Photopatterned Multidomain Gels – Multi-Component Self-Assembled Hydrogels Based on Partially Self-Sorting 1,3:2,4-Dibenzylidene-D-sorbitol Derivatives

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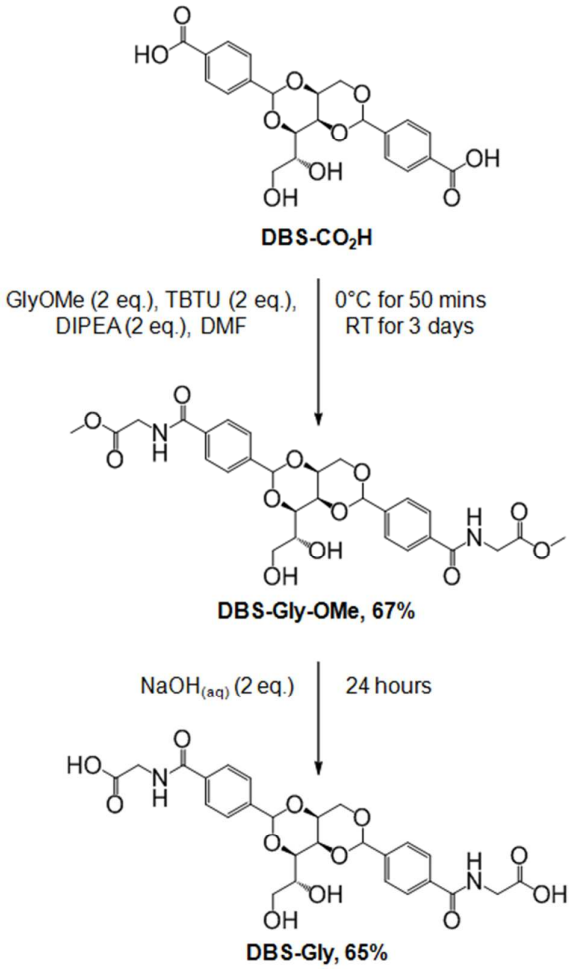
1. General Experimental Methods

All compounds required in synthesis and analysis were purchased from standard commercial suppliers. Proton and carbon NMR spectra were recorded on a Jeol 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). A Bruker 500 (^{1}H 500 MHz) was used for kinetics experiments. Samples were recorded as solutions in deuterated NMR solvents as stated and chemical shifts (δ) are quoted in parts per million. Coupling constant values (*J*) are given in Hz. The level of assignment of ^{1}H NMR spectra was achieved using model compounds, literature data and standard knowledge of ^{1}H NMR. DEPT experiments were used to assist in the assignment of ^{13}C NMR spectra. Positive and negative ion electrospray mass spectra were recorded on a Bruker Daltonics MicroTOF mass spectrometer. IR spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer. Melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected. Rheological measurements were recorded using a Malvern Instruments Kinexus Pro+ rheometer fitted with a parallel plate geometry. SEM was carried out on a JEOL JSM-7600F. Circular dichroism spectra were recorded on a Jasco J810 CD spectrophotometer. UV-vis absorbance was measured on a Shimadzu UV-2401 PC spectrophotometer.

2. Synthesis and Characterisation of DBS-Gly-OMe and DBS-Gly



Scheme 1. Synthesis of DBS-Gly

Synthesis and Characterisation of DBS-Gly-OMe: DBS-CO_2H[S1] (500 mg) was dissolved in DMF (12 ml) with addition of DIPEA (780 µl). The solution was cooled to 0°C, then TBTU (720 mg) was added as a solid, with DMF (4 ml) used to wash residual TBTU into the flask. The reaction was stirred for 20 minutes, then glycine methyl ester hydrochloride (282 mg) was added with a further portion of DMF (4 ml). The reaction was stirred for a further 30 minutes at 0°C, then at room temperature for 3 days. DMF was removed by rotary evaporation to give an orange residue. A solid was precipitated by the addition of water (50 ml), and collected over a sinter funnel. The solid was washed with water (3 × 50 ml) and DCM (2 × 50 ml), and then dried under high vacuum for 1 d, followed by drying to constant weight in a vacuum oven at 70°C for 1 d. Yield: 440 mg (67%) as yellow-brown solid.

M.p.: 228-231°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.99 (t, N*H*, *J* = 5.8, 2H), 7.89-7.87 (m, Ar*H,* 4H), 7.58-7.54 (m, Ar*H*, 4H), 5.74 (s, Ar-C*H*, 2H), 4.94 (d, CHO*H*, *J* = 5.6, 1H), 4.47 (t, CH_2O*H*, *J* = 5.4, 1H), 4.23-4.19 (m, sugar, 3H (overlap)), 4.01-3.99 (m, sugar and CH_2, 5H (overlap)), 3.88 (d, sugar, *J* = 9.2 , 1H), 3.80-3.79 (m, sugar, 1H), 3.65 (s, CH_3, 6H), 3.63 (s, sugar, 1H), 3.47-3.46 (m, sugar, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 170.92 (*C*ONH), 166.80 (*C*OO), 142.22 (aromatic *p*-*C*), 141.95 (aromatic *p*-*C*), 134.31 (aromatic *p*-*C*), 134.23 (aromatic *p*-*C*), 127.62 (aromatic *o*-*C*), 127.54 (aromatic *o*-*C*), 126.73 (aromatic *m*-*C*), 126.66 (aromatic *m*-*C*), 99.26 (Ph-*C*), 99.21 (Ph-*C*), 78.13 (*C*H), 70.72 (*C*H), 69.82 (*C*H_2), 69.07 (*C*H), 68.15 (*C*H), 63.11 (*C*H_2), 52.30 (*C*H_3), 41.76 (*C*H_2). ν_{max} (cm^{-1}) (solid): 3333*m*, 1739*s*, 1643*s*, 1544*s*, 1505*w*, 1400*w*, 1369*w*, 1342*w*, 1215*s*, 1165*w*, 1094*s*, 1018*s*, 850*m*, 751*m*. ESI-MS (*m/z*) calc. for $C_{28}H_{33}N_2O_{12}$ 589.2028; found 589.2035 (5% $[M+H]^+$), 611.1839 (100% $[M+Na]^+$).

