Electronic Supplementary Material (ESI) for Biomaterials Science.

In Situ Gelling and Dissolvable Hydrogels for Use as On-Demand Wound Dressings for Burns

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Supplemental Information:

I. Synthesis and characterization of compounds

PEG Diacid. The synthesis of the PEG diacid compound was based on a previously reported protocol.[14] ^{1}H and ^{13}C NMR spectra were similar to the literature.[14]

^{1}H NMR (500MHz), $CDCl_3$: δ 1.93 (q, *J* = 7.21 Hz, 4H), 2.4 (tt, *J* = 7.21, 8H), 3.62 (m, 292H), 4.22 (tt, *J* = 4.73 Hz, 4H) ppm; **^{13}C NMR (500 MHz), $CDCl_3$:** 175.3, 172.8, 70.6, 68.9, 63.4, 33.1, 32.6, 19.9 ppm.

Compound 1. The synthesis of the starting material was based on a previously reported protocol.[14] ^{1}H and ^{13}C NMR spectra were similar to the literature.[14]

^{1}H NMR (500MHz), $CDCl_3$: δ 4.15 (tt, *J* = 3.3, 1.5, 4H), 3.54 (m, 296H), 2.8 (b, 8H), 2.6 (t, *J* = 7.3, 4H), 2.4 (t, *J* = 7.3, 4H), 2.0 (q, *J* = 7.3, 4H) ppm; **^{13}C NMR (500 MHz), $CDCl_3$:** 172.3, 169.0, 168.0, 70.5, 69.0, 63.6, 32.4, 29.9, 25.5, 19.7 ppm

Compound 2. The synthesis of compound **2** was based off of a previously reported protocol.[13,16]

^{1}H NMR (500MHz), $CDCl_3$: δ 4.21 (m, *J* = 4.6, 4.9, 4H), 3.62 (m, 296H), 2.68 (t, *J* = 7.3, 4H), 2.40 (t, *J* = 7.2, 4H), 1.98 (t, *J* = 7.2, 4H) ppm; **^{13}C NMR (500 MHz), $CDCl_3$:** 196.8, 172.6, 169.8, 70.6, 69.0, 63.6, 42.3, 32.8, 31.0, 20.5 ppm;

Compound 3. In a flame dried flask, 1,8-Diazabicyclo(5.4.0)undec-7-ene (265μL) and 6-mercaptohexanoic acid (122μL) were added to a solution of **1** (1g) in anhydrous DMF (5mL). The solution was stirred at room temperature for 16 hours. The organic phase was extracted with a 1M HCl solution, water, and brine. The organic phase was dried over sodium sulfate, filtered, and precipitated in diethyl ether. The precipitate was filtered and dried under vacuum to afford compound **3** as a white solid (96% yield).

^{1}H NMR (500MHz), $CDCl_3$: δ 4.22 (t, *J* = 4.8, 4H), 3.63 (m, 308H), 2.86 (t, *J* = 7.2, 4H), 2.61 (t, *J* = 7.3, 4H), 2.38 (t, *J* = 7.4, 4H), 2.30 (t, *J* = 7.4, 4H), 1.97 (t, *J* = 7.3, 4H), 1.60 (m, 8H), 1.39 (m, 4H), ppm; **^{13}C NMR (500 MHz), $CDCl_3$:** 198.6, 176.1, 172.7, 70.7, 69.0, 42.8, 33.5, 32.9, 29.2, 28.5, 28.1, 24.2, 20.6 ppm;

Compound 4. Synthesis of compound 4 follows the above procedure using 11-mercaptoundecanoic acid (0.190g) as the thiol source (92% yield).

^{1}H NMR (500MHz), $CDCl_3$: δ 4.22 (t, *J* = 4.9, 4H), 2.85 (t, *J* = 7.4, 7.3, 4H), 2.60 (t, *J* = 7.3, 4H), 2.38 (t, *J* = 7.3, 4H), 2.30 (t, *J* = 7.5, 4H), 1.97 (t, *J* = 7.3, 4H), 1.60 (m, 8H), 1.39 (m, 24H) ppm; **^{13}C NMR (500 MHz), $CDCl_3$:** 198.7, 176.5, 172.7, 70.5, 69.0, 63.5, 33.8, 32.9, 29.4, 29.3, 29.2, 29.1, 29.0, 28.95, 28.8, 28.7, 24.7, 20.6 ppm;

Compound 5, 6 and 7. The synthesis of compounds 5, 6 and 7 are based off of a previously reported protocol (yield 96-98%).[13,16] The HNMR and CNMR spectra are similar to those previously reported.

Compound 5:

^{1}H NMR (500MHz), **$CDCl_3$:** δ 4.16 (t, *J* = 4.3, 4H), 3.92 (s, 4H), 3.57 (m, 257H), 2.78 (b, 8H), 2.67 (t, *J* = 7.3, 4H), 2.34 (t, *J* = 7.3, 4H), 1.95 (q, *J* = 7.3, 4H) ppm;

^{13}C NMR (500 MHz), $CDCl_3$: δ ppm;

MALDI-TOF (pos): M_w: 3763 m/z

GPC: M_n: 5077; **M_w:** 5312; **PDI:** 1.05;

Mp (DSC): 46.06°C

Compound 6:

^{1}H NMR (500MHz), $CDCl_3$: δ 4.21 (tt, *J* = 1.5, 3.4, 4H), 3.63 (m, 290H), 2.86 (t, *J* = 7.3, 4H), 2.81 (b, 8H), 2.60 (tt, *J* = 2.5, 4.9, 8H), 2.37 (t, *J* = 7.3, 4H), 1.96 (q, *J* = 7.3, 7.4, 4H), 1.74 (q, *J* = 7.4, 7.7, 4H), 1.59 (m, 4H), 1.46 (m, 4H) ppm;
^{13}C NMR (500 MHz), $CDCl_3$: δ 198.6, 172.7, 169.1, 168.4, 70.5, 69.1, 63.6, 42.9, 33.0, 29.1, 28.4, 27.8, 25.6, 24.1, 20.6 ppm;
MALDI-TOF (pos): M_w: 3807 m/z
GPC: M_n: 4999; **M_w:** 5196; **PDI:** 1.04;
Mp (DSC): 45.80°C

Compound 7:
^{1}H NMR (500MHz), $CDCl_3$: δ 4.22 (m, 4H), 3.62 (m, 278H), 2.85 (m, 8H), 2.70 (t, *J* = 7.2, 7.3, 2H), 2.60 (tt, *J* = 7.3, 4H), 2.45 (t, *J* = 7.2, 7.4, 4H), 2.37 (t, *J* = 7.2, 7.3, 4H), 2.04 (q, *J* = 7.2, 7.4, 4H), 1.95 (m, 4H), 1.71 (m, 2H), 1.52 (m, 4H), 1.25 (m, 10H) ppm;
^{13}C NMR (500 MHz), $CDCl_3$: δ 198.8, 172.7, 169.2, 168.6, 70.5, 69.0, 63.5, 42.8, 32.9, 30.9, 29.5, 29.3, 29.2, 29.0, 28.8, 28.7, 25.6, 24.5, 20.6 ppm;
MALDI-TOF (pos): M_w: 4210 m/z
GPC: M_n: 6038; **M_w:** 6313; **PDI:** 1.05;
Mp (DSC): 47.42°C

