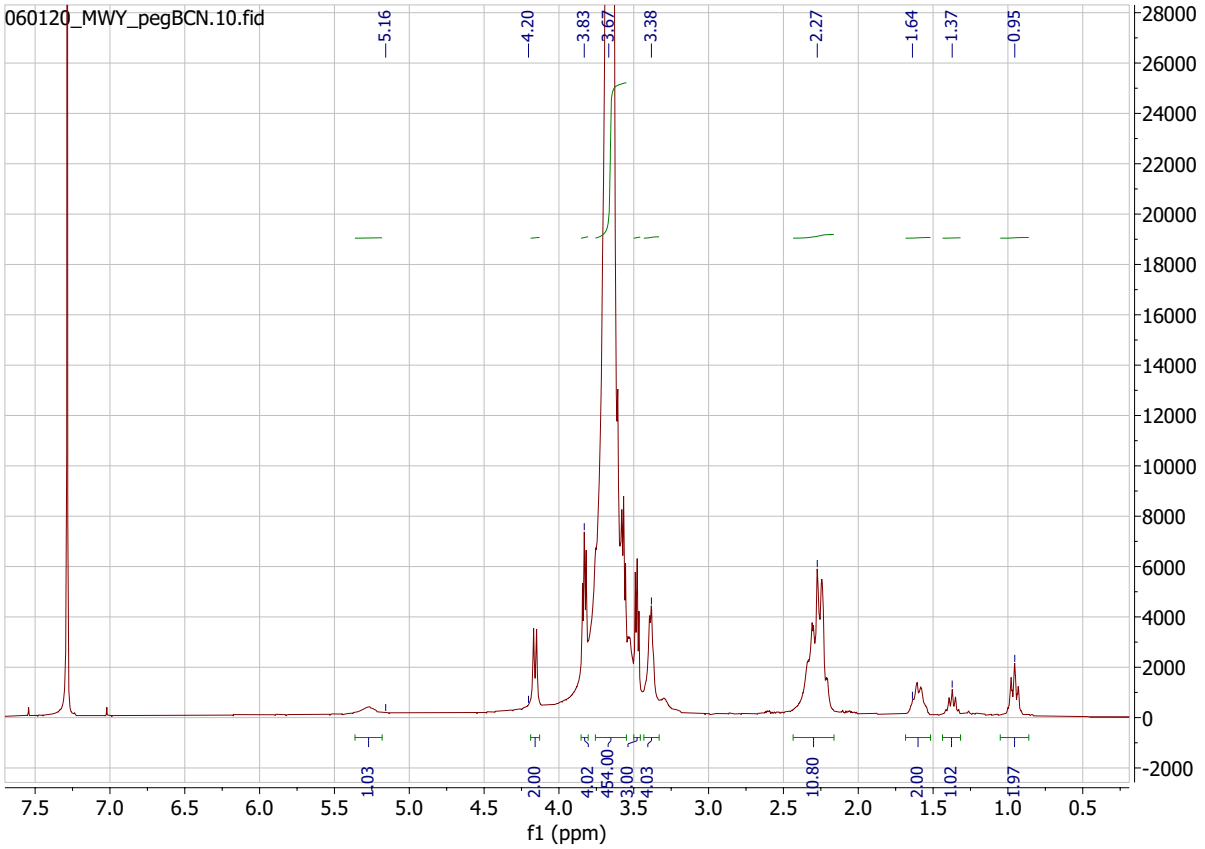


Figure S13. ^{1}H NMR characterization of Peg-BCN. The end group functionality of Peg-BCN was determined by using ^{1}H NMR (400 MHZ, $CDCl_3$) and comparing the integrated values of characteristic BCN peaks 5.16 ppm (1H), 1.64 ppm (2H), 1.37 ppm (1H), and 0.95 ppm (2H) to those of the PEG backbone 3.67 ppm (454H per Peg arm). Functionalization was found to >95%.

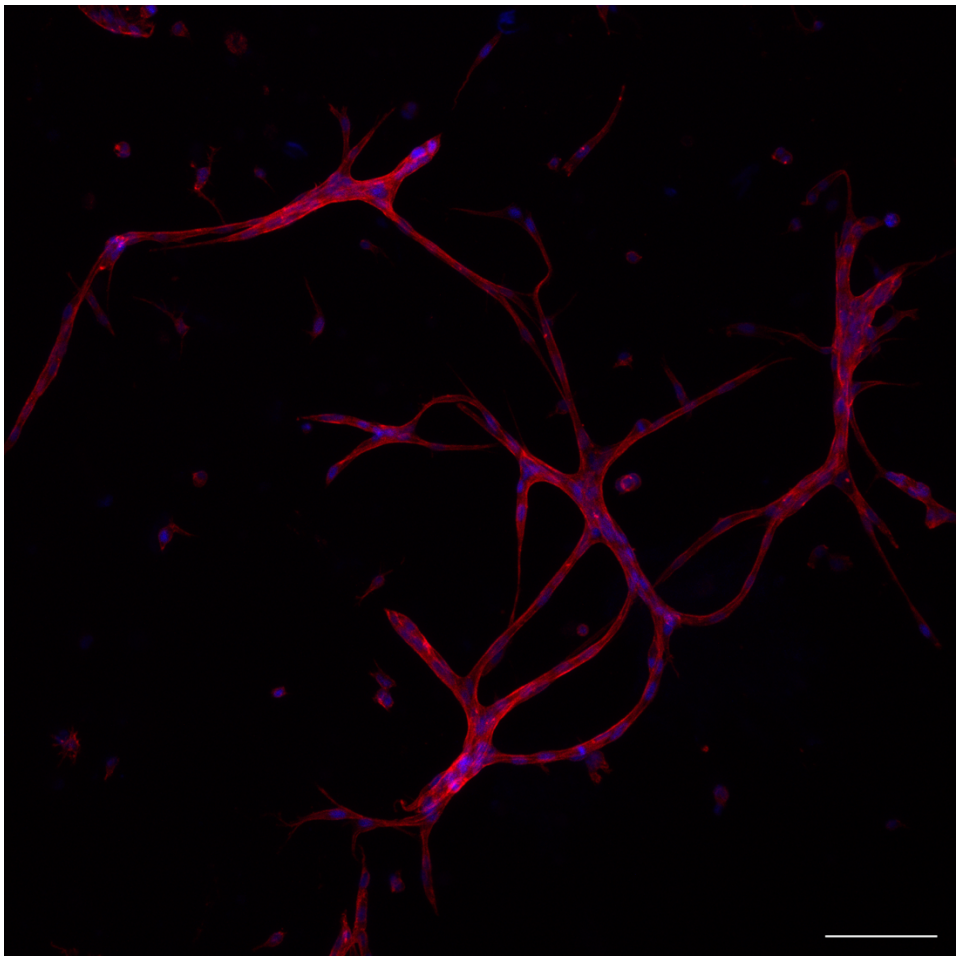


Figure S14. Low magnification image of a multi-cellular structure within the 88% alkyl hydrazone bonds condition. This is a 10x magnification image, showing the breadth of the multi-cellular structures, scale bar = 100 μm.