Synthesis and Characterisation of DBS-Gly: DBS-Gly-OMe (190 mg, 1 equiv.) was suspended in MeOH (25 ml) and H_2O (10 ml). NaOH (1.33 ml, 0.5 M, 2 equiv.) was added, and the reaction was stirred overnight at room temperature. MeOH was removed by rotary evaporation, and the mixture was acidified with $NaHSO_4$ until a white, stable gel formed (pH ≈ 2). The product was filtered using a sintered funnel, and washed thoroughly with water (4 × 50 ml). The product was dried under high vacuum, and then finally dried to constant weight in a vacuum oven at 70°C for 1 d. Yield: 117 mg (65%) as pale brown solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.80 (t, N*H*, *J* = 4.8, 2H), 7.89-7.87 (m, Ar*H,* 4H), 7.58-7.54 (m, Ar*H*, 4H), 5.74 (s, Ar-C*H*, 2H), 4.94 (br, CHO*H*, 1H), 4.48 (br, CH_2O*H*, 1H), 4.25-4.20 (m, sugar, 3H (overlap)), 4.00 (s, sugar, 1H), 3.92-3.88 (m, CH_2 and sugar, 5H (overlap)), 3.80 (m, sugar, 1H), 3.62 (m, sugar, 1H), 3.49-3.45 (m, sugar, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.86 (*C*ONH), 166.65 (*C*OO), 142.08 (aromatic *p*-*C*), 141.81 (aromatic *p*-*C*), 134.58 (aromatic *p*-*C*), 134.50 (aromatic *p*-*C*), 127.58 (aromatic *o*-*C*), 127.51 (aromatic *o*-*C*), 126.62 (aromatic *m*-*C*), 99.30 (Ph-*C*), 99.24 (Ph-*C*), 78.15 (*C*H), 70.72 (*C*H), 69.84 (*C*H_2), 69.07 (*C*H), 68.15 (*C*H), 63.11 (*C*H_2), 41.82 (*C*H_2). ν_{max} (cm^{-1}) (solid): 3335*s*, 1713*m*, 1644*s*, 1539*s*, 1505*w*, 1398*m*, 1340*w*, 1218*s*, 1165*m*, 1093*s*, 998*s*, 805*m*, 749*m*. ESI-MS (*m/z*) calc. for $C_{26}H_{27}N_2O_{12}$ 559.1569; found 559.1592 (100% $[M-H]^-$), 581.1431 (30% $[(M-2H)+Na]^-$).

3. Preparation of Gels

For DBS-Gly gels: 1 ml H_2O was added to a known weight of DBS-Gly in 2.5 ml sample vials. The vials were then sonicated to disperse the solid, and 10 µL aliquots of 0.5 M $NaOH_{(aq)}$ were added to dissolve (pH ≈ 11). The solutions were then transferred to vials containing ≥10 mg of GdL, followed by shaking to dissolve. The vials were then left overnight for gelation to occur.

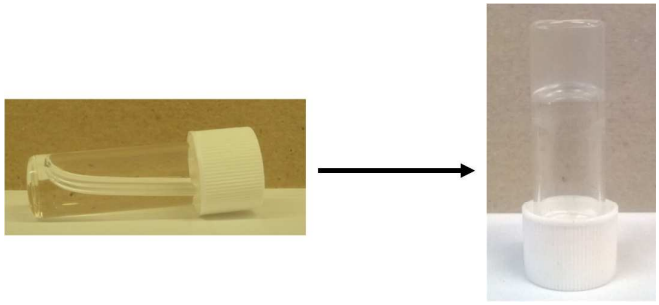


Figure S1. Formation of DBS-Gly hydrogel. Clear solution (left) changes to translucent gel (right) with decrease in pH brought about by hydrolysis of GdL.

For multi-component gels of DBS-Gly and DBS-CO_2H: 1 ml H_2O was added DBS-Gly (4.5 mg) and DBS-CO_2H (4.5 mg) in a 2.5 ml sample vial. The vial was then sonicated to disperse the solid, and 10 µL aliquots of 0.5 M $NaOH_{(aq)}$ were added to dissolve (pH ≈ 11). The solution was then transferred to a vial containing 18 mg of GdL, followed by shaking to dissolve. The vial was then left overnight for gelation to occur.

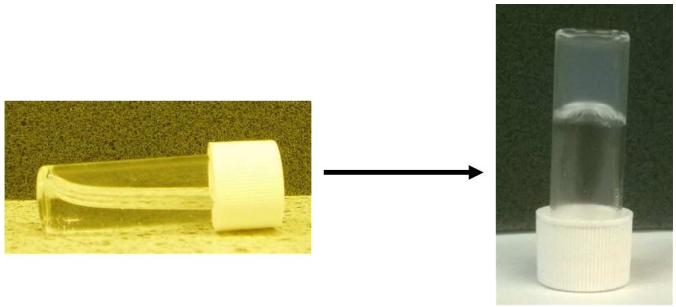


Figure S2. Formation of multi-component DBS-Gly and DBS-CO_2H hydrogel. Clear solution (left) changes to translucent gel (right) with decrease in pH brought about by hydrolysis of GdL.

