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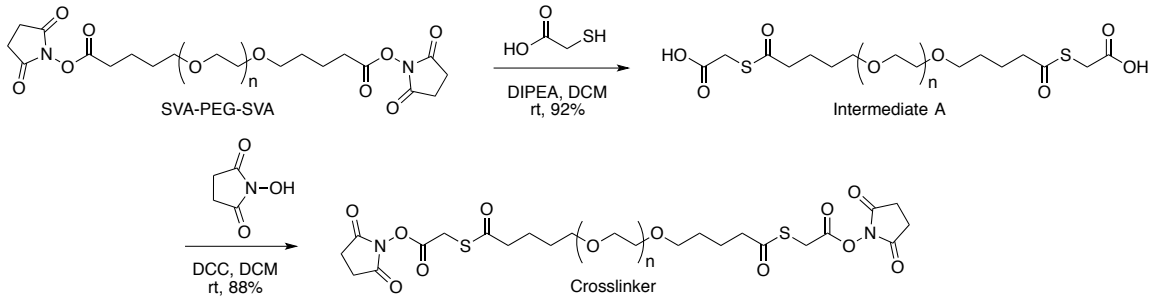
I. General information

Chemicals were either used as received or purified according to *Purification of Common Laboratory Chemicals*. All reactions were carried out under nitrogen using standard techniques, unless otherwise noted. SVA-PEG-SVA (Mw = 3.4 kDa) was purchased from Laysan Bio. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 500 MHz spectrometer. Chemical shifts for ^{1}H NMR were reported as δ, parts per million (ppm), relative to the signal of residual $CHCl_3$ in $CDCl_3$ at 7.26 ppm. Chemical shifts for ^{13}C NMR were reported as δ, ppm, relative to the center line signal of the $CDCl_3$ triplet at 77.0 ppm. Proton and carbon assignments were established using spectral data of similar compounds. The abbreviations s, t, and tt stand for the resonance multiplicity singlet, triplet and triplet of triplets, respectively. Infrared spectra were recorded on a Nicolet Nexus 670 FT-IR with ATR spectrophotometer. Absorptions are given in wavenumbers (cm^{-1}). Thermogravimetric Analysis (TGA) measurements were performed using TGA Q50. Samples were heated from 20 to 500 °C at a heating rate of 10 °C/min. Samples were also tested with Differential Scanning Calorimetry (DSC) at a heating rate of 10 °C/min and a cooling rate of 10 °C/min from -50 to 150 °C. The weight of samples was between 2 to 10 mg and the samples underwent three heat-cool-heat cycles. Polymer molecular weights were determined by gel permeation chromatography (GPC) versus polystyrene standards using DMF as the eluent at a flow rate of 1.0 mL/min through Styragel column (HR4E THF, 7.8 x 300 mm) with a refractive index detector. Adhesion measurements were obtained using an Instron 5848 Microtester with a 100 N load cell.

II. Synthesis and characterization of compounds

Dendron. The dendron was synthesized following a previously reported procedure.[1]



Intermediate A. A flame-dried 10 mL round bottom flask was equipped with a rubber septum and magnetic stir bar and was charged with SVA-PEG-SVA ($5.88x10^{-4}$ mol, 1.0 equiv), thioglycolic acid ($1.76x10^{-3}$ mol, 3.0 equiv), and DIPEA ($2.35x10^{-3}$ mol, 4.0 equiv). The flask was purged with a stream of nitrogen and dry DCM (4.0 mL) was added with a syringe. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 h. Then, the mixture was poured into a separatory funnel containing 25 mL of DCM and 50 mL of saturated citric acid solution. The layers were separated, and the organic layer was washed with water, brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure, and the residue was purified by precipitation in diethyl ether to afford the desired intermediate A as a white solid (1.811 g, 92% yield).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.80-3.44 (overlap, 210H), 2.65 (t, J = 7.4 Hz, 4H), 1.77 (tt, J = 7.4, 7.4 Hz, 4H), 1.62 (tt, J = 7.6, 6.2 Hz, 4H) ppm;
^{13}C NMR ($CDCl_3$, 125 MHz): δ 197.3, 169.9, 70.5, 70.1, 43.2, 31.0, 28.6, 22.2 ppm.

