Supporting Information

Tuning Stiffness of Cell-laden Hydrogel via Host-Guest Interactions

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Experimental

Materials: 4-arm PEG-OH (10 and 20 kDa) was purchased from JenKem Technology USA. Poly(vinyl alcohol) (PVA, 6 kDa), 3-mercaptopropionic acid (MPA), βCD, 1-adamantaneacetic acid and all other chemicals were obtained from Fisher Scientific unless noted otherwise.

Synthesis of βCDAE, LAP, TPVA and PEG macromers: Poly(ethylene glycol)-tetra-allylether (PEG4AE, 20 kDa), poly(ethylene glycol)-adamantane (PEG4AD, 10 kDa), allylether functionalized βCD (βCDAE),[1] and photoinitiator lithium acylphosphinate (LAP) were synthesized using established protocols without modifications.[2] Functionalized βCDAE was analyzed with ^{1}H NMR (**Figure S1A**) and mass spectrometry (Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS, **Figure S1B**). TPVA was synthesized followed established protocol with modified purification method.[3] Briefly, mixture of hydrochloric acid (5 ml, 12 N) and MPA (25 mL) was added slowly into PVA (5 g) pre-dissolved in 50 mL ddH_2O at 60 ^{o}C. After 24 hours of reaction (60 ^{o}C), the product was transferred to a dialysis bag (MWCO: 3600 Da, Spectrum lab) and dialyzed for 3 days in slightly acidic ddH_2O (pH 6.5). The TPVA product was recovered and lyophilized. The degree of thiolation was quantified by Ellman's assay to be about 16 mM of thiol per wt% of TPVA (**Figure S8**).

4-arm PEG-allylether (PEG4AE, 20 kDa) was synthesized following a published protocol. Briefly, 4-arm PEG (20 kDa) was dissolved in anhydrous toluene, and dried with evaporation by reduced pressure. Dried PEG was re-dissolved in anhydrous tetrahydrofuran (THF). Sodium hydride (1.5-fold excess of hydroxyl group) was added slowly to the PEG solution under continuous nitrogen purging at 40 °C. Upon complete liberation of hydrogen

gas, allylbromide (6-fold excess of hydroxyl group) was added slowly to the solution. After overnight reaction in dark, sodium bromide salt was filtered off to obtain a PEG4AE solution. PEG4AE was precipitated in cold ethyl ether, filtered and dried *in vacuuo*. The purity of PEG4AE product was verified with H^1 NMR in $CDCl_3$ (**Fig. S9A**, 90 %, Bruker 500). 4-arm PEG-adamantane (PEG4AD, 10 kDa) was synthesized following an established protocol. In brief, 4-arm PEG and glassware were dried overnight in vacuum oven. Under continuous stirring and purging with nitrogen, dried PEG was dissolved in anhydrous dichloromethane (DCM). A required amount of 1-adamantaneacetic acid (2-fold excess of hydroxyl group), 4-dimethylaminopyridine (DMAP, 1-fold of hydroxyl group) and N,N-diisopropylcarbodiimide (DIC, 4-fold excess of hydroxyl group) were added to PEG solution. After an overnight reaction, PEG4AD was filtered, precipitated in cold ethyl ether and dried in vacuuo. Dried PEG4AD was redissolved in ddH_2O, filtered and transferred to a dialysis bag. After two days of dialysis in slightly acidic ddH_2O (~pH 6.8), PEG4AD were freeze-dried. The purity of PEG4AD product was verified with H^1 NMR in $CDCl_3$ (**Fig. S9B**, >95 %, Bruker 500).

Hydrogel fabrication and reversible stiffening: Thiol-allylether hydrogels (45 μL/gel) were formed by light mediated photo-click reactions using precursor solution prepared in the order of mixing pH 7.4 PBS, 1 mM LAP, PEG4AE, βCDAE and TPVA. Immediately after mixing the components, solution was injected in between two glass slides separated by 1 mm thick spacers. Hydrogel slabs were prepared by exposing the solution to 365 nm light at 10 mW/cm^2 for 5 minutes. The elastic moduli (G') of hydrogels were measured after 48 hours of swelling in PBS at 37 ^oC. Hydrogel stiffening was achieved by incubating βCD-immobilized hydrogels in PEG4AD solution (48-well plate, 200 μL/well/gel) and placed on an orbital shaker (130 rpm) for 40 hours unless mentioned otherwise. After measuring the stiffness of hydrogels, gels were placed in either pH 7.4 PBS or 1 wt% of βCD (48-well plate, 400 μL/well/gel) for softening.