I. NMR spectra of crosslinkers

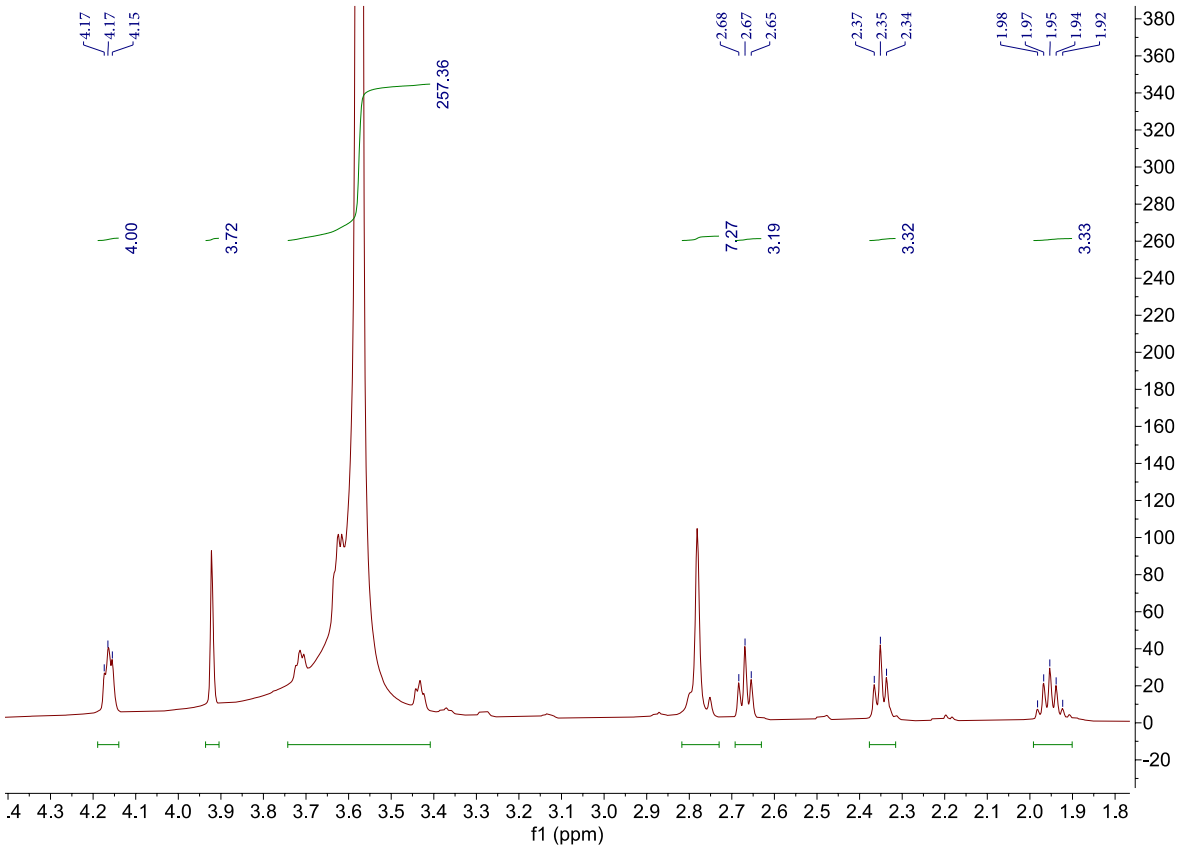

Figure S1. Representative ^{1}H NMR spectrum of crosslinker **5**

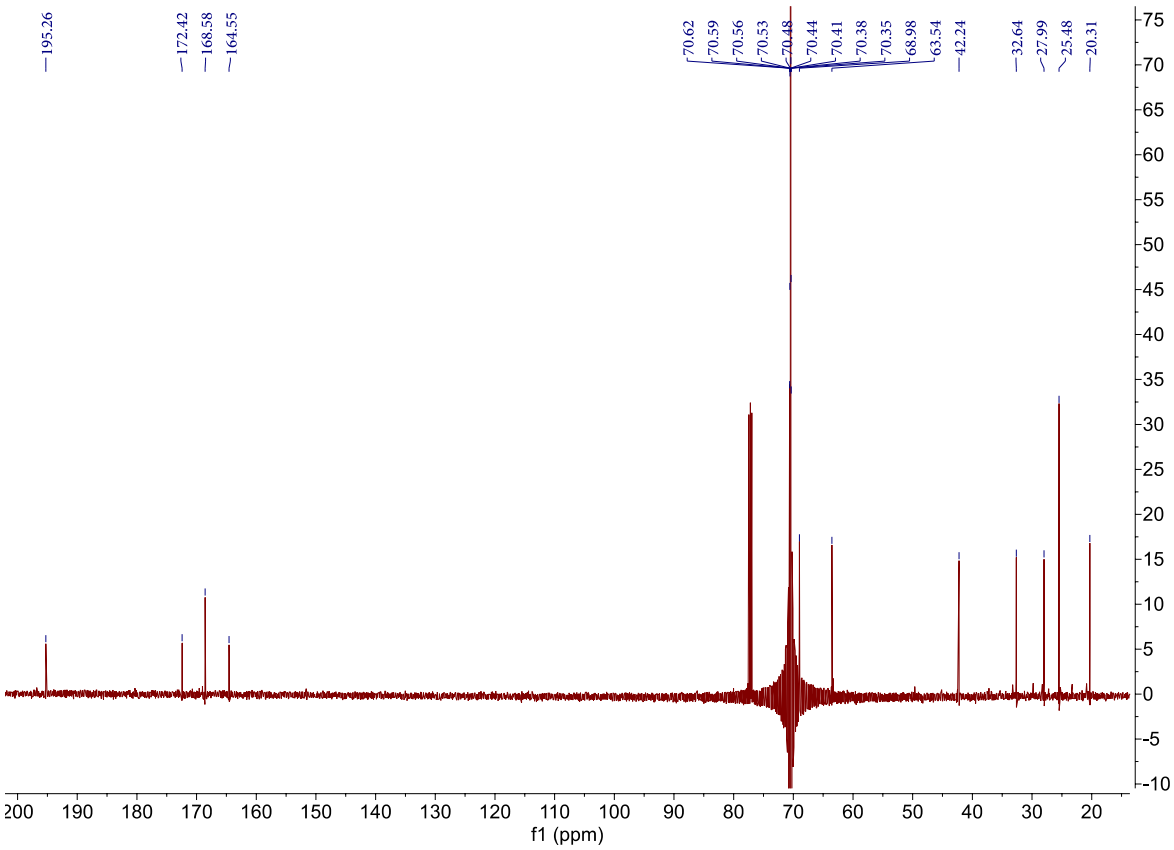

Figure S2. Representative ^{13}C NMR spectrum of crosslinker **5**

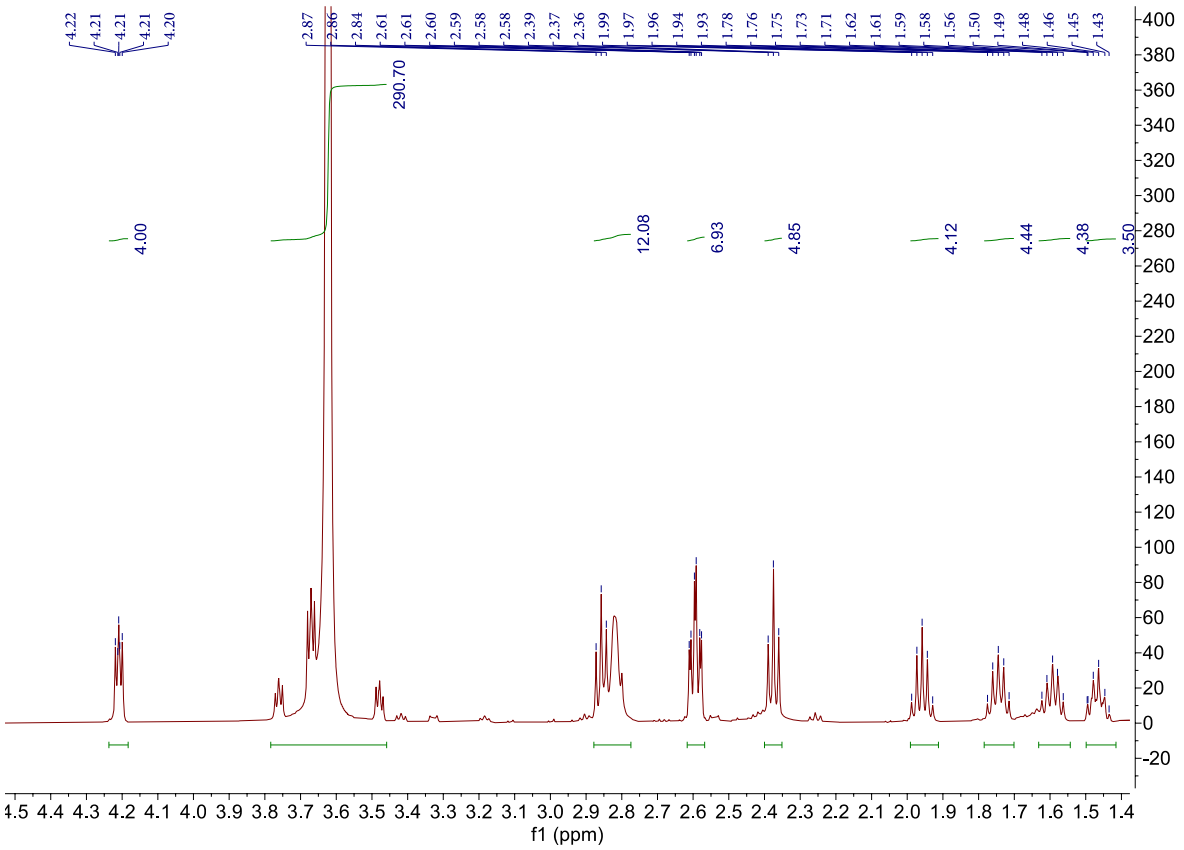