4. pH Monitoring in DBS-Gly Gel

1 ml H_2O was added to 4.5 mg of DBS-Gly in a 2.5 ml sample vial. The vial was then sonicated to disperse the solid, and 10 μL aliquots of 0.5 M $NaOH_{(aq)}$ were added to dissolve. The pH was then recorded with a pH meter, with the recorded value being referred to as pH at 0 minutes. 10 mg of GdL was then added, followed by shaking to dissolve, and the pH was recorded again (pH at 1 min). Further pH readings were then taken at 30 minute intervals, up to 6 hours, then a final reading was taken after 24 hours.

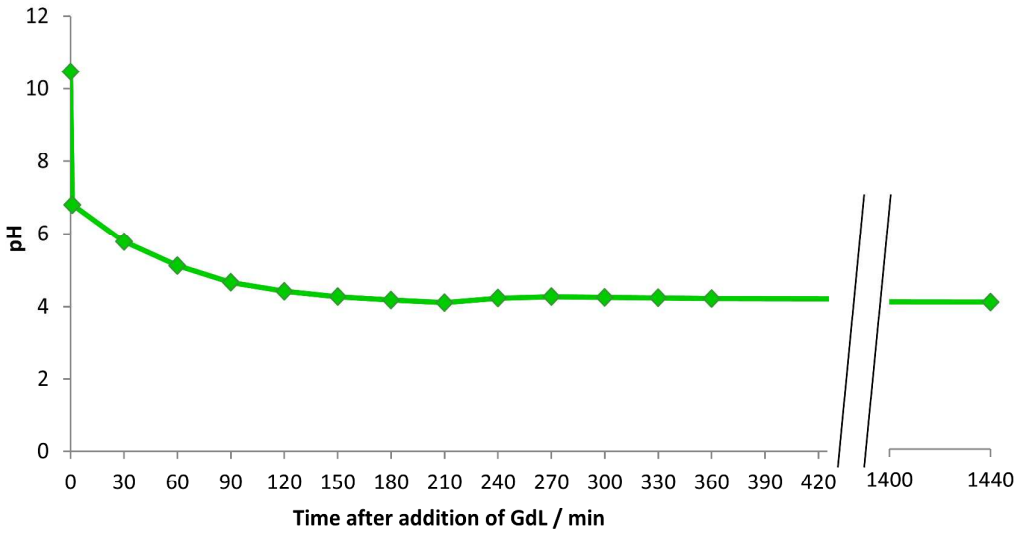


Figure S3. pH change over time after addition of GdL for a DBS-Gly gel system; there is a rapid drop in pH immediately after addition of GdL, suggesting rapid conversion of GdL to gluconic acid by excess NaOH present.

5. ^{1}H NMR Kinetics for DBS-Gly and Avrami Equation

Preparation of DBS-Gly gels for NMR characterisation of kinetics: Gels prepared as described above, replacing H_2O with D_2O, and with addition of 2 μl ml^{-1} DMSO to act as an internal standard. After addition of GdL (10 mg ml^{-1}), 0.7 ml of the solution was immediately transferred to NMR tube, and placed in the spectrometer, with spectra recorded every 30 minutes for a period of 14 hours. Zero time is taken to be the time at which the GdL is added, with the concentration at zero time being the initial concentration of DBS-Gly in the solution (for 0.45% wt/vol this was 10.08 mM).

Avrami equation: The Avrami model [1],[S2] in which $X(t)$ represents the volume fraction of the gel phase, K is a temperature dependent constant (similar to a rate constant), n is the Avrami exponent (which reflects the dimensionality of 'crystal' growth) and t is time, can be rearranged into equation [2]:

$$1 - X(t) = \exp(-Kt^n) \qquad [1]$$

$$\ln(\ln(1/1-X(t)) = \ln K + n\ln(t) \qquad [2]$$

$$X(t) = (I(\infty) - I(t))/(I(\infty)-I(0)) \qquad [3]$$

The volume fraction of the gel phase $X(t)$ can be expressed in terms of NMR signal intensity at equilibrium ($I(\infty)$), at time t ($I(t)$) and at the start of the experiment ($I(0)$) using equation [3]. This relationship enables linear fitting of equation [2] to calculate the Avrami exponent n by plotting $\ln[-\ln(I(\infty) - I(t))/(I(\infty) - I(0))]$ versus ln(t), where n is the gradient of the line of best fit.[S3]