Crosslinker. A flame-dried 10 mL round bottom flask was equipped with a rubber septum and magnetic stir bar and was charged with intermediate A (5.40×10^{-4} mol, 1.0 equiv), NHS (1.19×10^{-3} mol, 2.2 equiv), and DCC (1.19×10^{-3} mol, 2.2 equiv). The flask was purged with a stream of nitrogen and dry DCM (18.0 mL) was added with a syringe. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 h. Then, the reaction mixture was filtered twice to remove DCU. Solvent was evaporated under reduced pressure, and the residue was purified by precipitation in diethyl ether to afford the desired crosslinker as a white solid (1.688 g, 88% yield).
^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.98 (s, 4H), 3.81-3.43 (overlap, 210H), 2.84 (s, 8H), 2.68 (t, J = 7.5 Hz, 4H), 1.78 (tt, J = 7.4, 7.4 Hz, 4H), 1.62 (tt, J = 6.8, 6.8 Hz, 4H) ppm;
^{13}C NMR ($CDCl_3$, 125 MHz): δ 195.9, 168.6, 164.8, 70.7, 70.2, 43.3, 28.8, 28.1, 25.6, 22.3 ppm;
IR (neat): ν_{max} 3573, 2883, 1742, 1467, 1343, 1107, 961, 842 cm^{-1};
mp (DSC): 21 °C;
T_{decomp}5/50 (TGA): 233/372 °C;
GPC (DMF): Mn = 3237 g/mol, Mw = 3733 g/mol, PDI = 1.15.

III. Mechanical properties of the hydrogel

The rheological measurements were obtained on a TA Instruments RA 1000 rheometer. To prepare the hydrogel samples, the dendron was dissolved in borate buffer at pH 8.6, and was reacted with the crosslinker which was dissolved in phosphate buffered saline (PBS) buffer at pH 6.5. The ratio of amine (dendron) to the NHS (crosslinker) was 1:1, and the total concentration of the polymer in solution was 40 wt%. Hydrophilic gels formed spontaneously within 3 to 10 sec upon mixing the two aqueous solutions. Cylindrical hydrogel samples of 9 mm diameter and 3 mm thickness were prepared in a precast Teflon mold and analyzed after sitting in a moisture chamber at 23 °C for 1 h. All rheological measurements were performed at 20 °C to avoid evaporation. The oscillatory stress sweeps of the hydrogel samples were recorded at a frequency of 1 Hz. The frequency sweeps were measured at frequencies from 0.1 to 10 Hz with a controlled oscillatory stress of 50 Pa. A normal force of 0.2 N was applied to the hydrogel using 8 mm steel plate geometry. Data are expressed as mean ± standard deviation (n = 5). The hydrogel exhibited viscoelastic properties.

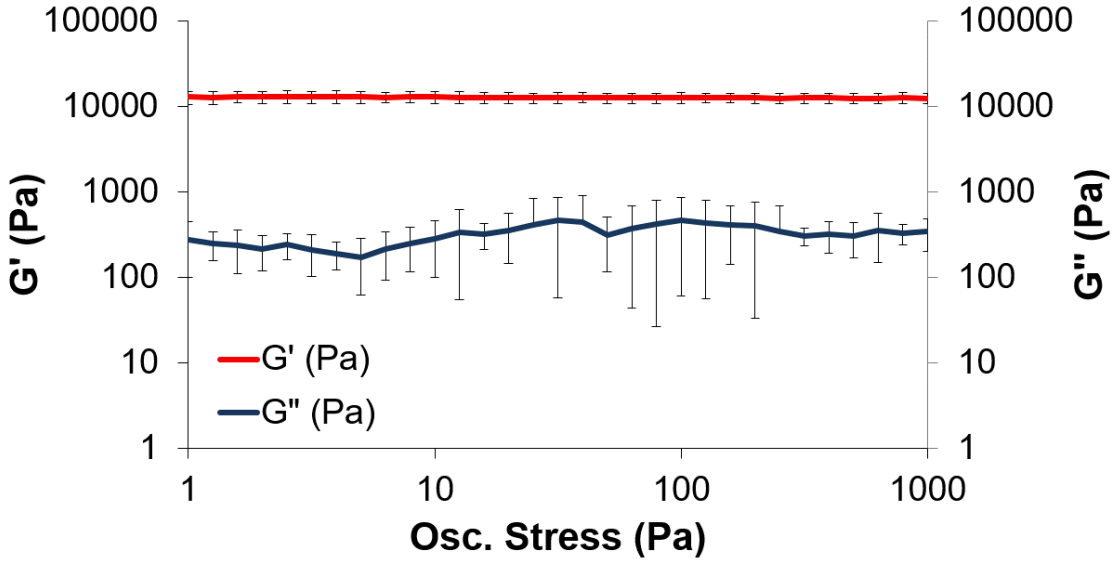


Figure S1. Oscillatory stress sweep of the hydrogel. Data are expressed as mean ± standard deviation (n = 5)