Figure S3. Representative ^{1}H NMR spectrum of crosslinker **6**

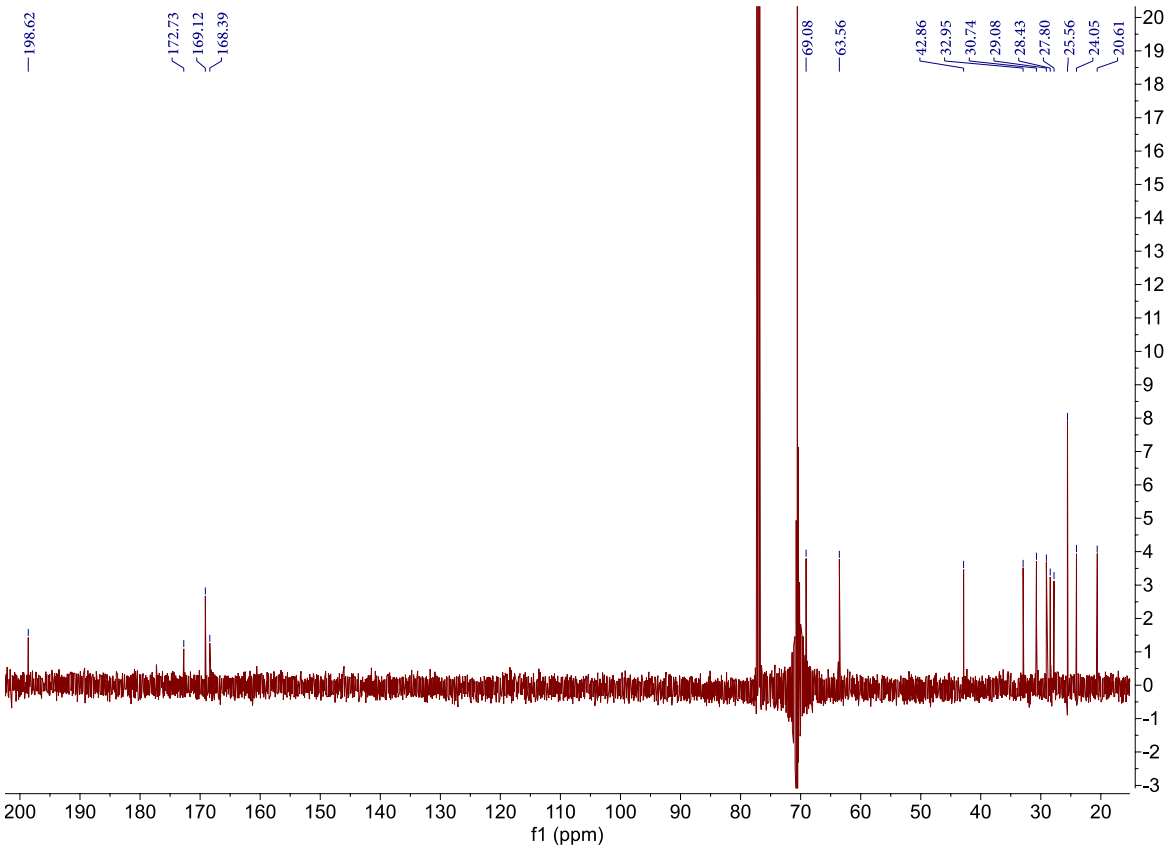

Figure S4. Representative ^{13}C NMR spectrum of crosslinker **6**

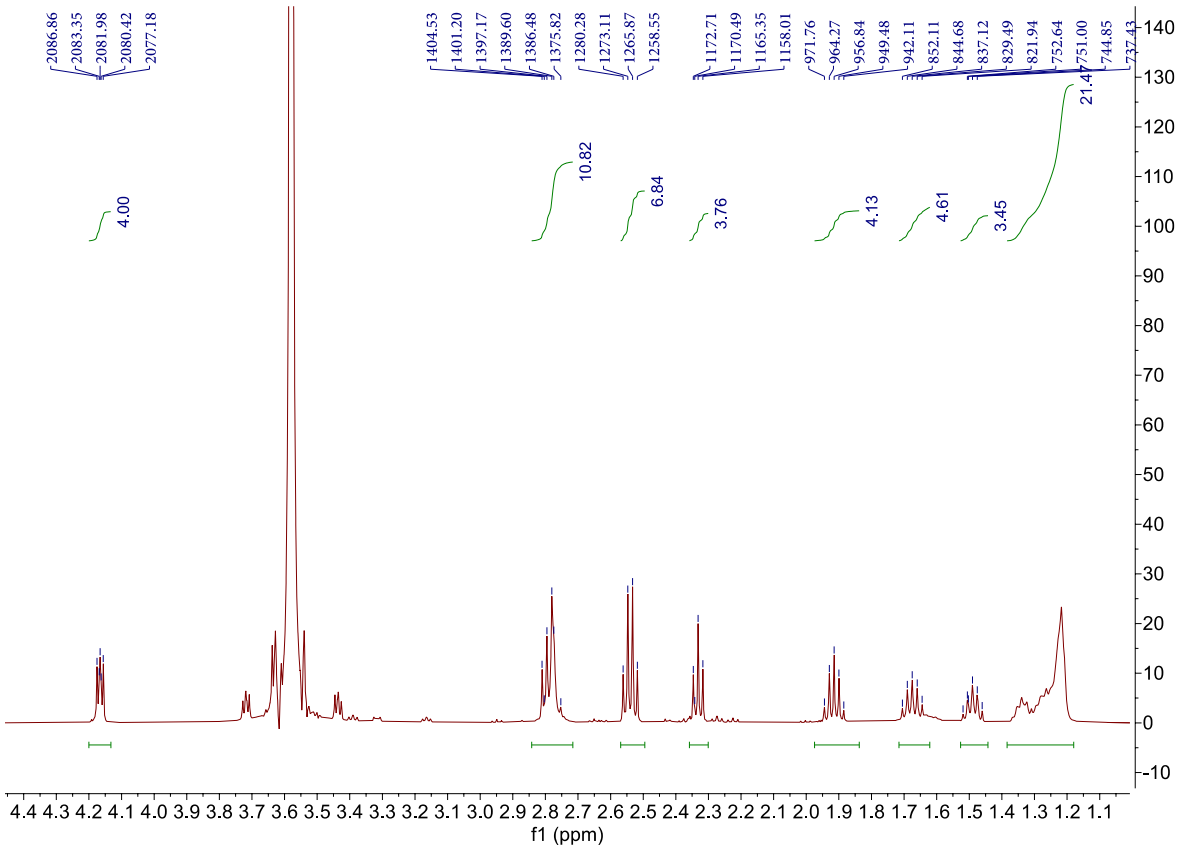

Figure S5. Representative ^{1}H NMR spectrum of crosslinker **7**