Rheometry: For rheometrical property measurements, hydrogel discs (8 mm in diameter and 1 mm in height) were fabricated as described previously and placed in pH 7.4 PBS for 48 hours. Frequency sweep (0.01 to 10 Hz, at 1 % strain) and strain sweep (0.1 % to 5 %, at 1 Hz frequency) oscillatory rheometry were performed on a Bohlin CVO 100 digital rheometer. Shear moduli of the hydrogels were measured using a parallel plate geometry (8 mm) with a gap size of 750 μm. The average of elastic moduli obtained from the linear viscoelastic region (LVR) was reported. *In situ* gelation rheometry for hydrogels was conducted in a light cure cell using a parallel plate geometry (25 mm) at room temperature. Briefly, the macromer solution was placed on a quartz plate in the light cure cell, and irradiated with liquid light guide (365 nm, 10 mW/cm^2). Light was turned on 30 seconds after the time sweep *in situ* rheometry (5 % strain, 1 Hz frequency, and a gap size of 90 μm). Gel point was determined at the time when G' surpassed loss modulus (G").

βCDAE immobilization efficiency: Following hydrogel preparation procedure as described previously, hydrogels (30 μL/gel) were fabricated using syringes with cut-off tips as molds. Hydrogels were synthesized in the presence of βCDAE or non-modified βCD. To detect the concentration of non-reacted thiol after photopolymerization reaction, immediately after gel polymerization, hydrogels were individually placed in 48-well plate containing Ellman's reaction buffer (300 μL/well/gel). After 30 minutes of incubation at room temperature, these collected Ellman's reaction buffer was compared against a standard curve with known cysteine concentrations. In a separate experiment, immediately after polymerization, hydrogels were dried in a desiccator and the first dry mass were obtained. After incubating gels in ddH_2O for a day to allow sol fraction to leach out, gels were dried and the second dry mass were measured. Gel fraction of hydrogel was reported as the percentage of the second dry weight to the first dry

weight. Furthermore, ATR-FTIR was used to detect the presence of immobilized βCDAE in the hydrogel.

Cytocompatibility of βCD and PEGAD: MIN6 β-cells were cultured on 96-well tissue culture treated plate for two days at 37 ^{o}C and 5 % of CO_2. β-cells were treated with different concentrations of soluble βCD and PEGAD (10 kDa) dissolved in media (high-glucose DMEM with L-glutamine and sodium pyruvate, 10 % FBS, β-mercaptoethanol and antibiotic-antimycotic) at 37 ^{o}C and 5 % of CO_2. After incubating for 40 hours, βCD and PEGAD containing media were removed, cells were supplemented with media containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 37 ^{o}C and 5 % of CO_2 (100 μL/well). After 4 hours of incubation, dimethyl sulfoxide (DMSO, 100 μL/well) was added to wells, and plate was further incubated in oven for 10 minutes (at 60 ^{o}C). The absorbance was measured at 540 nm and DMSO was used as a blank.

Cell encapsulation and cytocompatibility: MIN6 β-cells (final density 2×10^6 cells/mL) was suspended in polymer solutions (i.e., PBS, LAP, PEG4AE, βCDAE and TPVA) and exposed to light for 5 minutes (365 nm at 5 mW/cm^2). These MIN6 cell-laden hydrogels (20 μl/gel) were cultured in growth media (high-glucose DMEM with L-glutamine and sodium pyruvate, 10 % fetal bovine serum (FBS), β-mercaptoethanol and antibiotic-antimycotic) and maintained at 37 ^{o}C and 5 % of CO_2. Fresh media was changed every two days. After a week, cell-laden hydrogels were transferred to 48-well plate (2 gels/well), supplemented with media containing 5 wt% of PEG4AD (200 μL/well), and placed on a rotary platform in 37 ^{o}C and 5 % of CO_2 for 3 days. For quantitative cell viability study, cell-laden hydrogels were incubated in 500 μL Alamarblue® reagent (10 % in cell culture medium; AbD Serotec) at 37 ^{o}C and 5 % of CO_2. After 16 hours of incubation, 200 μl of incubated Alamarblue® media was transferred to a transparent 96-well plate. Using a microplate reader, the Alamarblue® fluorescence (excitation:

560 nm and emission: 590 nm) due to cell metabolic activity was determined. Cell viability was quantified by CellTiter Glo® assay (Promega). In addition, confocal imaging (FV1000 Laser Scanning Biological Microscope) of cell-laden hydrogels were stained with Biotium live/dead kit (Calcein AM stained live cells green and Ethidium homodimer-1 stained dead cells red) for an hour and washed with HBSS for 10 minutes. The confocal imaging was performed with a step size of 10 μm for a total image thickness of 100 μm. In preparation for RNA isolation, cell-laden gels were rapidly frozen using liquid nitrogen and stored in -80 ^{o}C until use. Collected samples were processed following NucleoSpin® RNA protocols (Clontech). The concentrations of pure RNA were determined using NanoDrop 2000 Spectrophotometer (Thermo Scientific). Reverse transcription PCR (rt-PCR) was performed on Applied Biosystems 2720 Thermal Cycler to convert RNA into cDNA using PrimeScript^{TM} RT Reagent Kit (Clontech). cDNA samples were further prepared with SYBR Premix Ex Taq II kit (Clontech) and primers (Sigma-Aldrich, Table S1) for quantitative real time PCR (Applied Biosystems 7500 Fast Real-Time PCR machine).