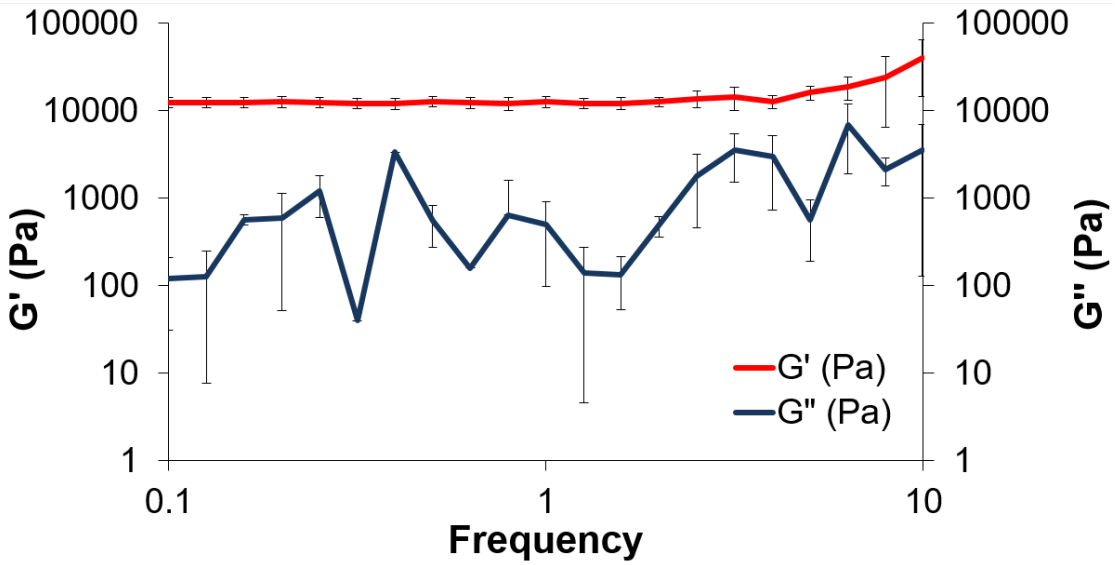


Figure S2. Frequency sweep of the hydrogel. Data are expressed as mean ± standard deviation (n = 5)

Equilibrium swelling degree. Cylindrical hydrogels (9 mm diameter, 3 mm thickness) were immersed in 6 mL of PBS (10 mM, pH 7.4) for 18 h. Equilibrium weights were measured using a milligram precision scale. The equilibrium conditions were obtained after three consecutive measurements and were averaged. After swelling for 18 h, the G' of the hydrogel was 430 Pa at frequency of 1 Hz and oscillatory stress of 50 Pa (40 wt%, n = 3). The equilibrium swelling degree (ESD) was calculated by dividing the difference in weight between hydrogel at equilibrium (W_{eq}) and hydrogel right after gelation (W_0) by its weight right after gelation (W_0).

$$ESD = \frac{(W_{eq} - W_0)}{W_0} \cdot 100\%$$

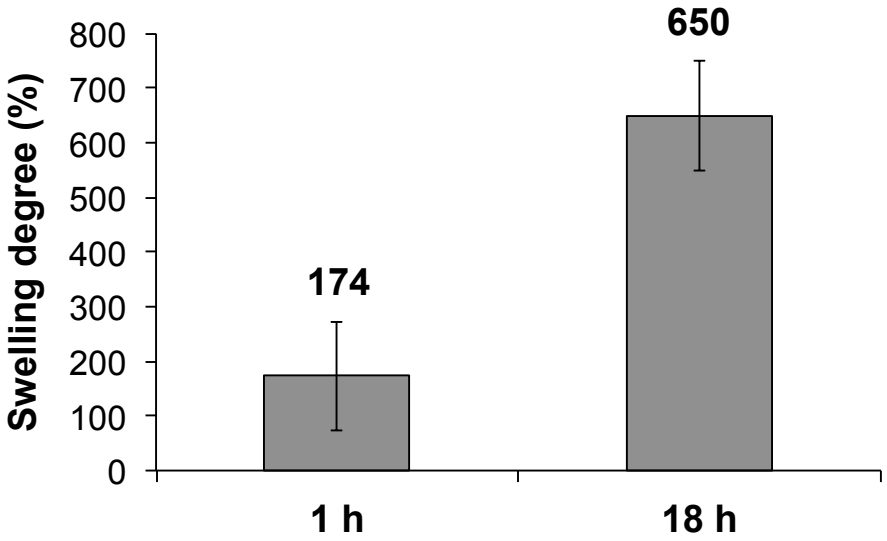


Figure S3. Equilibrium swelling degree of the hydrogel

After exposure to PBS buffer (10 mM, pH 7.4), hydrogel swells to 650% and reaches equilibrium after 18 h. Data are expressed as mean ± standard deviation (n = 3). Frequency sweep of the hydrogel was measured after 18 h of swelling at 50 Pa oscillatory stress using a 12 mm diameter steel plate.