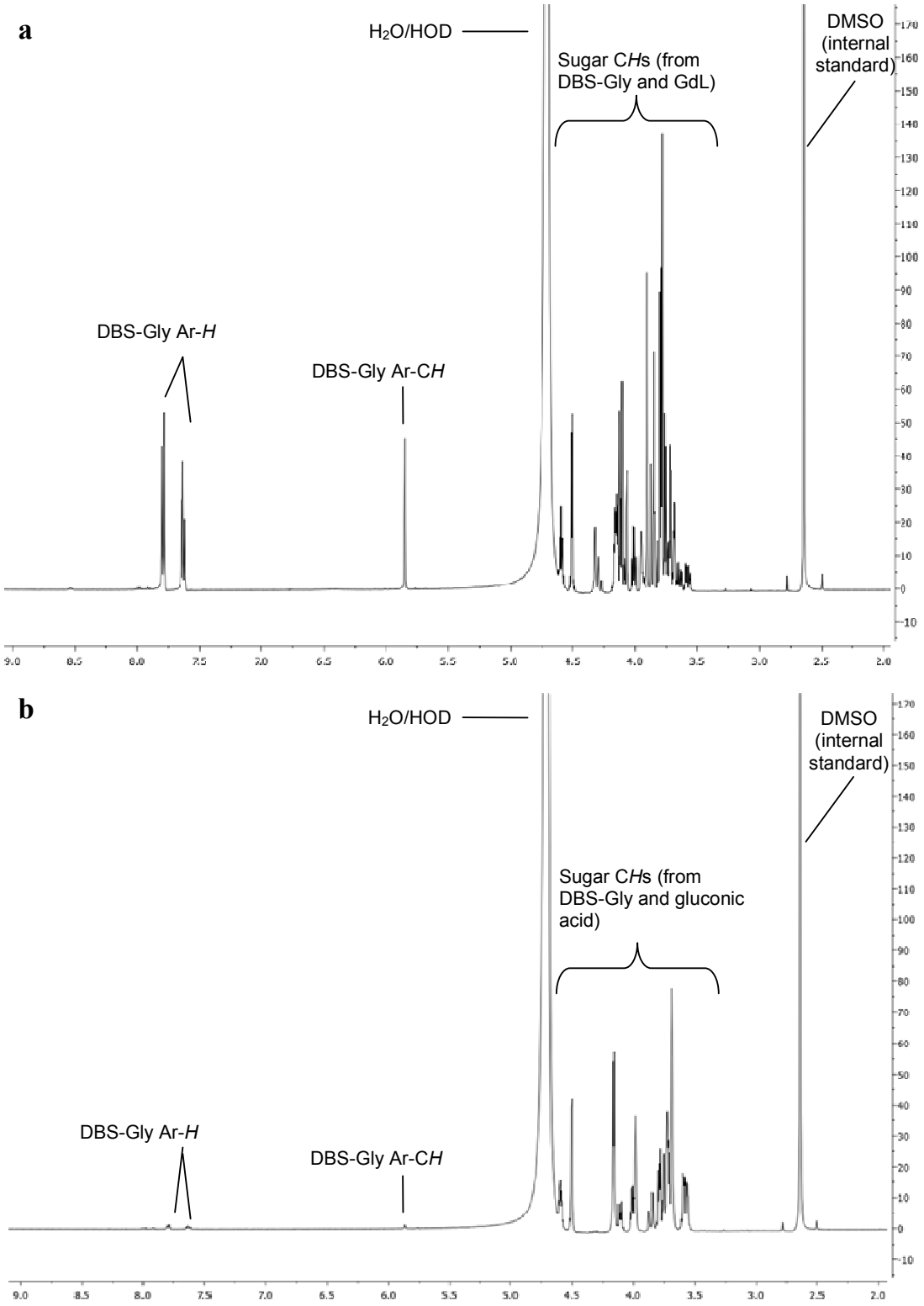


Figure S4. ^{1}H NMR spectra (400 MHz, D_2O) of DBS-Gly gel (0.45% wt/vol) at (a) start of gelation and (b**)** end of gelation; the near absence of signals related to DBS-Gly in (b) indicates that most of the gelator has been incorporated into a sample-spanning solid-like network.

6. pK_a Titrations[S4]

A stock solution of each gelator at 0.2% wt/vol was prepared, using the minimum amount of $NaOH_{(aq)}$ (0.5 M) to dissolve the solid. 5 mL of these solutions were used for each titration. To these solutions, aliquots of HCl (0.1 M) were added, in 15 µL volumes for DBS-Gly and 20 µL volumes for DBS-CO_2H. The pH values were recorded from a pH meter once the readings had stabilised (*ca*. 10 minutes).

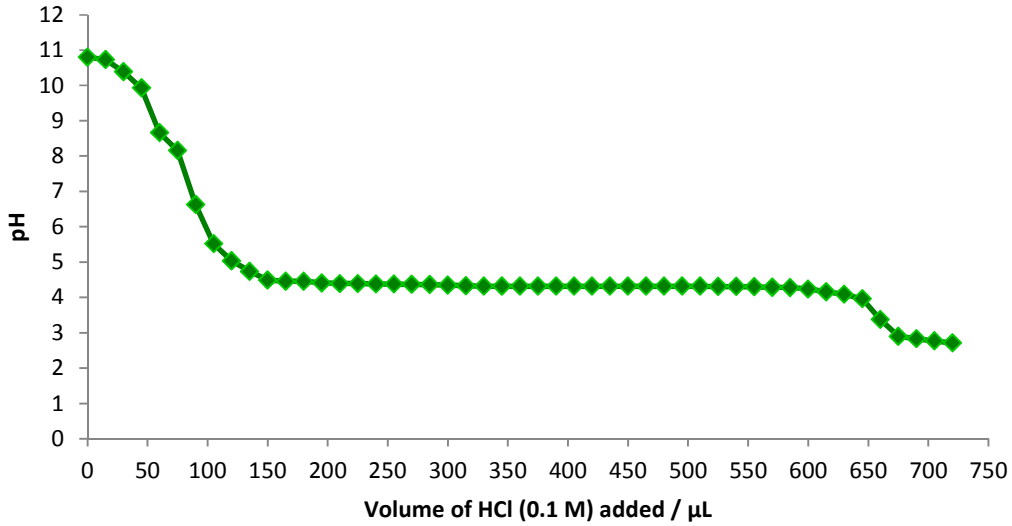


Figure S5. Titration curve for DBS-Gly; from this, pK_a was extrapolated to be *ca*. 4.3.