References

[1] H. Shih, C.-C. Lin, Biomacromolecules 2015, 16, 1915.
[2] B. D. Fairbanks, M. P. Schwartz, C. N. Bowman, K. S. Anseth, Biomaterials 2009, 30, 6702.
[3] R. M. Dicharry, P. Ye, G. Saha, E. Waxman, A. D. Asandei, R. S. Parnas, Biomacromolecules 2006, 7, 2837; B. Gupta, S. Anjum, S. Ikram, Journal of Applied Polymer Science 2013, 129, 815.

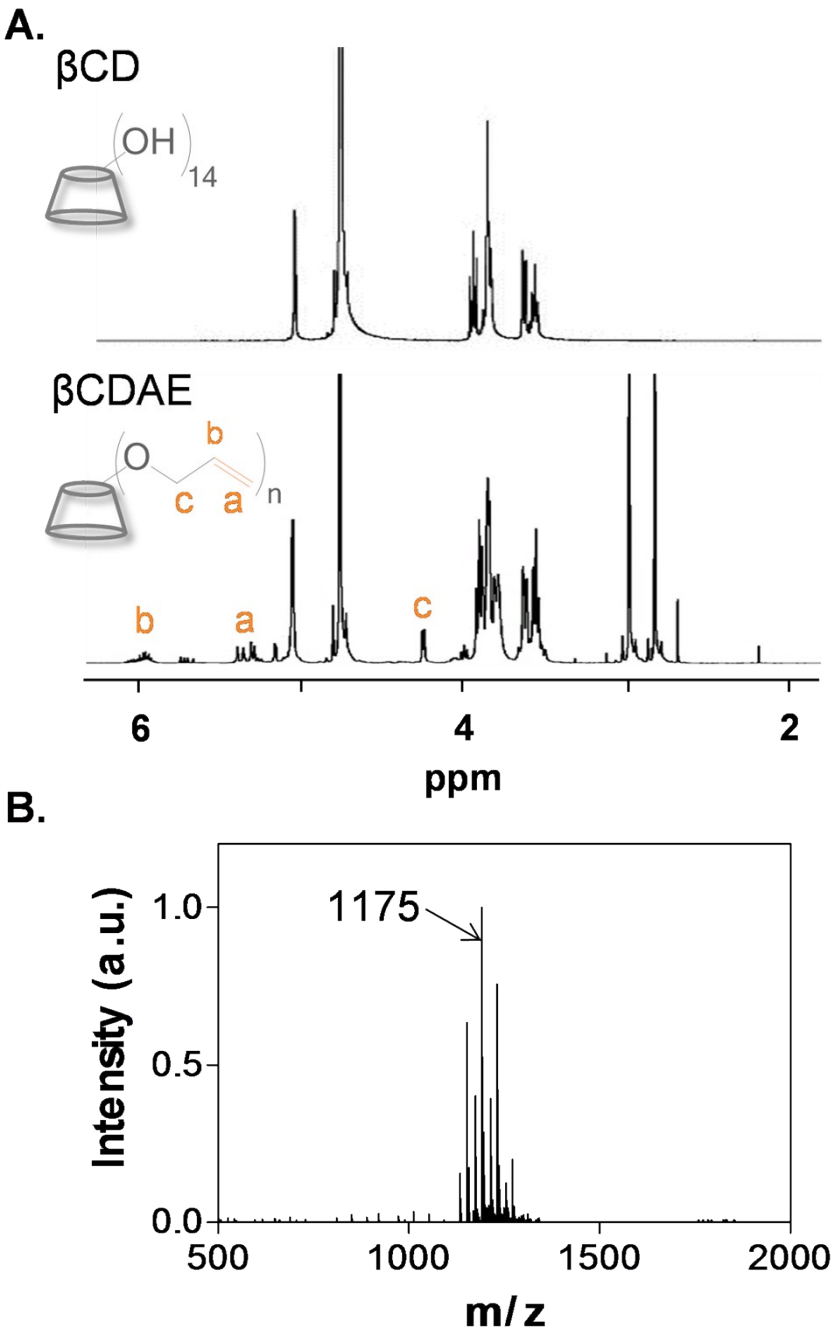
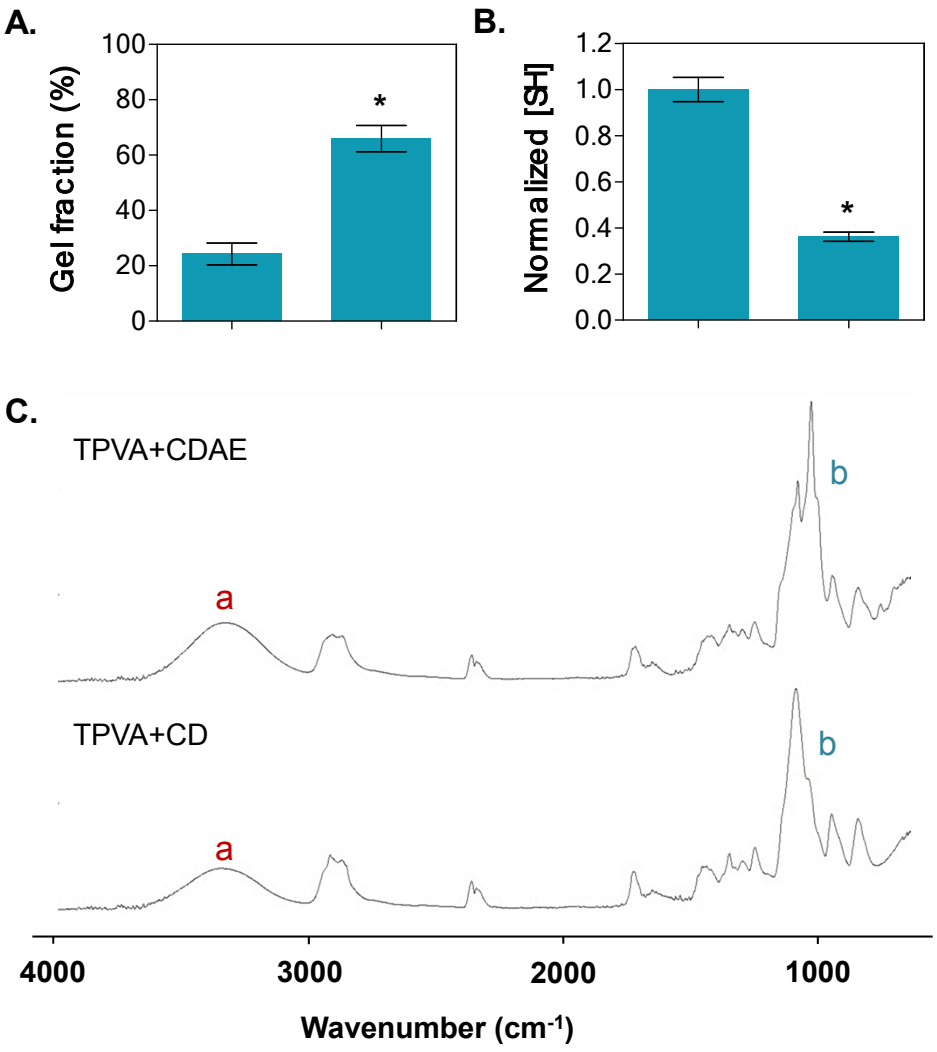


