ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Thermoreversible Hyaluronic Acid-PNIPAAm Hydrogel Systems for 3D Stem Cell Culture

*Barbara L. Ekerdt, Christina M. Fuentes, Yuguo Lei, Maroof M. Adil, Anusuya Ramasubramanian, Rachel A. Segalman, and David V. Schaffer**

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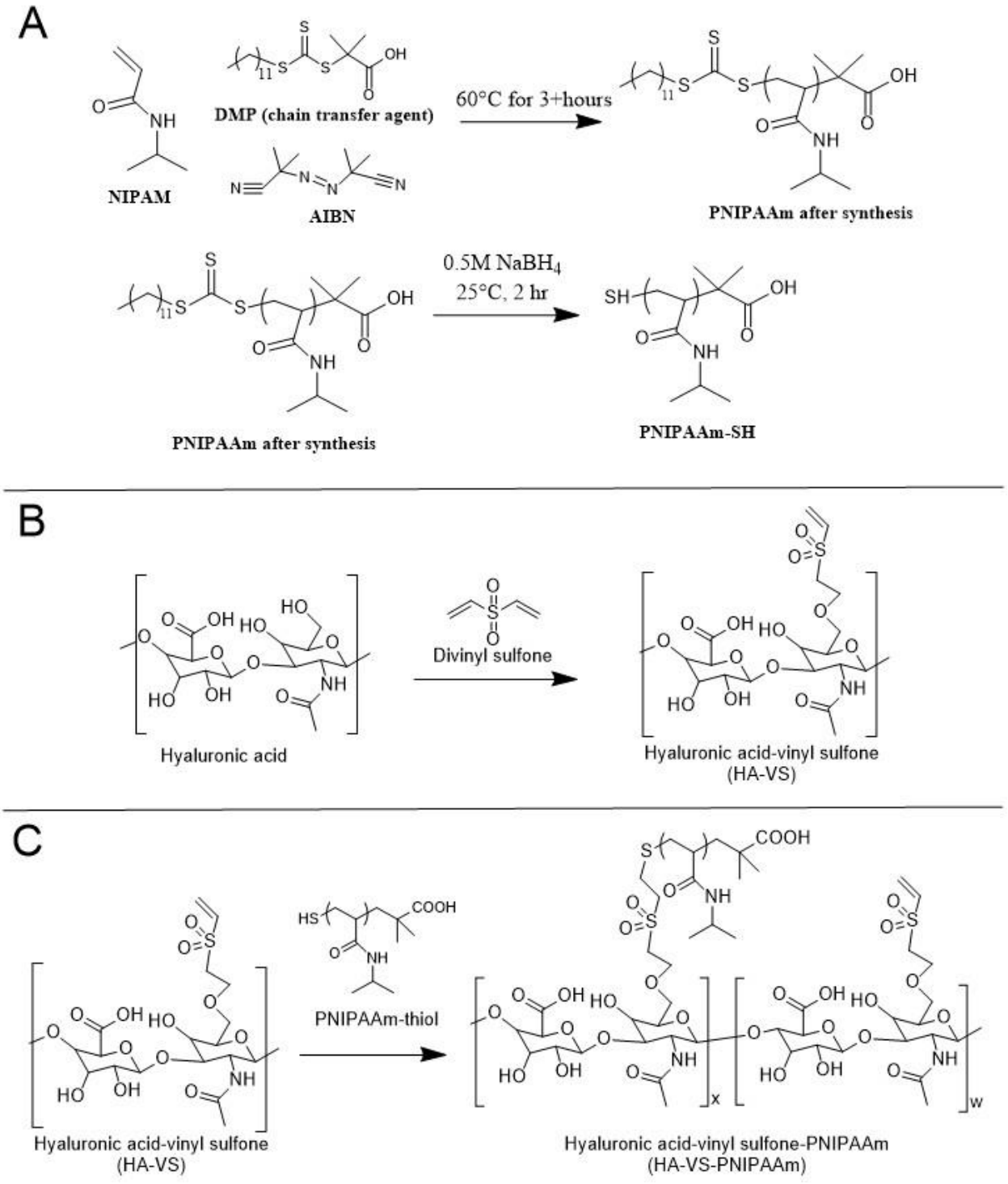

Figure S1. Polymer synthesis schemes: A. PNIPAAm synthesis via RAFT polymerization followed by thiocarbonythio reduction with $NaBH_4$ to yield thiols on chain termini. Butyl methacrylate monomer can also be included in the polymerization to create a random copolymer P(NIPAAm-r-BMA)-SH. B. Synthesis scheme of functionalizing HA with divinyl sulfone C. Conjugation of PNIPAAm-SH to vinyl sulfone groups on the HA.