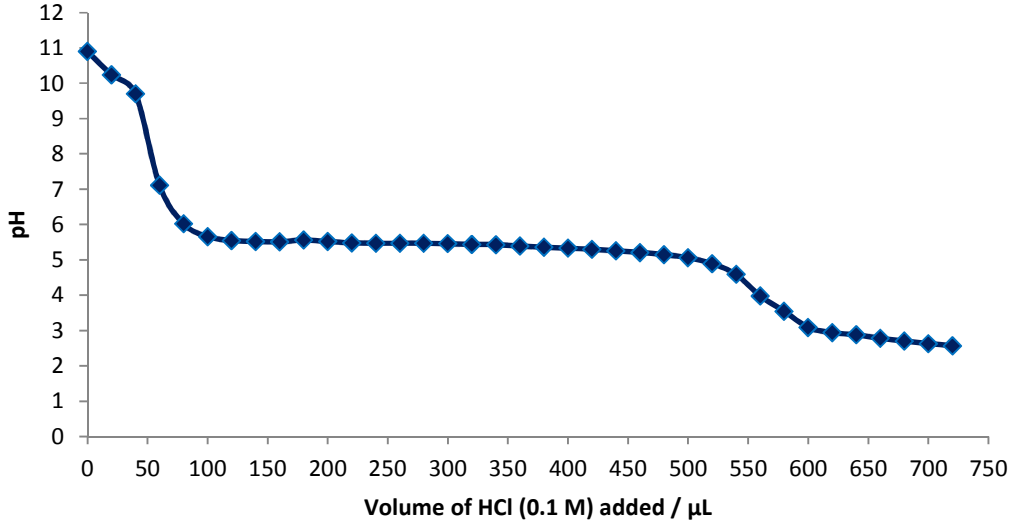


Figure S6. Titration curve for DBS-CO_2H; from this, pK_a was extrapolated to be *ca*. 5.4.

7. Rheological Data

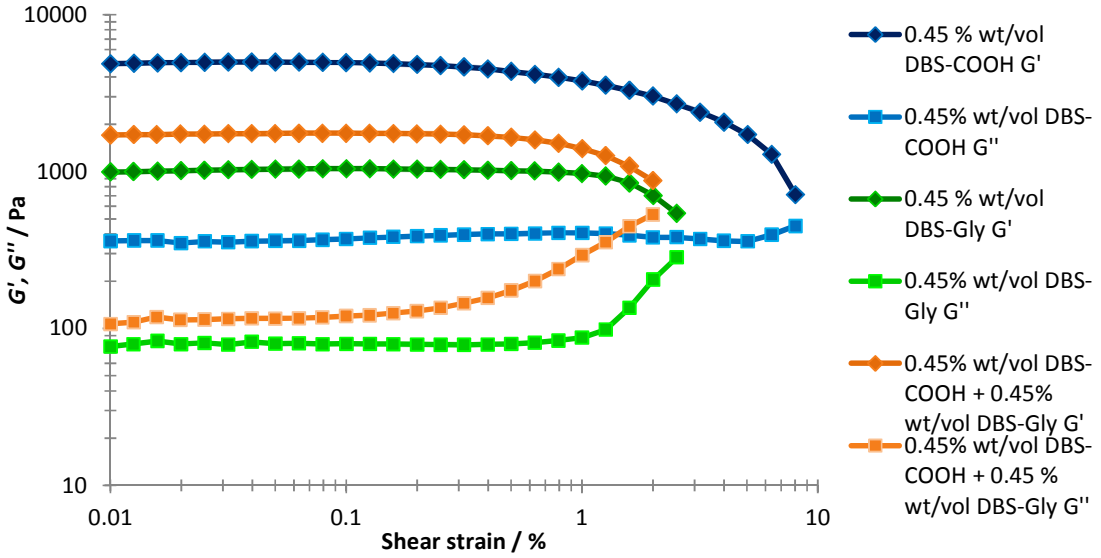


Figure S7. Amplitude sweeps for gels of 0.45% DBS-CO_2H, 0.45% DBS-Gly, and multi-component system with 0.45% of both LMWGs. Frequency = 1 Hz.

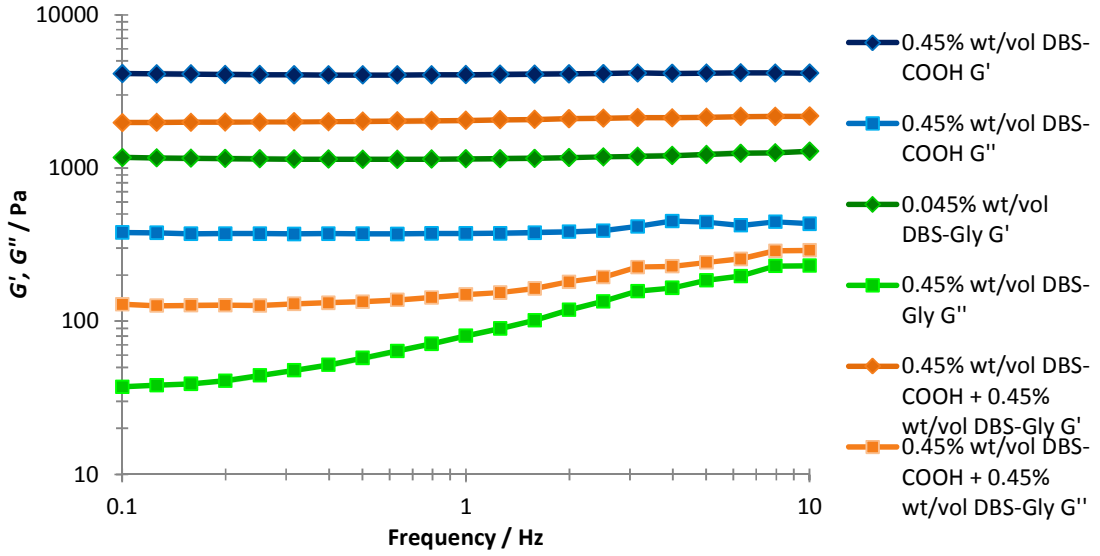


Figure S8. Frequency sweeps for gels of 0.45% DBS-CO_2H, 0.45% DBS-Gly, and multi-component system with 0.45% of both LMWGs. Shear strain = 0.3%.