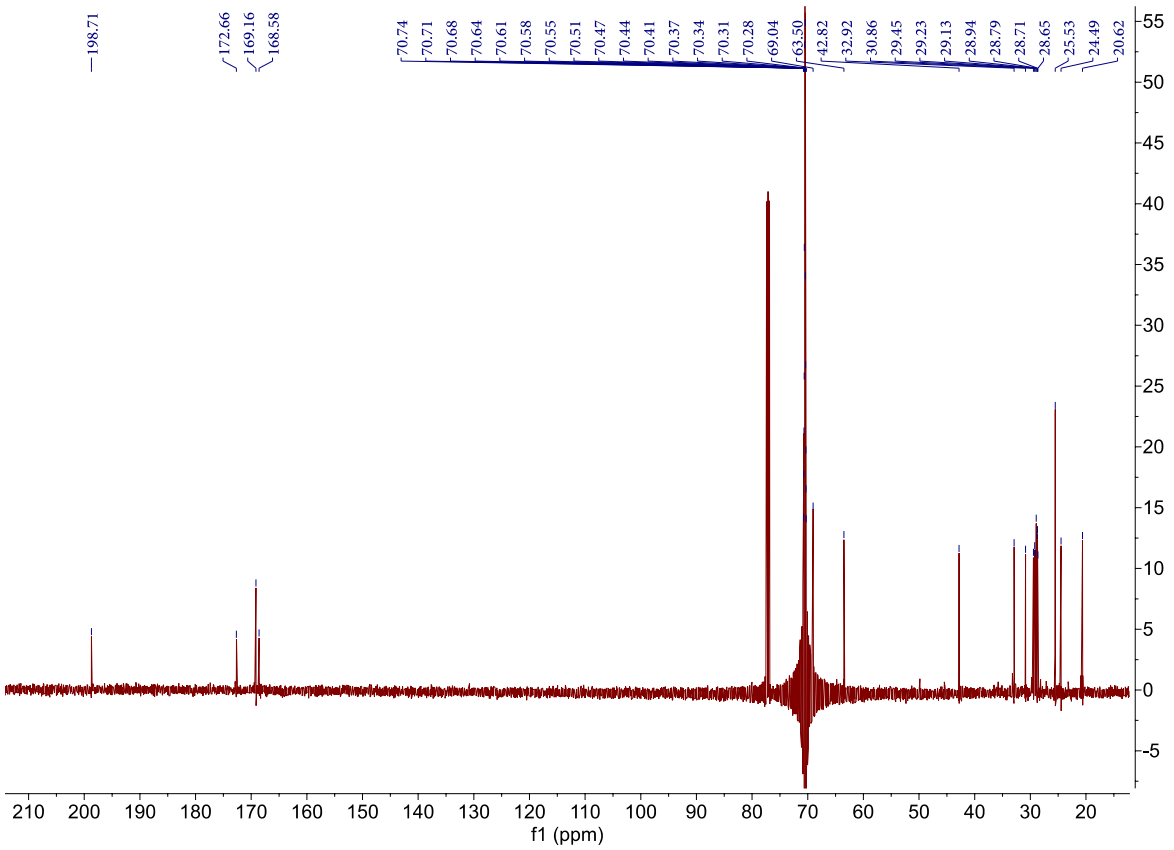

Figure S6. Representative ^{13}C NMR spectrum of crosslinker **7**

II. MALDI spectra

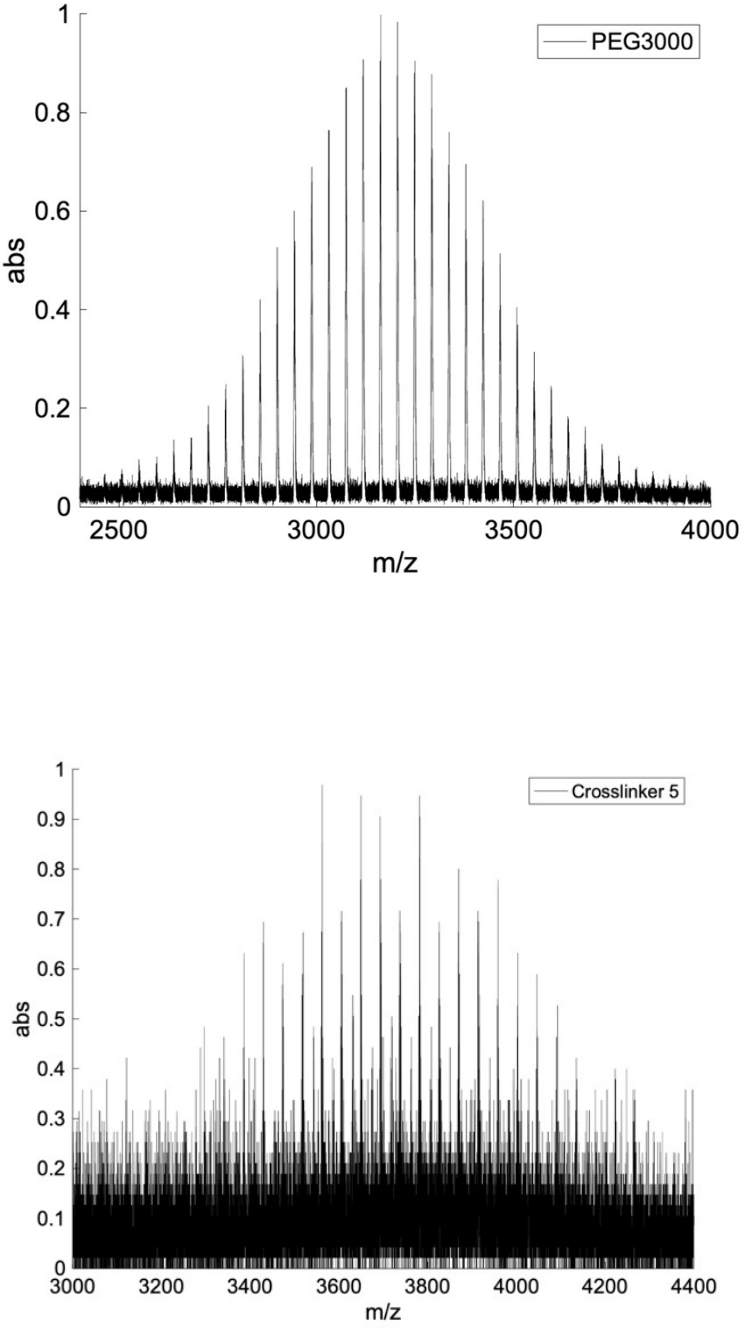

Figure S7. Representative MALDI spectrum of PEG starting material, 3000Da (top) crosslinker **5** (bottom)