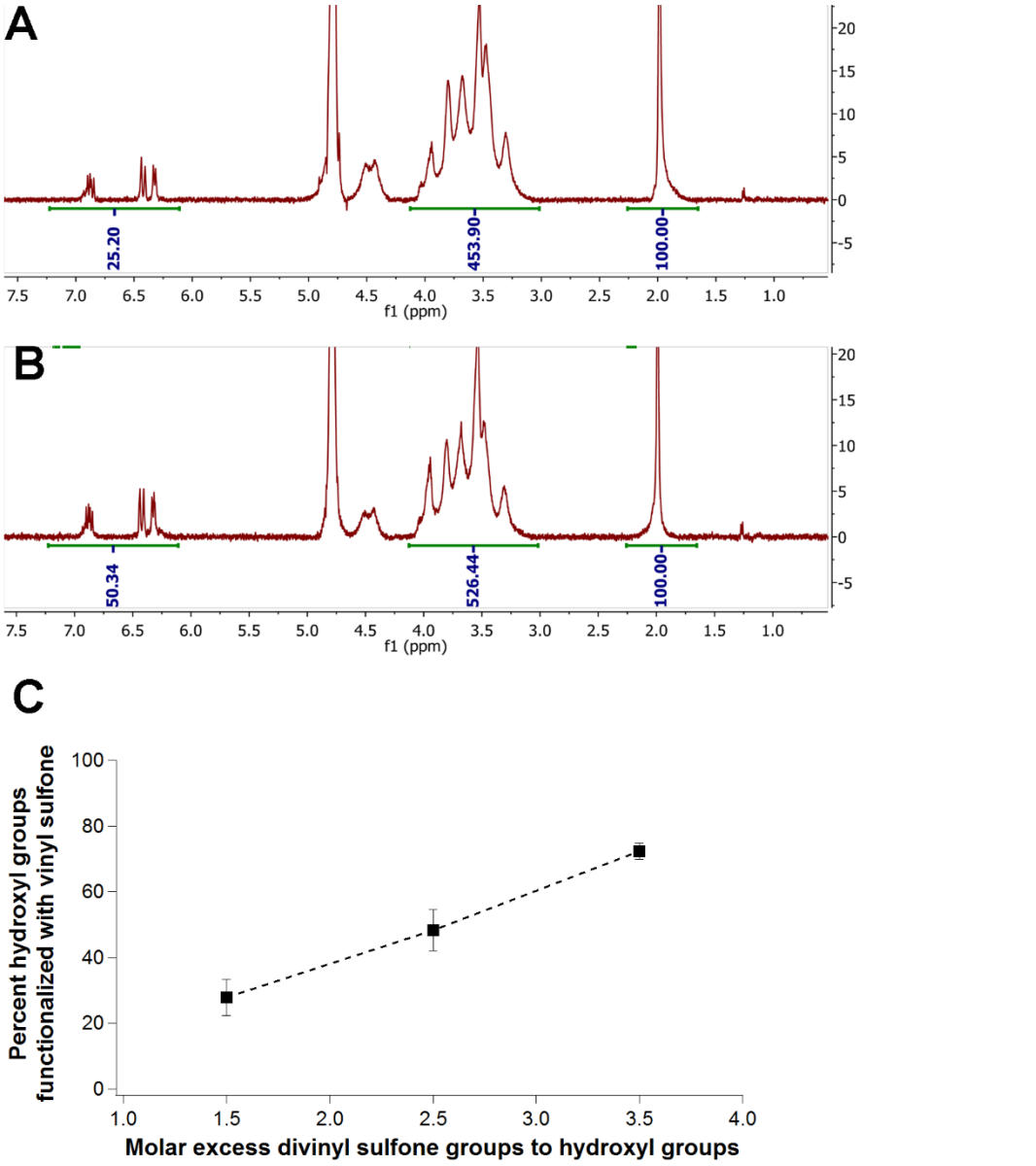

Figure S2. ^{1}H NMR spectra of typical Hyaluronic acid-vinyl sulfone (HA-VS) polymers. The peak around 2.0 ppm reflects three H's in Hyaluronic acid and the peaks between 6.0 and 7.5 represent three H's for vinyl sulfones; setting the integral around 2.0 ppm to a value of 100 allows quantification of vinyl sulfone functionalization. A. 1.5x molar excess divinyl sulfone (DVS) to hydroxyl groups produced HA-VS polymers that were approximately 25% functionalized. B. 2.5x molar excess DVS to hydroxyl groups

produced approximately 50% functionalized polymers. C. Percent functionalization of HA hydroxyl groups with divinyl sulfone. Error bars indicate standard deviation (n=3)