8. SEM Images of Multi-Component Gels Prepared with GdL

Preparation of samples for SEM: a small portion of the gel was removed with a spatula and placed on a copper support, then freeze-dried by immersing in liquid nitrogen and then lyophilising overnight. Excess solid material was broken off with a spatula and then the sample was sputter coated with a thin layer (about 12 nm) of gold/palladium to prevent sample charging, before placing the sample on a metal SEM stub and imaging.

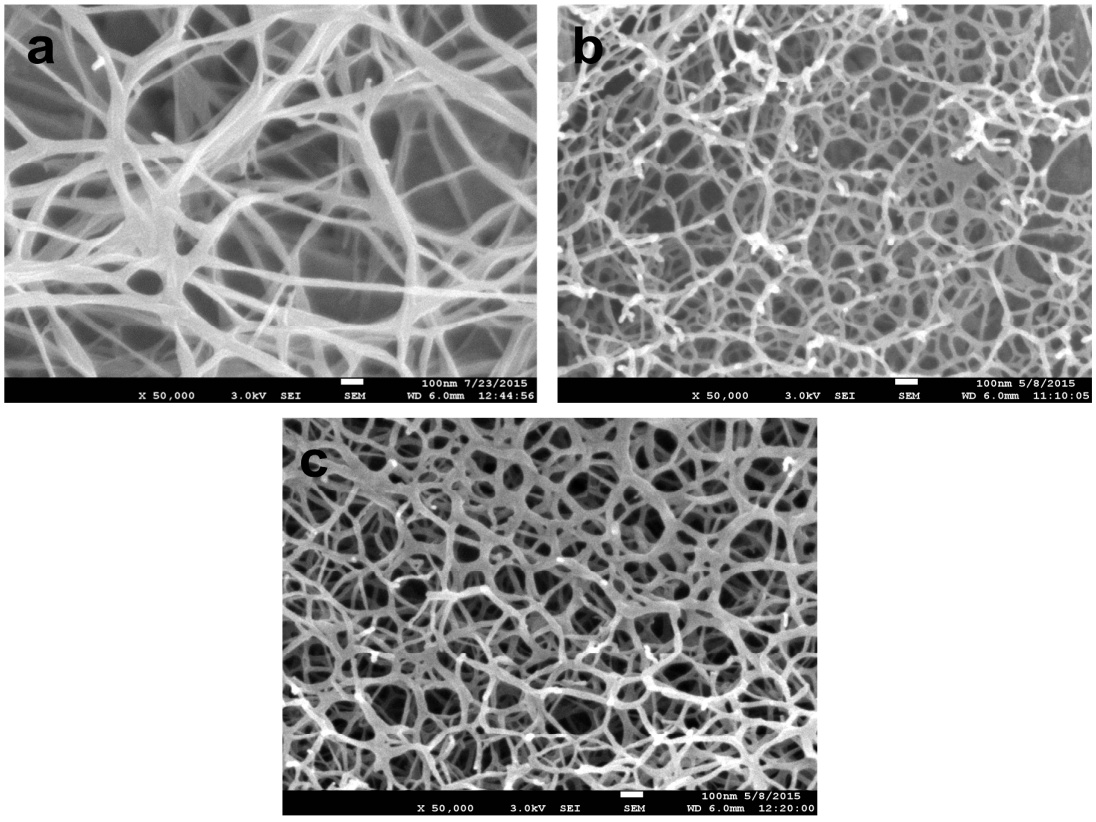


Figure S9. SEM images of a) DBS-CO_2H (0.40% wt/vol); b) DBS-Gly (0.45% wt/vol); c) multi-component gel of DBS-CO_2H and DBS-Gly (0.45% wt/vol each). Scale bars = 100 nm.

9. UV/Vis Spectrum of DPIN

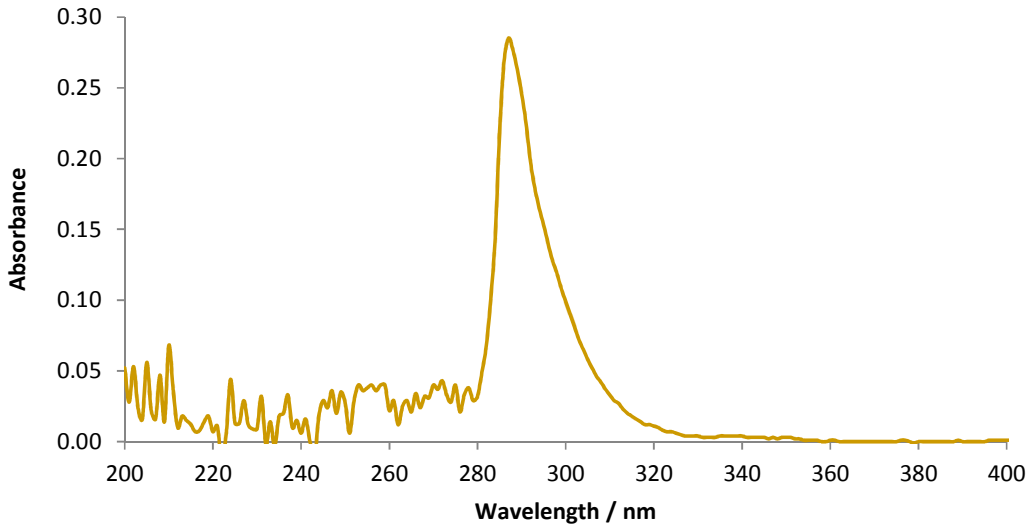


Figure S10. UV/Vis spectrum of photoacid generator diphenyliodonium nitrate (DPIN), 1.5 mM in H_2O. From this spectrum, λ_{max} = 287 nm.