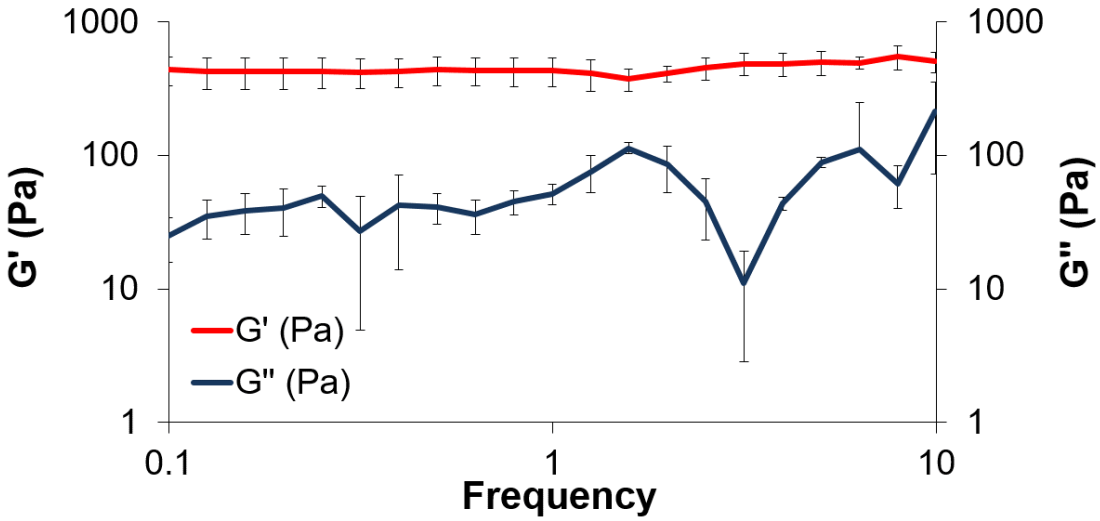


Figure S4. Frequency sweep of the hydrogel after swelling in PBS buffer (10 mM, pH 7.4) until equilibrium (18 h). Data are expressed as mean ± standard deviation (n = 3)

IV. *In vitro* cytotoxicity studies of the hydrogel

NIH3T3 cells, a murine fibroblast line was cultured in DMEM media supplemented with 10% fetal calf serum and penicillin/streptomycin (1%, 10,000 IU/mL penicillin; 10,000 mg/mL streptomycin) and grown at 37 °C with 5% CO_2. Hydrogel samples (n = 3) were left to gel for 30 min at room temperature in transwell inserts. Prior to cell exposure, the samples were soaked in PBS overnight. After 24 h exposure, cell viability was judged via the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3- carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay, with absorbance read at 490 nm. Exposure to the hydrogel did not result in a statistically lower viability ($P > 0.05$).

To assess *in vitro* cytotoxicity of the dissolution agent and dissolved hydrogel products, NIH3T3 murine fibroblast cells were cultured in DMEM supplemented with 10% fetal calf serum at 37 °C with 5% CO_2. Hydrogel samples (n = 5) were left to gel for 30 min at room temperature and were subsequently dissolved in low serum OPTI-MEM containing 50 mM CME. The NIH3T3 fibroblasts were treated with both media containing 50 mM CME with and without dissolved hydrogel sample at 37 °C for five min, the time it took for the hydrogel samples to fully dissolve in media. Cytotoxocity was then measured via a multiplexed, protease-based, fluorescence assay (Promega, Madison, WI). Treatment with CME only, and CME + hydrogel dissolution products resulted in 65.4 ± 5.4 ($P = 0.0010$) and 72.3 ± 4.6 ($P = 0.0024$) percent viability compared to a media-only control. There is no significant difference between the viability of the CME and CME + hydrogel dissolution products treatment groups ($P > 0.3$). The decrease in cell viability may reflect the limitations of this *in vitro* assay where cysteine and its analogs, including CME, act as metal chelators,[2,3,4] leading to cytotoxic effects.[5] CME further affords a hypertonic shock due to its high osmolarity[6] as the commercially available hydrochloride salt. In the clinic, the CME solution and the dissolution products would be irrigated with saline and these contributions would be minimized.

Additionally, CME is generally considered safe. The oral LD50 (mouse) of the compound is 2,300 mg/kg and intraperitoneal LD50 (mouse) is 1,340 mg/kg, as reported in the MSDS. In the United Kingdom, tablets containing CME are sold under the name Visclair or Mecysteine Hydrochloride 100 mg Gastro-resistant Tablets. This medication is indicated as an adjunct in the management of conditions such as chronic obstructive pulmonary disease, when characterized by thick viscid or glutinous mucus, including the symptomatic relief of cough with sputum. Moreover, CME has been used in shaving compositions (Patent 5,902,574, The Gillette Company) and products for hair waving (Patent 4,218,435, Yamanouchi Pharmaceutical Co.). In the literature, CME has been reported to protect cultured rodent lung tissue from sulfur mustard. It was assessed that CME is non-toxic to cultured rat lung slices at 5 mM after 30 min of treatment.[7]

V. Macrophage activation

RAW 264.7 cells, a murine macrophage line was cultured in DMEM media supplemented with 10% fetal bovine serum and penicillin/streptomycin (1%, 10,000 IU/mL penicillin; 10,000 mg/mL streptomycin) grown at 37 °C with 5% CO_2. The hydrogel samples (n = 3) were left to gel for 30 min at room temperature in transwell inserts. Prior to cell exposure they were soaked in PBS overnight. Macrophages were exposed to hydrogels for 24 h via a transwell insert, or 1 μg/mL LPS dissolved in the media. Media samples were taken after 24 h exposure and tested for IL-6, a cytokine secreted by macrophages indicating macrophage activation. Presence was quantified via an ELISA assay with absorbance read at 450 nm. LPS had a calorimetric signal, statistically higher than the hydrogel ($P < 0.01$) indicative of the presence of IL-6, whereas the hydrogel's signal was statistically indistinguishable ($P > 0.05$) to that of a media control. Therefore the lack of IL-6 presence demonstrates that the hydrogel does not activate macrophages.