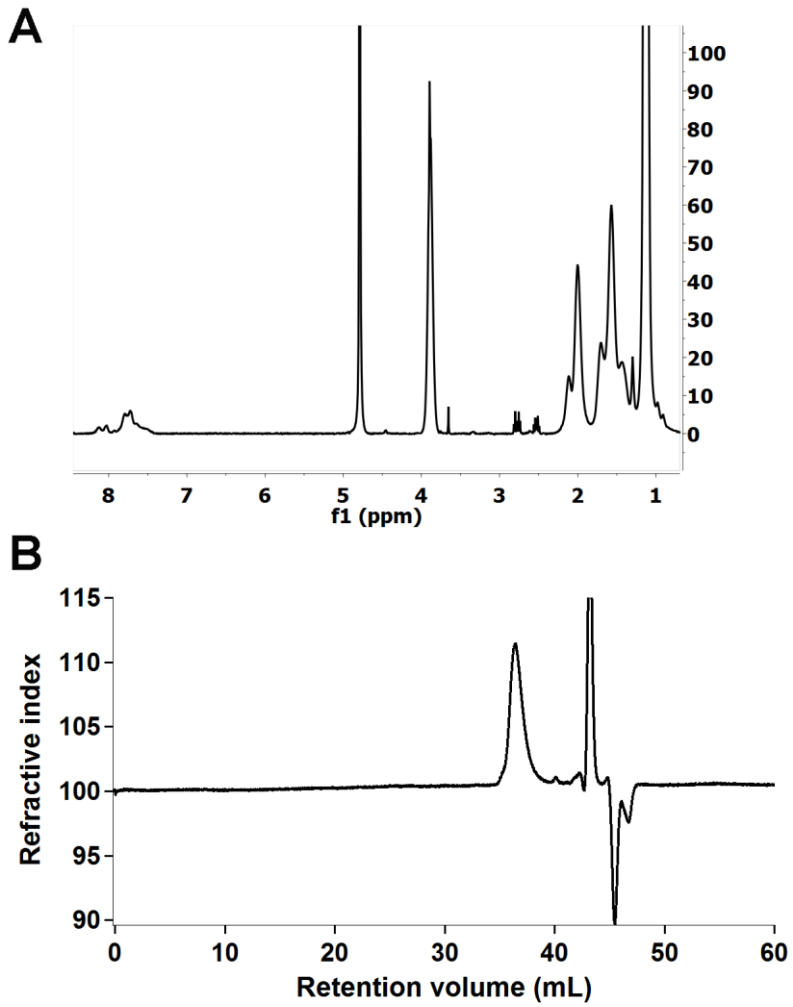

Figure S3. Characterization of synthesized PNIPAAm-SH. A. ^{1}H NMR spectrum. B. Gel permeation chromatography (GPC) trace in THF.