10. ^{1}H NMR of Multi-Component Gels

Preparation of multi-component gels for NMR characterisation of kinetics: 3.15 mg of both DBS-Gly and DBS-CO_2H were suspended by sonication in 0.65 mL D_2O (with 2 μL mL^{-1} DMSO as an internal standard) in 2.5 mL vials, followed by addition of 51 μL of $NaOH_{(aq)}$ (0.5 M) to dissolve. GdL was then added in known amounts, and the solutions were immediately transferred to NMR tubes and placed in the spectrometer. Spectra were recorded every 30 minutes for a period of 14 hours. Zero time is taken to be the time at which the GdL is added, with the concentration at zero time being the initial concentration of the gelators in the solution (10.08 mM DBS-Gly and 8.03 mM DBS-CO_2H).

11. Preparation of DBS-Gly Gels with Photoacid Generator DPIN

Preparation of DBS-Gly gels using DPIN for NMR characterisation: Varying amounts of DPIN (4-10 mg) were dissolved in 497.5 µL D_2O (with 2 µL mL^{-1} DMSO as internal standard), followed by addition of of 2.5 µL $HCl_{(aq)}$ (0.5 M). Separately, 4.5 mg amounts of DBS-Gly were dissolved in 461 µL D_2O (with 2 µL mL^{-1} DMSO as internal standard) through the addition of 39 µL $NaOH_{(aq)}$ (0.5 M). The two solutions were mixed, and 700 µL volumes were transferred to NMR tubes. An initial ^1H NMR spectra was recorded, after which the tubes were cured for 30 minutes under UV light. A second spectra was then recorded. From these experiments the optimum amount of DPIN in the final solution was found to be 8 mg mL^{-1} (*ca.* 3 equivalents).

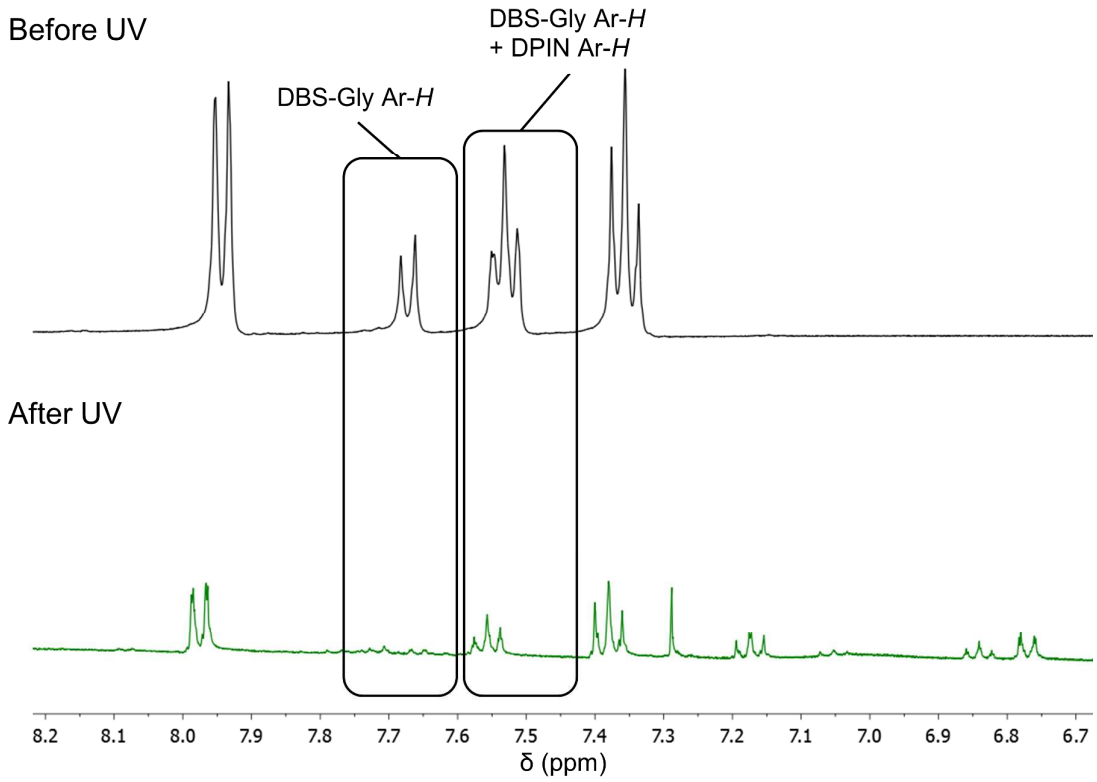


Figure S11. ^1H NMR of DBS-Gly system using 8mg mL^{-1} equivalents DPIN as acidifying agent, before and after UV exposure of 1 hour. A significant reduction in the intensity of the DBS-Gly Ar-*H* peaks can be observed.

Preparation of DBS-Gly gels using DPIN for NMR characterisation of kinetics: 96 mg DPIN dissolved in 5.97 mL D_2O, followed by addition of 30 µL $HCl_{(aq)}$ (0.5 M). Separately, 45 mg DBS-Gly dissolved in 4.66 mL D_2O with the addition of 340 µL $NaOH_{(aq)}$ (0.5 M). 5 mL of the DPIN solution added to the DBS-Gly solution, along with 20 µL of DMSO to act as internal standard. 13 x 700 µL volumes of the solution transferred to 13 x NMR tubes. One tube left uncured, whilst the other 12 were cured under UV light, with one tube being removed every 5 minutes for 60 minutes, for a ^{1}H NMR spectrum to be recorded.