Figure S1. (A) ^{1}H NMR of βCD (top), and βCD-AE (bottom). Prepared with D_2O (at 4.79 ppm). (B) Mass spectroscopy of βCDAE.



	Wavenumber (cm^{-1})	TPVA + CD	TPVA + CDAE
a	3700-3000	27.3 ± 1.5	39.8 ± 0.8
b	1050-970	0.4 ± 0.1	13.5 ± 0.5

Figure S2. Estimation of βCD-immobilization post-gelation: (A) gel fraction; (B) normalized thiol concentration measured by Ellman's assay; and (C) ATR-FTIR characterization. 2.5 wt% TPVA at 0.8 mM PEG4AE and 27.6 mM βCDAE ($R_{[allylether]/[thiol]}$ = 0.8) or βCD in the precursor solution (1 mM LAP, 365nm light at 10 mW/cm^2).

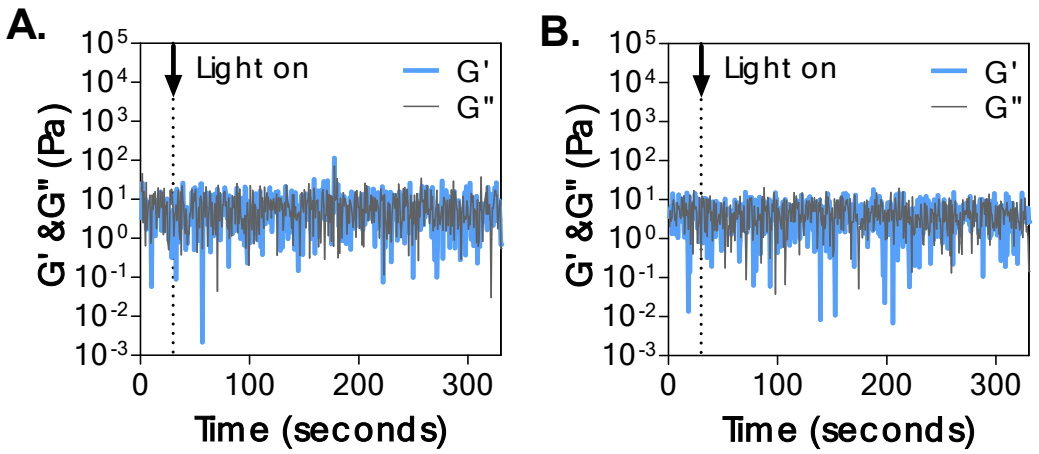


Figure S3. *In situ* photorheometry showing storage modulus (G') and loss modulus (G") of thiol-allylether photopolymerization (A) at 2.5wt% TPVA and 27.6 mM βCDAE; and (B) at 0.8 mM PEG4AE and 27.6 mM βCDAE in the precursor solution (1 mM LAP, 365nm light at 10 mW/cm^2). Light was turned on at 30 seconds (dotted line).

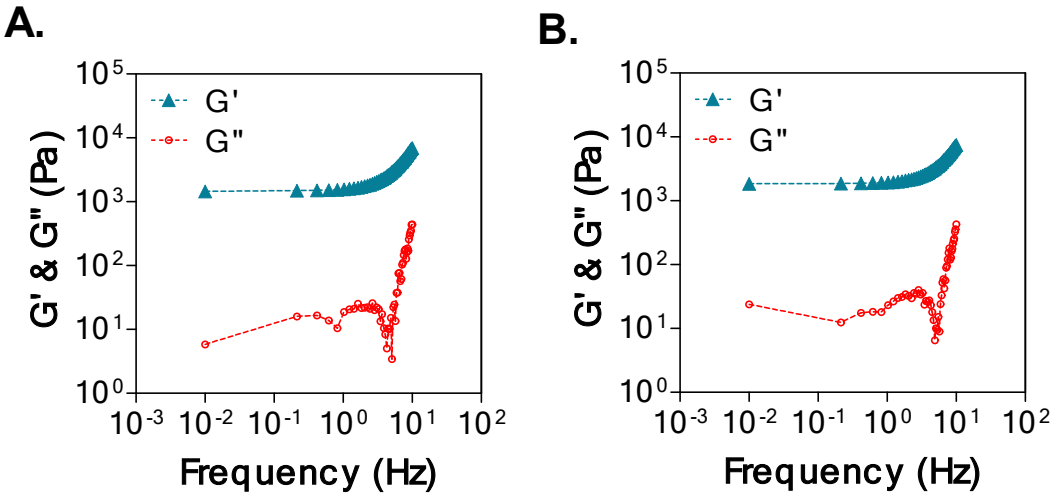


Figure S4. Frequency sweep rheometry of thiol-allylether hydrogels before (A), and after (B) incubating in PEG4AD. Error bars omitted for clarity.