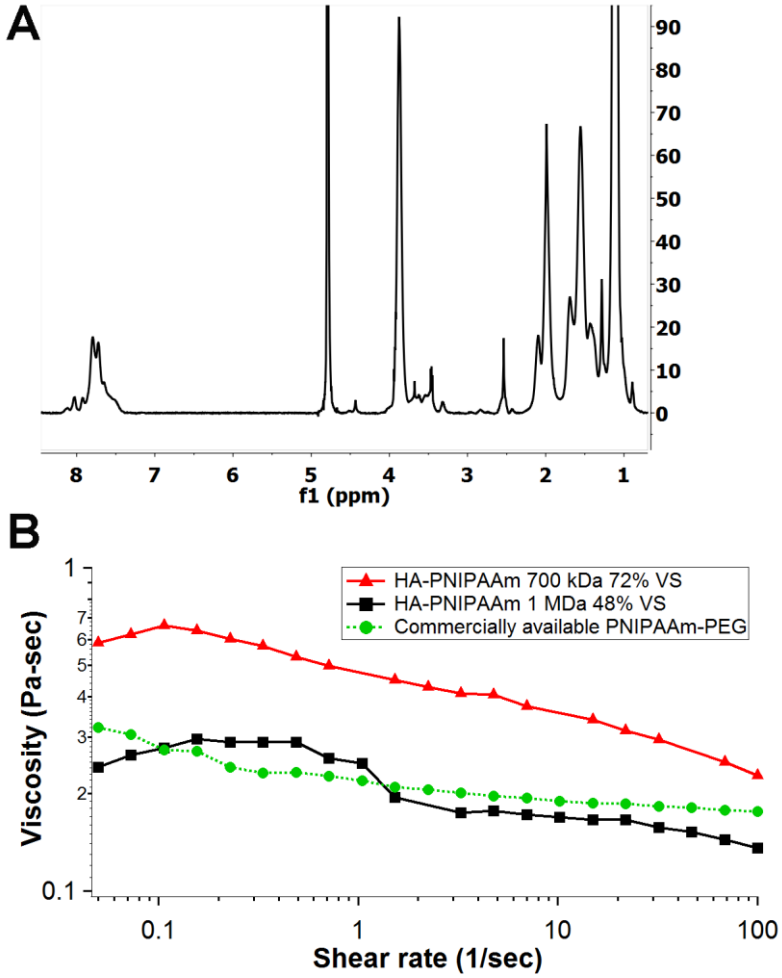

Figure S4. Characterization of HA-PNIPAAm polymer. A. ^{1}H NMR spectrum of HA-PNIPAAm. B. Viscosity sweep for HA-PNIPAAm and commercially available PNIPAAm-PEG at 4°C. Red triangle: HA-PNIPAAm 700 kDA functionalized 72% with vinyl sulfone groups; Black squares: HA-PNIPAAm 1 MDa functionalized 48% with vinyl sulfone groups; Green circles: Commercially available PNIPAAm-PEG. Hydrogels with 72% VS show higher viscosity through entire range of 0.1-100 Hz despite the smaller HA size, making the hydrogel solution harder to pipette and mix with cells. The 48% VS HA-PNIPAAm hydrogels show similar viscosity behavior as the commercially available PNIPAAm-PEG hydrogel.

Figure S5. Examples of polymers that were phase separated at 37°C. Phase separation causing water excretion as well as opaque solids were observed with polymer solutions generated with a high amount of butyl methacrylate, high concentrations of polymer, and high PNIPAAm attachment to the functionalized HA.

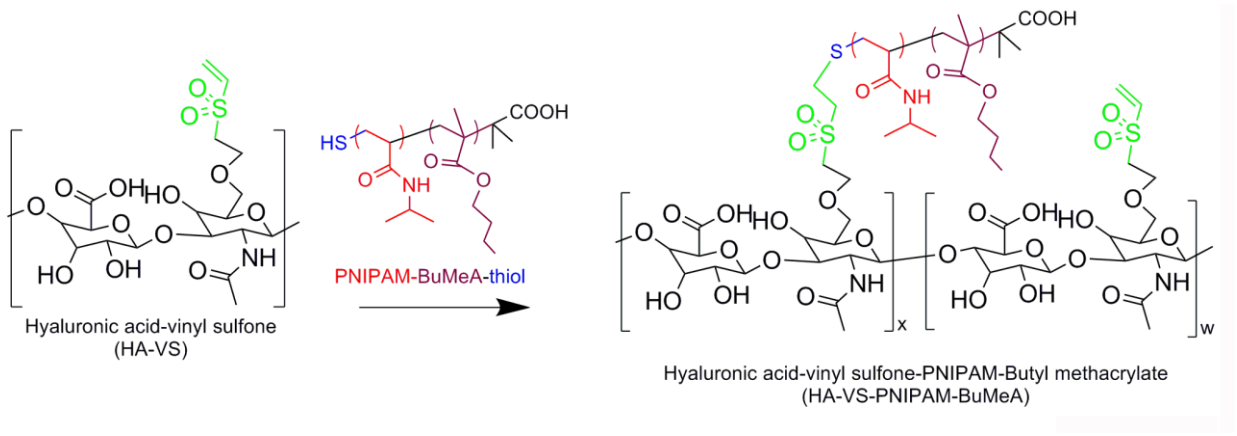

Figure S6. Polymer synthesis scheme of HA-VS reacted with P(NIPAAm-r-BMA)-SH to incorporate additional hydrophobic groups.