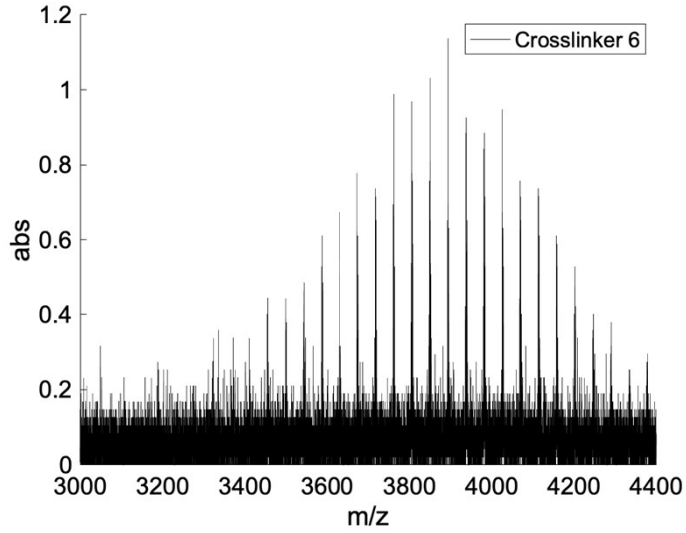

Figure S8. Representative MALDI spectrum of crosslinker **6**

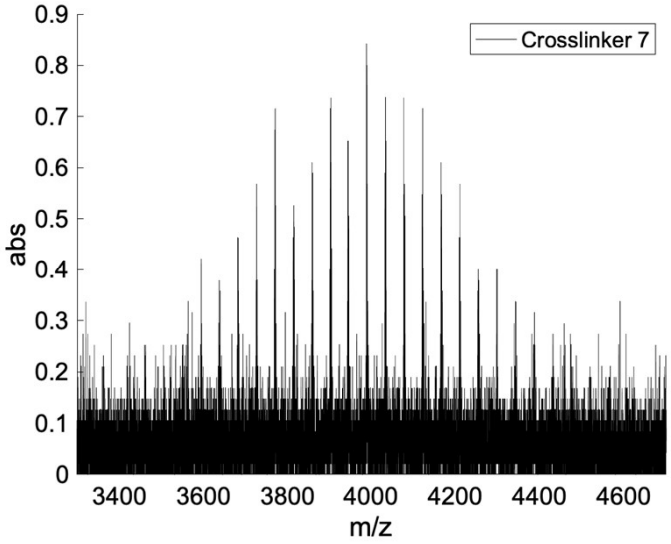

Figure S9. Representative MALDI spectrum of crosslinker **7**

III. Kinetics data

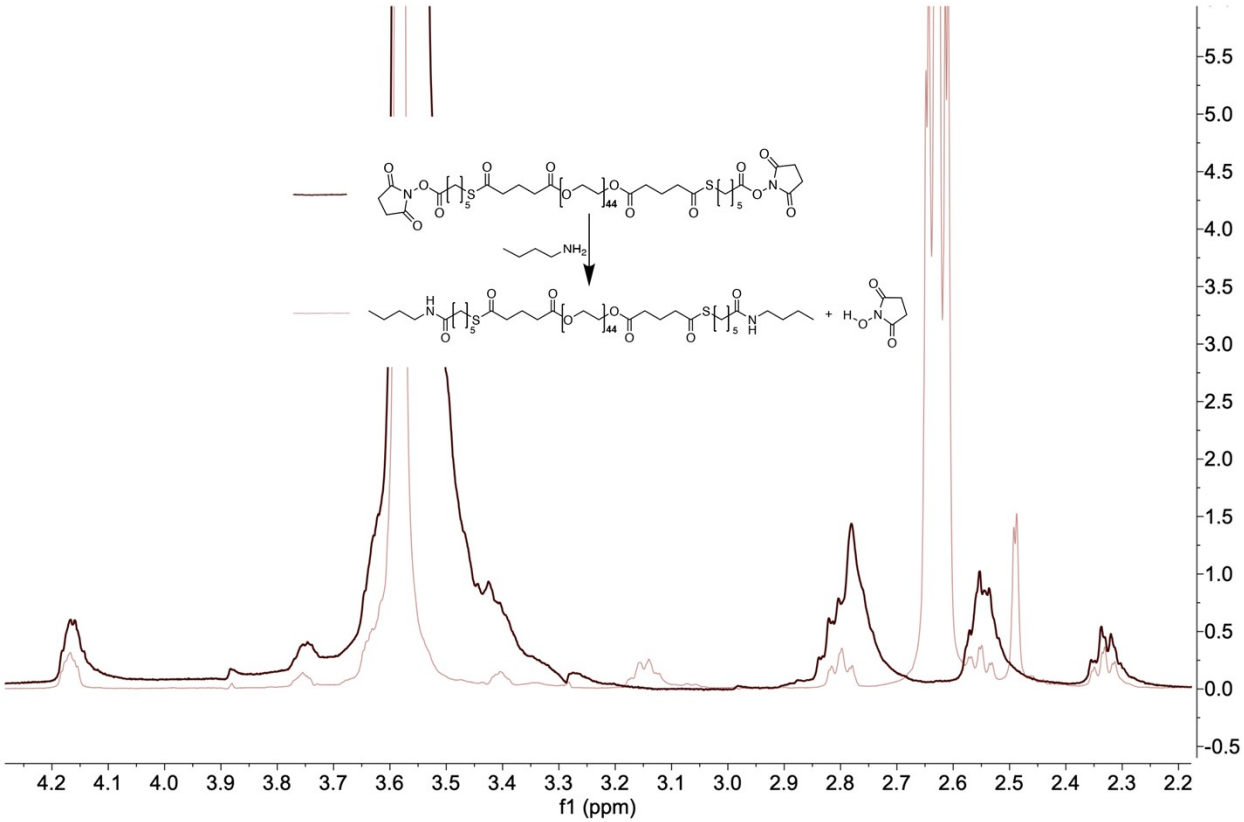

Figure S10. Representative ^{1}H NMR spectrum of crosslinker **6** before (red bold) and after (red narrow) reaction with PEI mimetic, *N*-butylamine. We observe a shift in the NHS peak that is conjugated to crosslinker **6** at 2.78ppm (red bold) to 2.49ppm (red) after the NHS ester is cleaved from crosslinker **6** when reacted with *N*-butylamine.

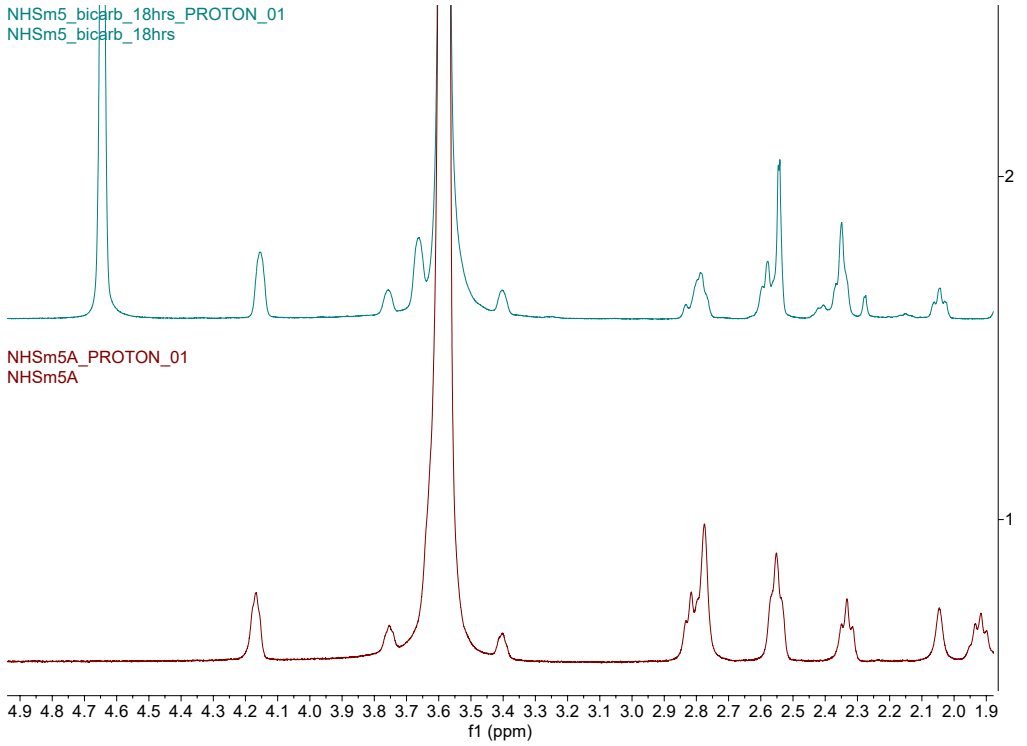

Figure S11. Representative ^{1}H NMR spectrum of intact crosslinker **6** (bottom) (NHS at 2.78ppm), and NHS-hydrolyzed (2.54ppm) crosslinker **6** in 0.3M sodium bicarbonate buffer, pH 8.0 (top).