VI. NMR spectra of the crosslinker

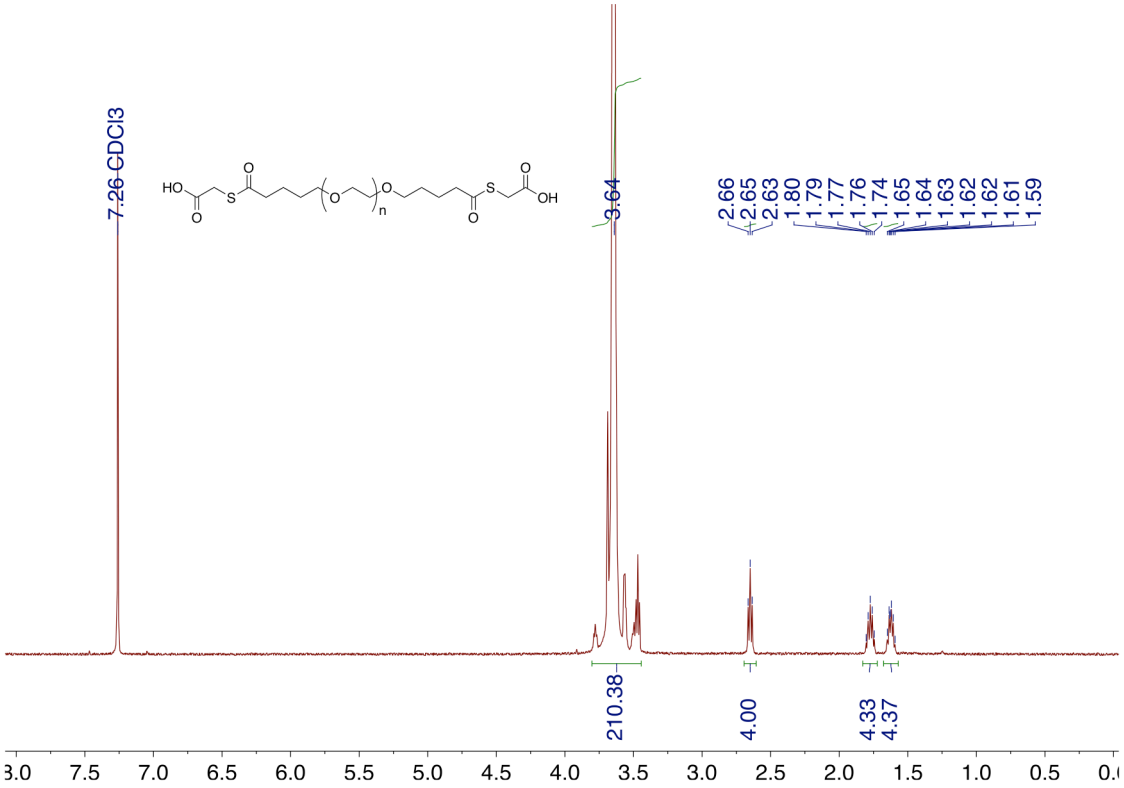


Figure S5. Representative ^{1}H NMR spectrum of intermediate A

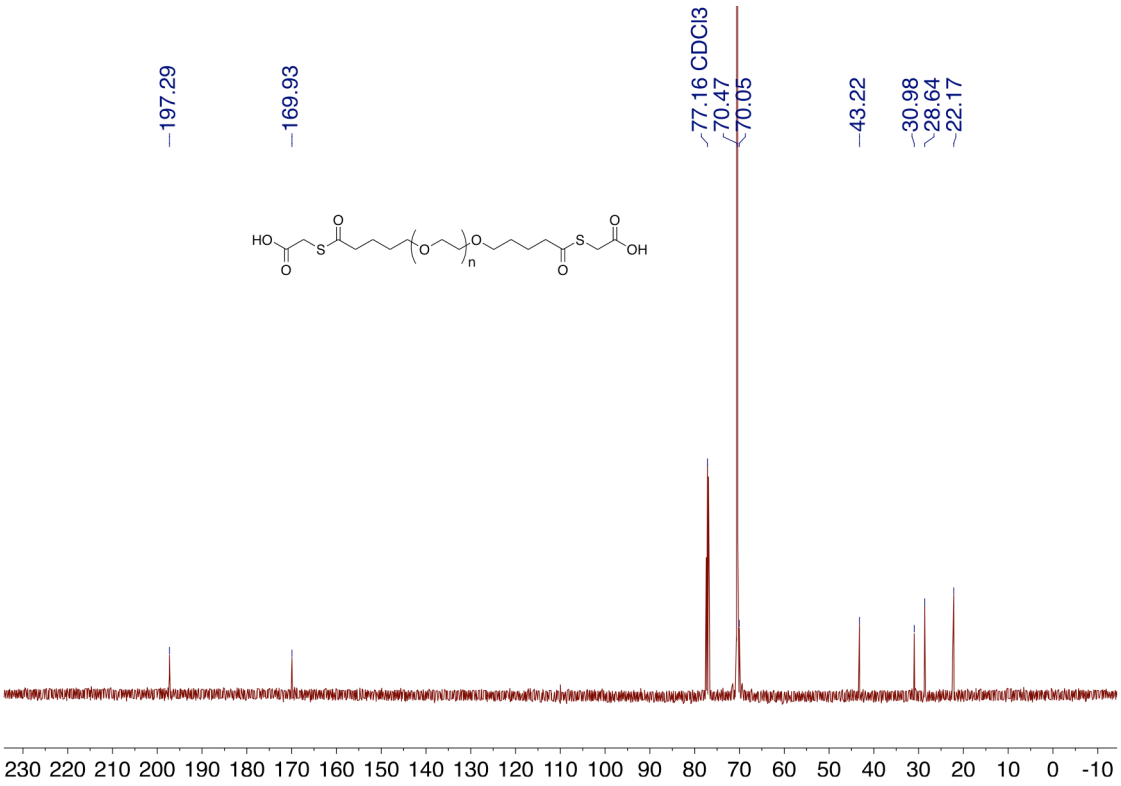


Figure S6. Representative ^{13}C NMR spectrum of intermediate A

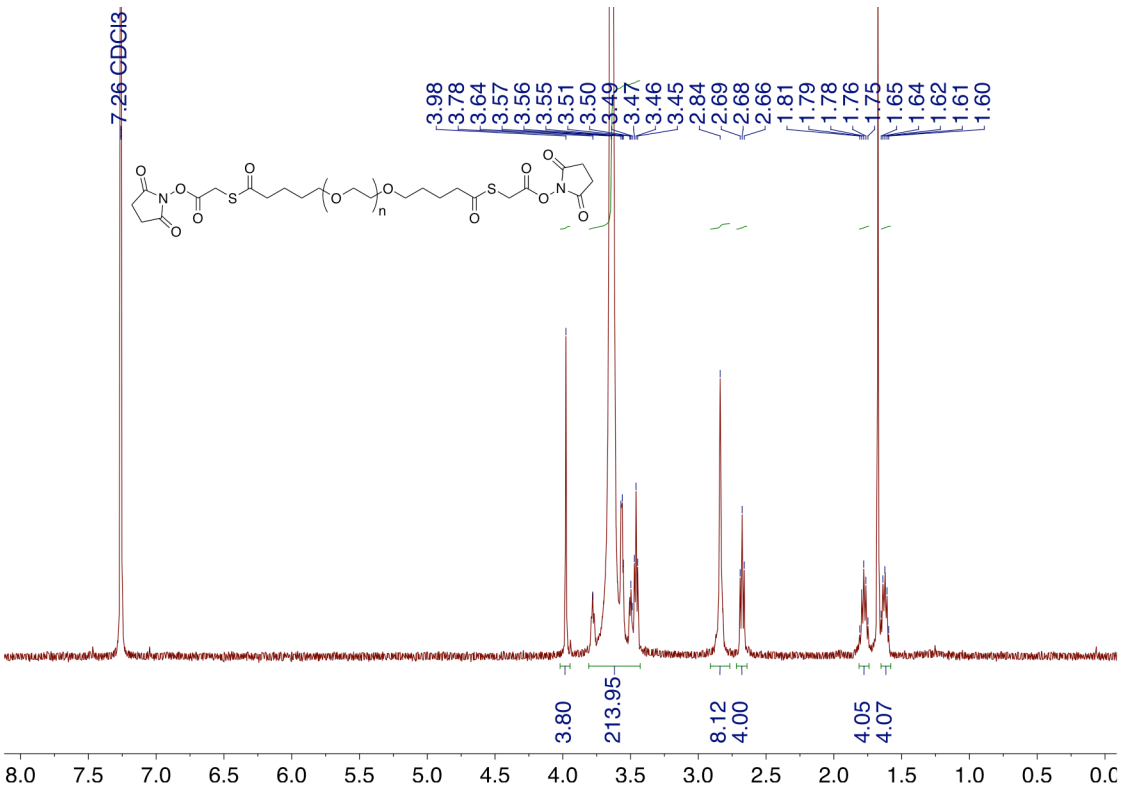


Figure S7. Representative ^{1}H NMR spectrum of the crosslinker

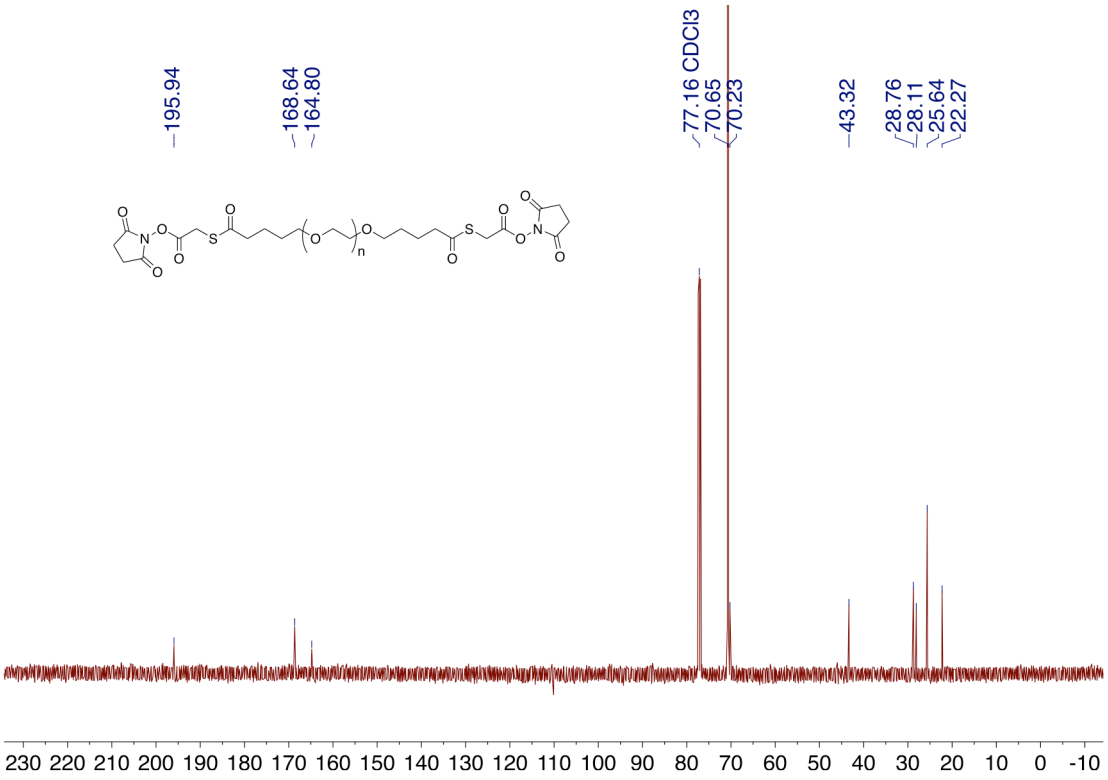


Figure S8. Representative ^{13}C NMR spectrum of crosslinker

VII. Evaluation of hydrogel's adhesive properties

The performed tests were based on the ASTM F2255-05 standard. The width of the tissue specimen was 2.5 ± 0.1 cm. The overlap area between two tissue specimens was 1.0 ± 0.1 cm. The thickness of the tissue specimens was 1.0-2.0 mm. The length of the tissue substrate attached to each specimen holder was 1.5 times the length of the overlap area in order to ensure that the failure occurs at the overlap bond and does not pull the tissue off the specimen holds. Ten specimens were tested.

Tissue preparation: The tissue substrate was kept moist at all times. The substrate was cut to the dimensions reported in ASTM F2255-05 standard using a template and a fresh scalpel blade.

Preparation of adhesive bond: 200 µL of hydrogel was used to uniformly coat the overlap area without overflow. The two test fixtures were bonded, taking care to keep the fixtures aligned to maintain the overlap. The hydrogel was allowed to set for 1 h and the samples were kept moist at 23 °C for 1 h.

Test procedure: The test specimens were placed in the grips of the testing machine so that the applied load coincides with the long axis of the specimen. The specimen was loaded to failure at a constant crosshead speed of 5 mm/min. The load at failure (maximum load sustained) was 0.62 ± 0.30 N and 100% cohesive failure was observed. The apparent shear strength is maximum load divided by the bond area and was calculated to be $2.48 \times 10^{-3} \pm 1.2 \times 10^{-3}$ MPa.