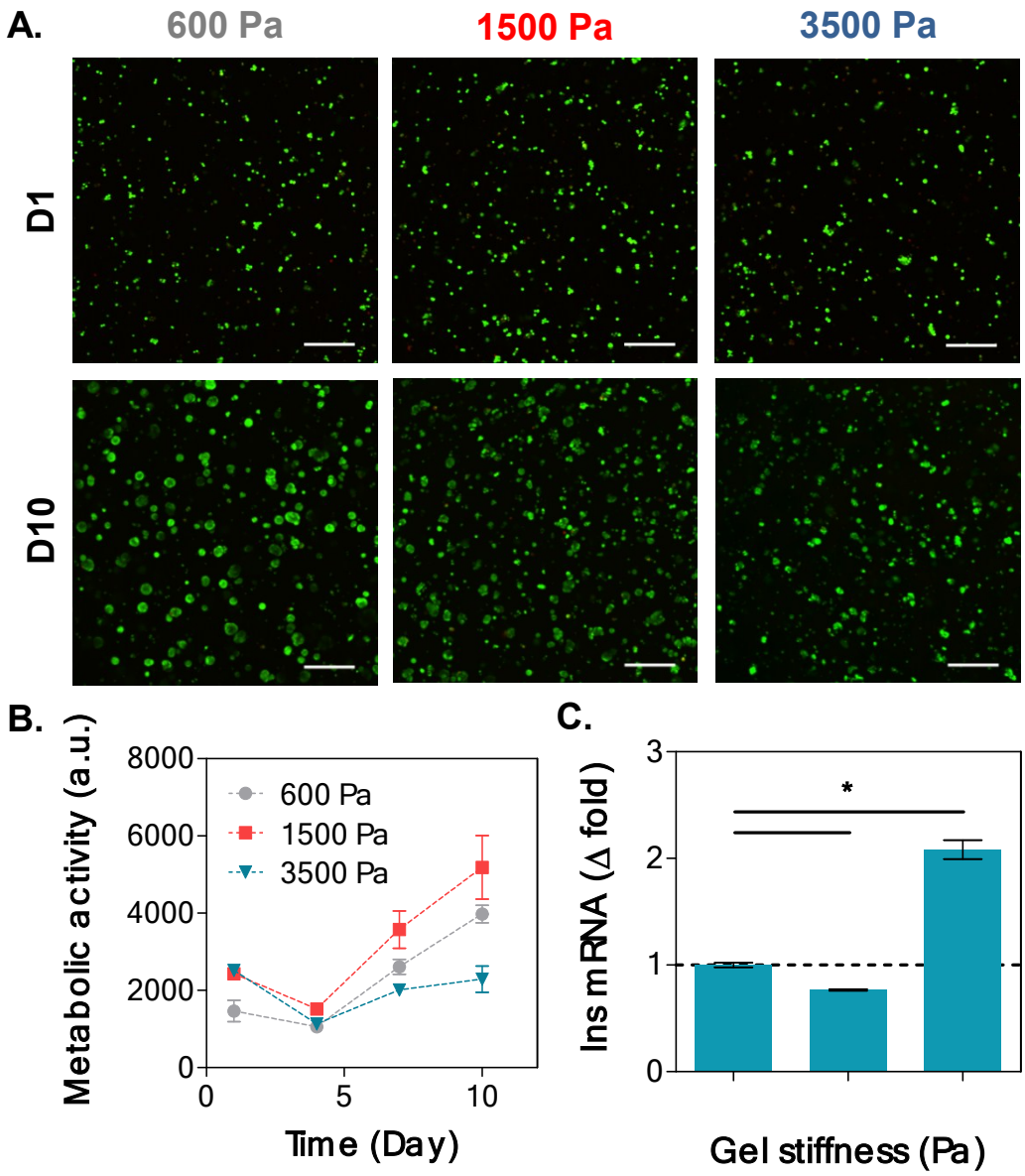


Figure S5. Effect of thiol-allylether hydrogel stiffness on the cytocompatibility of MIN6 β-cells. (A) Representative confocal z-stack images of MIN6 cells stained with live/dead staining kit on day 1 and day 10. MIN6 cells were encapsulated (2×10^6 cells/mL) in thiol-allylether hydrogels. (B) Cells viability as assessed by Alamarblue® reagent. (C) Normalized insulin mRNA expression on day 10. All gel formulations contained β-cells at 2×10^6 cells/mL, 2.5wt% TPVA, 0.6/0.8/1mM PEG4AE and 27.6 mM βCDAE, 1 mM LAP, and 365 nm light at 5 mW/cm^2 (Scales: 200 μm).

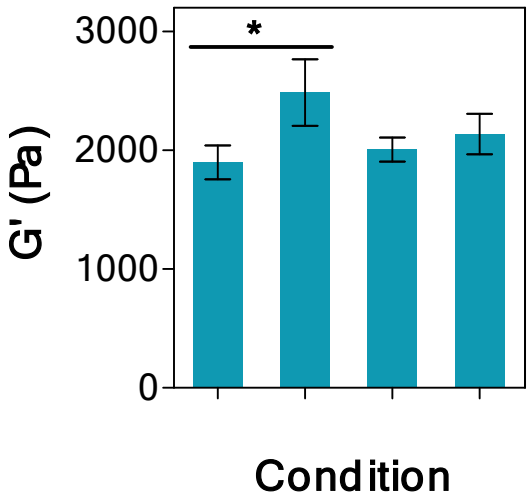


Figure S6. Effect of PEG4AD and βCD on the stiffening and softening of thiol-allylether hydrogel via CD-AD interactions. All gel formulations contained 2×10^6 cells/mL β-cells, 2.5 wt% TPVA, 0.8 mM PEG4AE and 27.6 mM βCDAE, 1 mM LAP, and 365 nm light at 10 mW/cm^2 (N = 3).