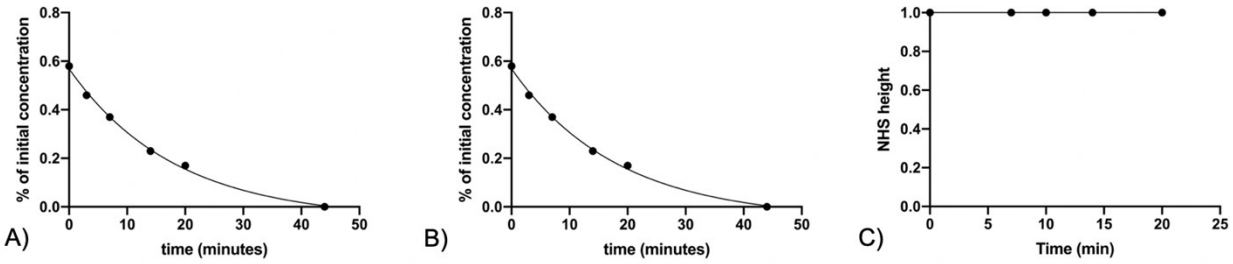

Figure S12. Rate order of A) thioester hydrolysis in crosslinker **5** in 0.3M Borate buffer, pH 8.0, B) thioester hydrolysis in crosslinker **6** in 0.3M Borate buffer, pH 8.0, C) **NHS** ester stability in 0.1M phosphate buffer pH 6.5.

IV. Rheology, swelling, and dissolution of hydrogels

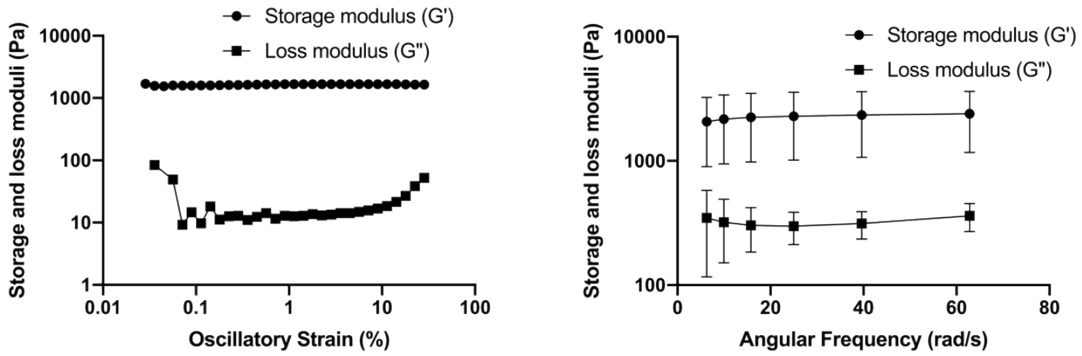

Figure S13. Strain sweep (left) and frequency sweep (right) of hydrogel **6**.

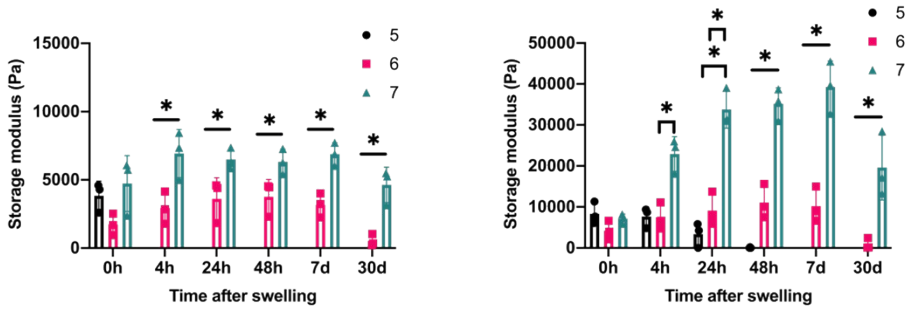

Figure S14. Storage modulus of hydrogels composed of crosslinkers **5, 6,** and **7** and 10 wt% (left) and 20 wt% (right)

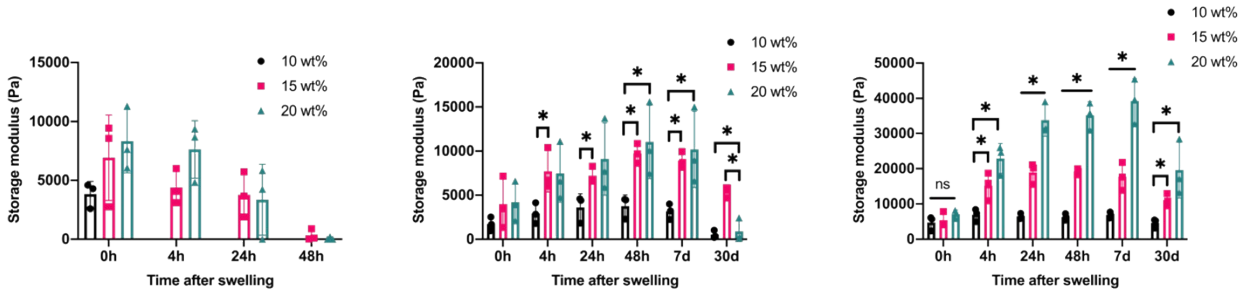

Figure S15. Storage modulus for crosslinkers **5** (left), **6** (middle), and **7** (right) at 10, 15, and 20 wt% over 30 days of swelling or until dissolution.

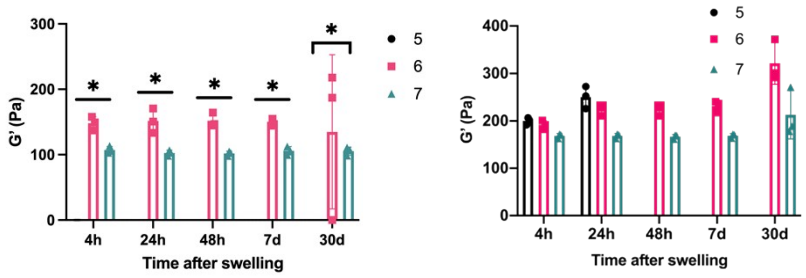

Figure S16. Swelling of hydrogels at 10 wt% (left) and 20 wt% (right).

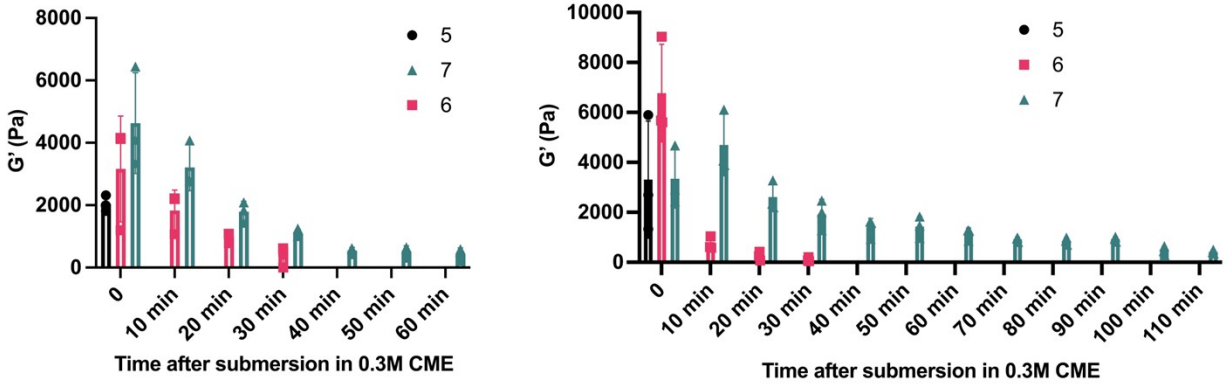

Figure S17. Dissolution of hydrogel formulations with crosslinkers **5, 6,** and **7** at 10 wt% (left) and 20 wt% (right) upon submersion in 0.3M CME solution, pH 8.6.