VIII. Design of *in vivo* animal experiments

General anesthesia was induced with 5% isoflurane in an induction chamber. Following induction, anesthesia was maintained with 2% isoflurane for the full length of the procedure. Sustained-release buprenorphine (1.2 mg/kg every 72 h) and meloxicam (1 mg/kg every 24 h) were administered subcutaneously at the time of anesthetic induction and at regular intervals thereafter for post-operative analgesia. Body temperature was maintained with a water-circulating heating pad. After confirming anesthetic plane, a 3.5 x 3.5 cm brass block, heated to 100 °C in an azeotropic boiling PEG:H_2O (80:20) solution, was placed on the shaved skin of the dorsum with 4 N of contact force for 10 sec. Fluid resuscitation with 0.5 mL of normal saline was administered subcutaneously. The burns covered approximately 5% of total body surface area (TBSA). This model results in a superficial partial-thickness burn with destruction of the epidermis and limited injury to the the superficial papillary dermis.[8]

Immediately after burn induction, the animals were divided into three groups: 1) burn only (negative control, n = 10), 2) burn + bacterial contamination (postitive control, n = 10), or 3) burn + hydrogel + bacterial contamination (intervention group, n = 10). Bacterial contamination in the positive control and intervention groups was achieved by covering the burn wound with a 2 × 2 cm gauze containing $2x10^8$ CFU (colony forming units) of log-phase *Pseudomonas aeruginosa* (Strain PAO1, ATCC 47085).[9] Injury was covered with Tegaderm (3M Health Care, St. Paul, MN). The infecting bacterial inoculum was prepared in brain heart infusion broth (BHI; Becton-Dickinson) at 37 °C for 5-6 h. The cells were harvested (optical density of 0.8 at 620 nm), centrifuged and resuspended in sterile BHI. The number of infecting bacteria was verified by plating serial dilutions of the injected inocula onto BHI agar plates.

The rats were euthanized 72 h after burn induction and bacterial counts were taken from the burn wound (as a measure of local proliferation) and from the spleen (as a measure of systemic dissemination).[10] Samples from each animal's burn wound and spleen were harvested with sterile technique, weighed, homogenized, and resuspended in sterile saline solution. Bacterial counts of *P. aeruginosa* per gram of tissue (burn wound or spleen) were determined by serial dilutions of the sample and by colony counting on the BHI agar plates after an overnight incubation. Samples that did not exhibit growth were assigned a value of half the minimum detection limit of the assay ($1x10^2$ CFU/g).

IX. Statistical analysis

Due to the small sample size in the *in vivo* burn study, we could not accept the normal distribution assumption for burn wound and spleen bacterial cultures and therefore utilized a Kruskal-Wallis test to compare bacterial counts across groups with *post hoc* Wilcoxon rank-sum tests and Bonferroni correction for mutiple comparisons. Values are reported as means ± standard error the mean (SEM). Significance was set at a two-sided *P* value of 0.05. Analyses were performed with Stata (Stata/IC 14.0, StataCorp LP, College Station, TX, USA).

Table 1. *Post hoc* comparisons of Pseudomonal burden in eschars		
	Negative Controls	Positive controls
Positive Controls	0.0001*	-
Hydrogel-treated	0.317	0.0003*

The * denotes statistical significance as these *P* values are less than the Bonferroni corrected *P* value of 0.017.

Table 2. *Post hoc* comparisons of systemic Pseudomonal burden		
	Negative Controls	Positive controls
Positive Controls	0.013*	-
Hydrogel-treated	0.999	0.013*

The * denotes statistical significance as these *P* values are less than the Bonferroni corrected *P* value of 0.017.

Table 3. Prevalence of detectable Pseudomonal infection by group			
	Negative controls	Positive controls	Hydrogel-treated
Local infection	0/10	9/10	1/10
Systemic infection	0/10	5/10	0/10

X. References

1. C. Ghobril, K. Charoen, E. K. Rodriguez, A. Nazarian, M. W. Grinstaff, *Angew. Chem. Int. Edit.* **2013**, *52*, 14070.
2. D. H. Hamer, *Annu. Rev. Biochem.* **1986**, *55*, 913.
3. E. Grill, E.-L. Winnacker, H. Meinhart, *Science* **1985**, *230*, 674.
4. B. R. White, B. T. Stackhouse, J. A. Holcombe, *J. Hazard. Mater.* **2009**, *161*, 848.
5. S. Hugenschmidt, F. Planas-Bohne, D. M. Taylor, *Arch. Toxicol.* **1993**, *67*, 76.
6. M. B. Friis, C. R. Friborg, L. Schneider, M.-B. Nielsen, I. H. Lambert, S. T. Christensen, E. K. Hoffmann, *J. Physiol.* **2005**, *567*, 427.
7. P. E. Wilde, D. G. Upshall, *Hum. Exp. Toxicol.* **1994**, *13*, 743.
8. H. Borman, T. Maral, B. Demirhan, M. Haberal, *Ann. Plast. Surg.* **1999**, *43*, 513.
9. L. Steinstraesser, O. Burkhard, M. H. Fan, F. Jacobsen, M. Lehnhardt, G. Su, A. Daigeler, H. U. Steinau, D. Remick, S. C. Wang*, BMC Surg* **2005**, *5*, 19.
10. Y. Barnea, Y. Carmeli, B. Kuzmenko, E. Gur, O. Hammer-Munz, S. Navon-Venezia, *Ann. Plast. Surg.* **2006***, 56*, 674.