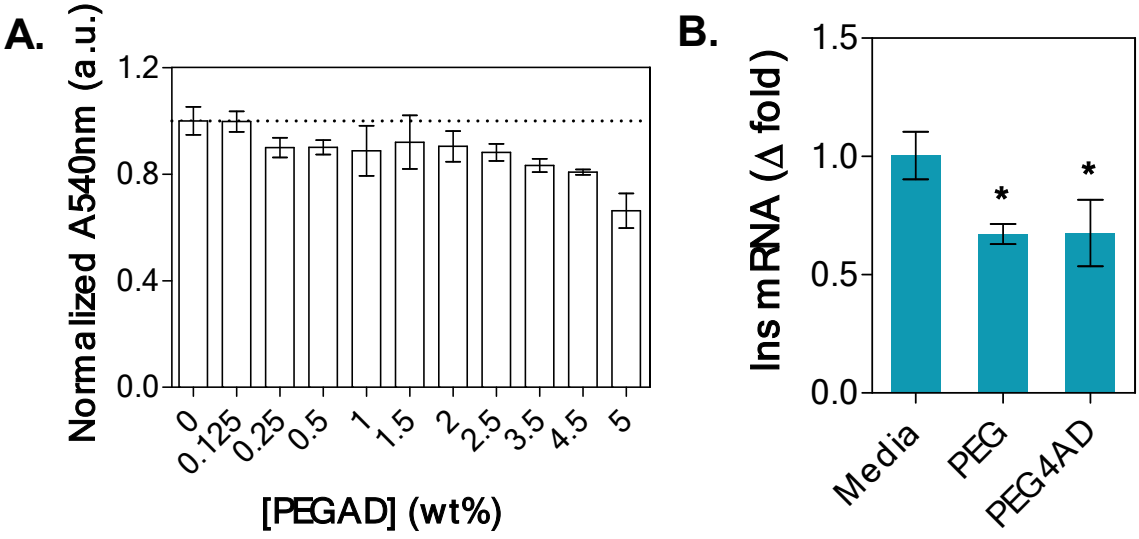


Figure S7. (A) Effect of PEGAD on the cytocompatibility of MIN6 β-cells. Cells viability as assessed by MTT. PEGAD (10 kda) was used. (B) Effect of PEG4AD (10 kDa) on the Ins mRNA expression. Cells were all cultured on 2D TCP.

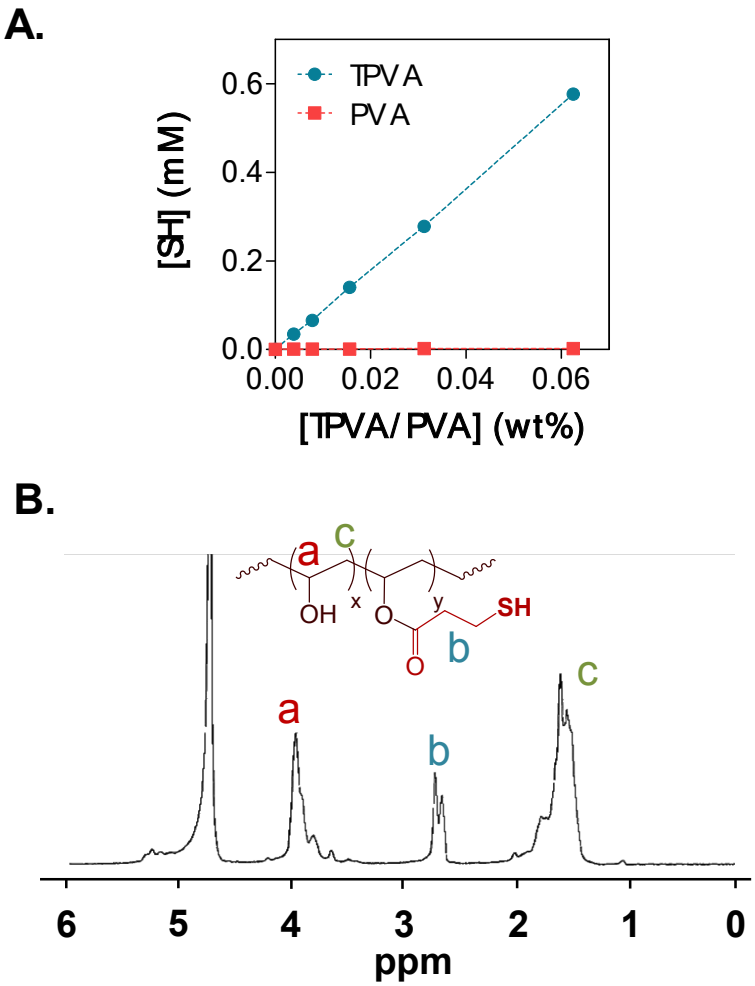


Figure S8. (A) Degree of TPVA thiolation measured by Ellman's assay. (B) ^{1}H NMR of TPVA prepared with D_2O (at 4.79 ppm).