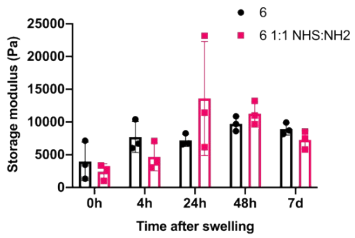

Figure S18. Rheological measurements on hydrogels from crosslinker **6** with 2:1 (black) or 1:1 (pink) NHS:NH_2 mole ratio.

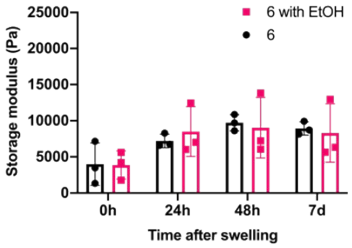

Figure S19. Rheological measurements of hydrogels made of crosslinker **6** with and without EtOH.

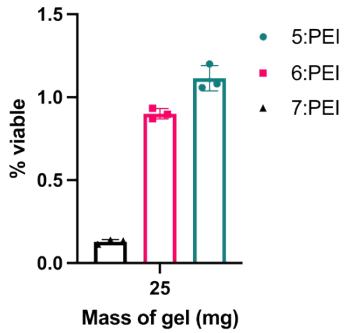

Figure S20. Cell viability of hydrogel formulations **5**:PEI, **6**:PEI and **7**:PEI against NIH3T3 fibroblasts. The cells were purchased from ACTT.

V. *In vivo* porcine study

Parameter	Ionic Hydrogel Dissolving (n=3)		Gauze Sponge (Sterile) (n=3)		No Material Used (n=3)	
Inflammation	1.00 ± 0.00 1.00	100%	1.00 ± 0.00 1.00	100%	1.33 ± 0.58 1.00	100%
Neutrophils	0.33 ± 0.58 0.00	33%	0.00 ± 0.00 0.00	0%	1.00 ± 1.00 1.00	67%
Histiocytes	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%
Lymphocytes	1.00 ± 0.00 1.00	100%	1.00 ± 0.00 1.00	100%	1.00 ± 0.00 1.00	100%
Multinucleated Giant Cells	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%
Plasma Cells	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%	0.00 ± 0.00 0.00	0%
Eosinophils	1.00 ± 0.00 1.00	100%	1.00 ± 0.00 1.00	100%	1.00 ± 0.00 1.00	100%

Table S1. Mean ± SD, median and incidence of inflammation and inflammatory cell types. Day 3, Group 1, no dressing changes.

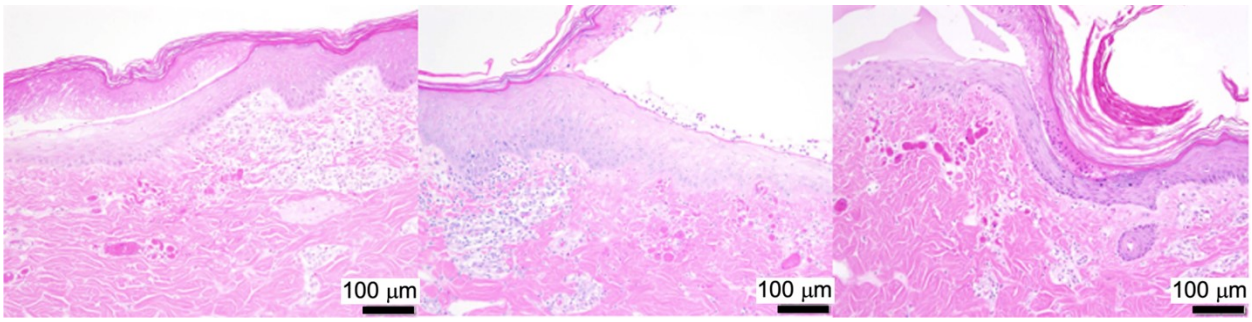

Figure S21. H&E of Group 1 for gauze (left), no dressing (middle), and hydrogel dressing (right).

Parameter	Ionic Hydrogel Dissolving (n=3)		Gauze Sponge (Sterile) (n=3)		No Material Used (n=3)	
Inflammation	1.33 ± 0.58	100%	1.33 ± 0.58	100%	1.33 ± 0.58	100%
	1.00		1.00		1.00	
Neutrophils	1.33 ± 0.58	100%	1.33 ± 0.58	100%	1.33 ± 0.58	100%
	1.00		1.00		1.00	
Histiocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Lymphocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Multinucleated Giant Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Plasma Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Eosinophils	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	

Table S2. Mean ± SD, median and incidence of inflammation and inflammatory cell types. Day 7, Group 3, no dressing changes.

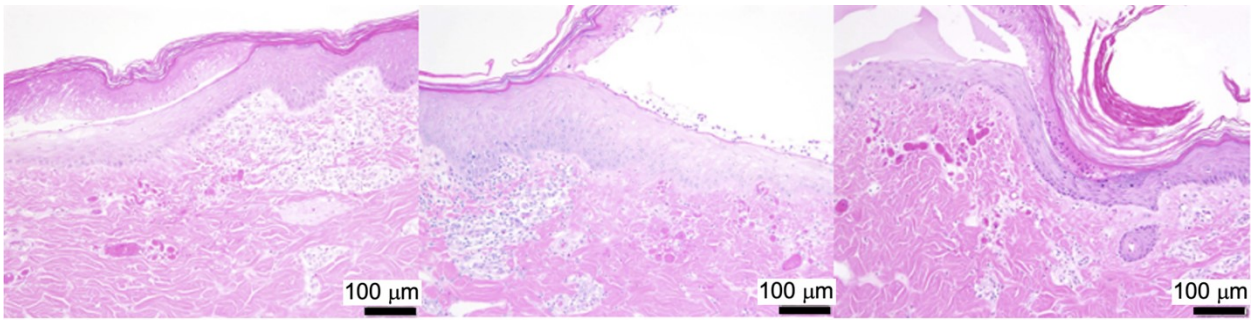

Figure S22. H&E of Group 1 for gauze (left), no dressing (middle), and hydrogel dressing (right).

Parameter	Ionic Hydrogel Dissolving (n=3)		Gauze Sponge (Sterile) (n=3)		No Material Used (n=3)	
Inflammation	1.67 ± 0.58	100%	1.67 ± 0.58	100%	2.00 ± 0.00	100%
	2.00		2.00		2.00	
Neutrophils	1.33 ± 0.58	100%	1.33 ± 0.58	100%	2.00 ± 0.00	100%
	1.00		1.00		2.00	
Histiocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Lymphocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Multinucleated Giant Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Plasma Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Eosinophils	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	

Table S3. Mean ± SD, median and incidence of inflammation and inflammatory cell types. Day 7, Group 2, 1 dressing changes.