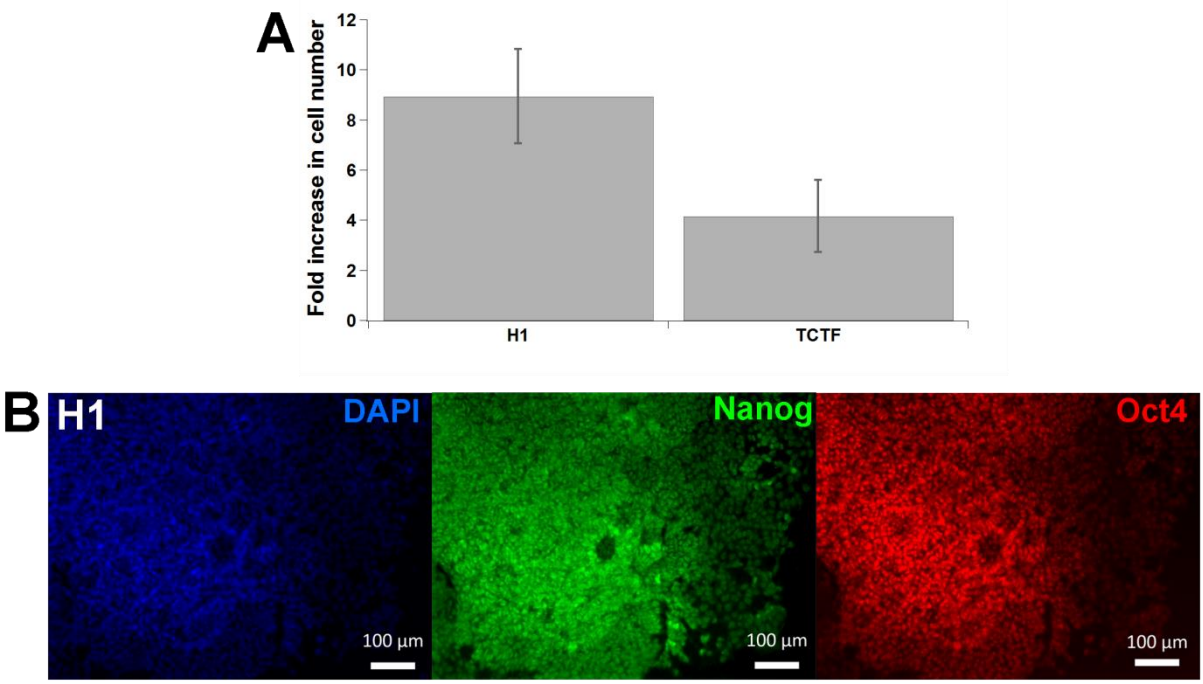

Figure S7. Cell expansion rates and antibody staining for first round in HA-PNIPAAm gels. A. Cell expansion rate for the first passage for H1 and TCTF hPSCs. Error bars indicate standard error (n=3). No statistical difference between cell types based on a t-test assuming unequal variance and with an α of 0.05 (p-value = 0.057). B. Antibody staining for pluripotency markers Nanog and Oct4 after the first passage within HA-PNIPAAm gels with H1 hESCs.

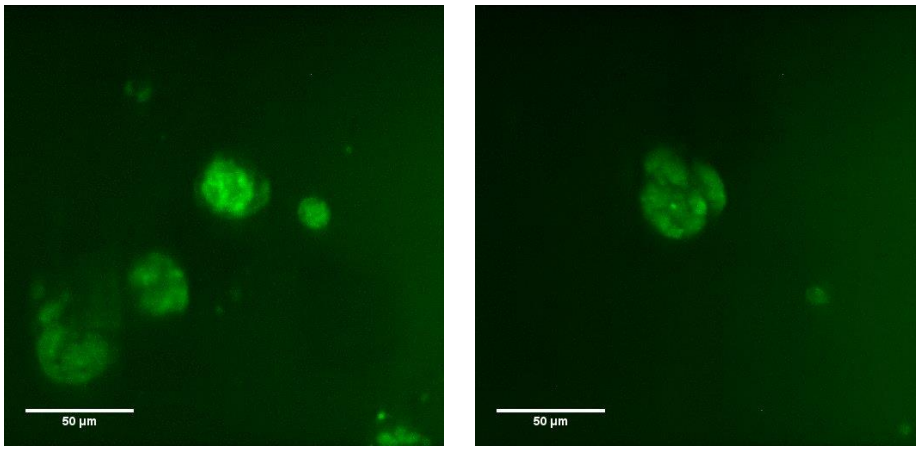

Figure S8. A GFP tagged Oct4 TCTF reporter cell line captures hPSC growth within the HA-PNIPAAm hydrogel. Images were taken using confocal. Histology of the cells within HA-PNIPAAm is difficult because the hydrogel will melt upon cooling, so a live reporter cell line was used to capture TCTF cell growth within the hydrogel.