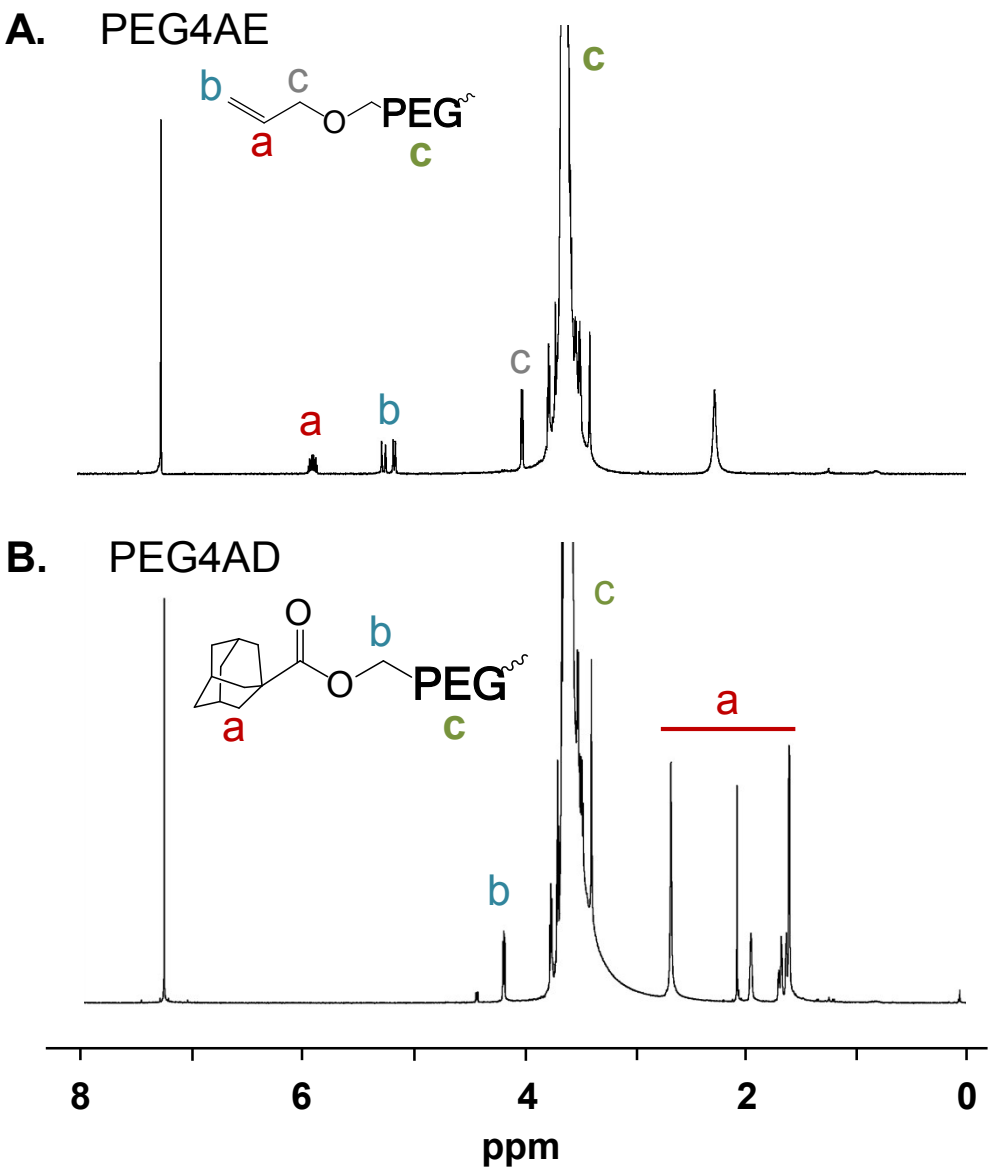


Figure S9. ^{1}H NMR of (A) PEG4AE (20 kDa), and (B) PEG4AD (10k Da). All dissolved in $CDCl_3$ (at 7.26 ppm).

Table S1. Mouse primer sequences used in real time PCR.

Gene Symbol	Primer Sequence	Reference
Tbp	ACCCTTCACCAATGACTCCTATG	[1]
	ATGATGACTGCAGCAAATCGC	
Ins	TGGCTTCTTCTACACACCCAAG	[2]
	ACAATGCCACGCTTCTGCC	

[1] Ejarque, M., et al., Neurogenin3 cooperates with Foxa2 to autoactivate its own expression. J Biol Chem, 2013. **288**(17): p. 11705-17. [2] Iype, T., et al., Mechanism of insulin gene regulation by the pancreatic transcription factor Pdx-1: application of pre-mRNA analysis and chromatin immunoprecipitation to assess formation of functional transcriptional complexes. J Biol Chem, 2005. **280**(17): p. 16798-807.