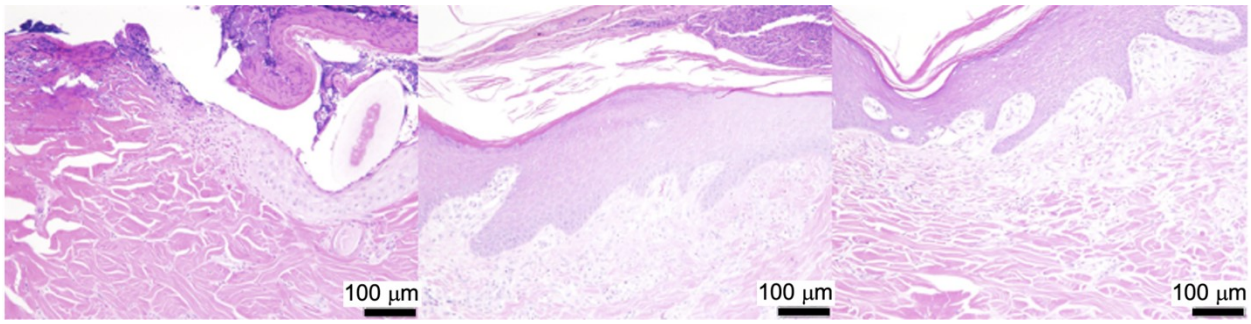

Figure S23. H&E of Group 2 for gauze (left), no dressing (middle), and hydrogel dressing (right).

Parameter	Ionic Hydrogel Dissolving (n=3)		Gauze Sponge (Sterile) (n=3)		No Material Used (n=3)	
Inflammation	1.33 ± 0.58	100%	2.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		2.00		1.00	
Neutrophils	1.33 ± 0.58	100%	2.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		2.00		1.00	
Histiocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Lymphocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Multinucleated Giant Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Plasma Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Eosinophils	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	

Table S4. Mean ± SD, median and incidence of inflammation and inflammatory cell types. Day 14, Group 4, 1 dressing changes.

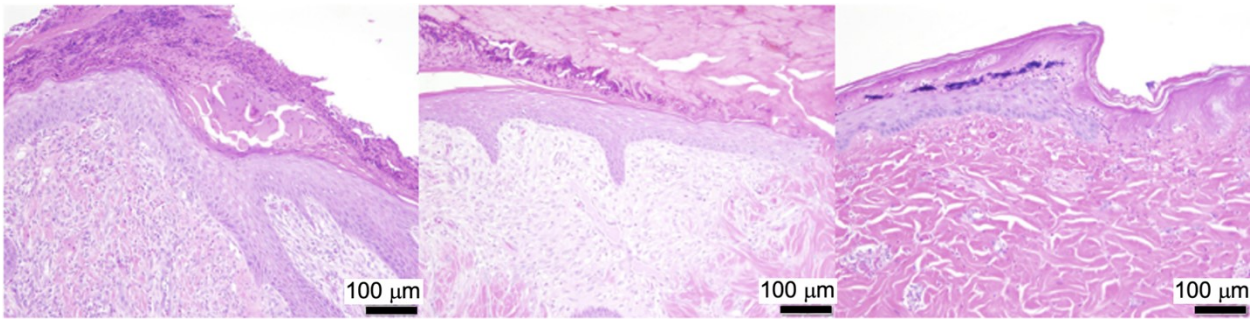

Figure S24. H&E of Group 4 for gauze (left), no dressing (middle), and hydrogel dressing (right).

Parameter	Ionic Hydrogel Dissolving (n=3)		Gauze Sponge (Sterile) (n=3)		No Material Used (n=3)	
Inflammation	1.33 ± 0.58	100%	2.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		2.00		1.00	
Neutrophils	1.33 ± 0.58	100%	2.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		2.00		1.00	
Histiocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Lymphocytes	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	
Multinucleated Giant Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Plasma Cells	0.00 ± 0.00	0%	0.00 ± 0.00	0%	0.00 ± 0.00	0%
	0.00		0.00		0.00	
Eosinophils	1.00 ± 0.00	100%	1.00 ± 0.00	100%	1.00 ± 0.00	100%
	1.00		1.00		1.00	

Table S5. Mean ± SD, median and incidence of inflammation and inflammatory cell types. Day 14, Group 4, 2 dressing changes.

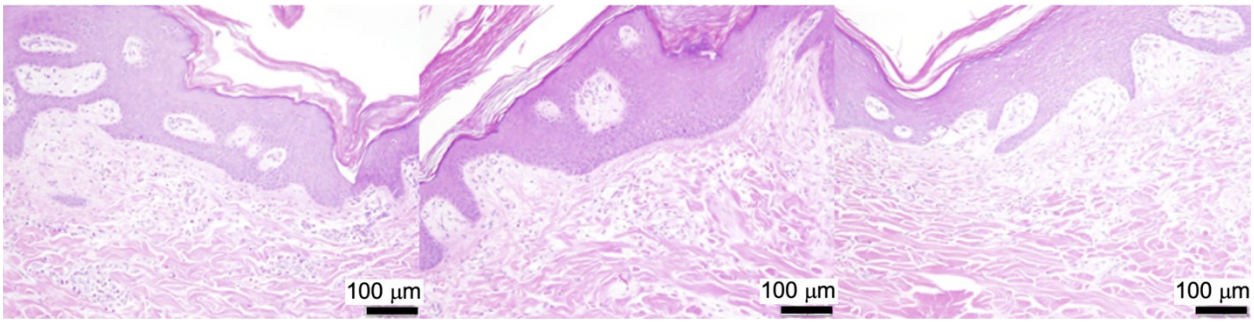

Figure S25. H&E of Group 5 for gauze (left), no dressing (middle), and hydrogel dressing (right).

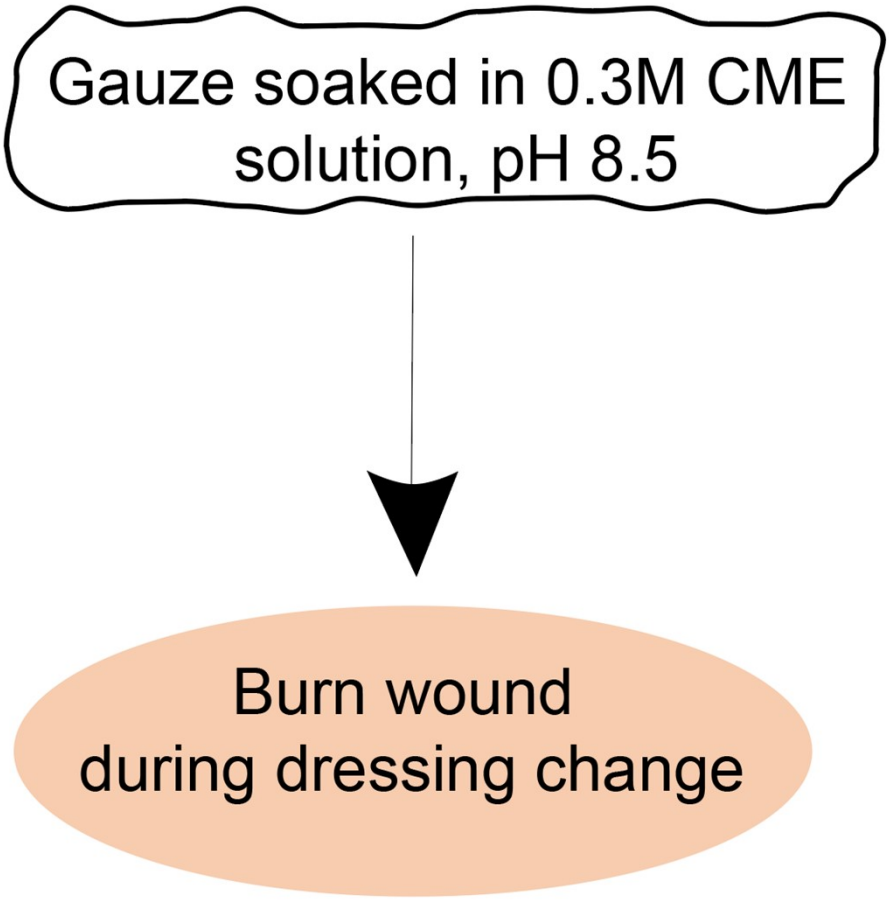

Figure S26. Gauze soaked in 0.3M CME solution, placed over the hydrogel **6** burn wound dressing for 10 minutes to induce dressing dissolution. Subsequently, burn wound was wiped with gauze soaked in H_2O and new hydrogel dressing was prepared on top of the wound.