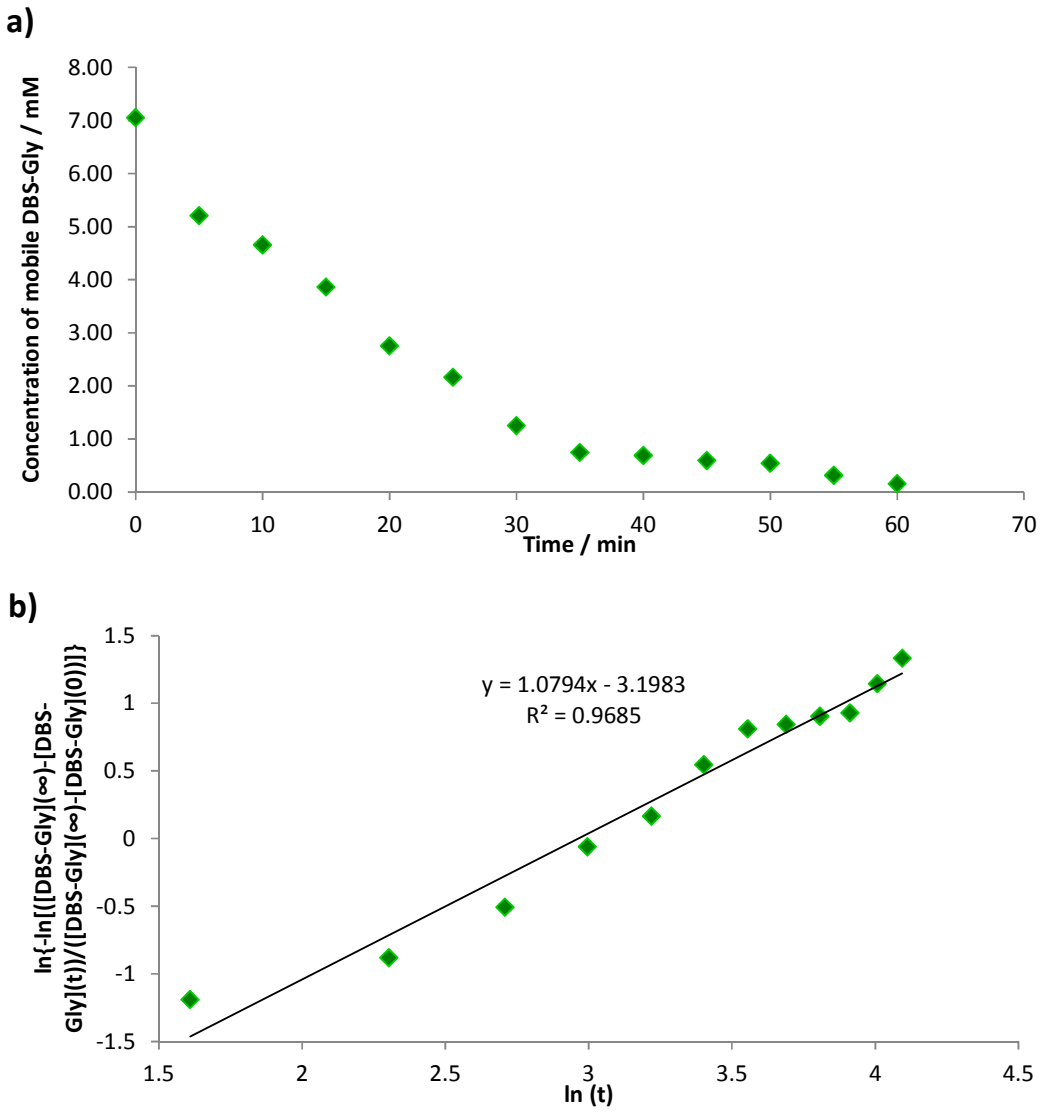


Figure S12. a) Kinetics of formation of DBS-Gly network using DPIN as acidfying agent, as monitored by NMR spectroscopy; b) Avrami plot for DBS-Gly network formation using DPIN as acidfying agent, where the Avrami exponent, n, is equivalent to the gradient of line of best fit.

Preparation of DBS-Gly suspensions using DPIN: 16 mg of DPIN was dissolved in 995 µL H_2O, followed by addition of 5 µL $HCl_{(aq)}$ (0.5 M). Separately, 9 mg of DBS-Gly was dissolved in 932 µL II_2O through the addition of 68 µL $NaOII_{(aq)}$ (0.5 M). The two solutions were mixed, and known volumes (250 – 1000 µL) were transferred to 2.5 mL sample vials, and cured under UV light for 2 hours until opaque suspensions of partial gel were formed.

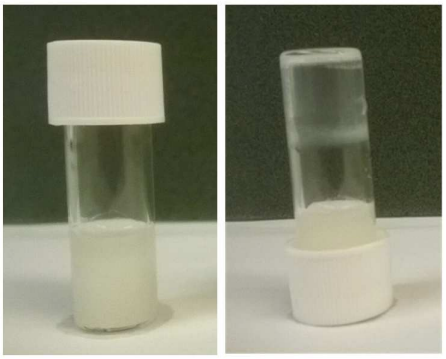


Figure S13. Suspensions of partial gel of DBS-Gly using DPIN as acidifying agent, formed in vials.

Preparation of DBS-Gly gel using DPIN: 64 mg of DPIN dissolved in 3.98 mL H_2O, followed by addition of 20 µL $HCl_{(aq)}$ (0.5 M). Separately, 22.5 mg DBS-Gly dissolved in 2.33 mL H_2O through the addition of 170 µL $NaOH_{(aq)}$ (0.5 M). 2.5 mL of the DPIN solution added to the DBS-Gly solution, mixed, then solution transferred to 5 cm x 5 cm glass mould. Solution cured under UV light for 2 hours, after which an opaque, weak gel was observed.

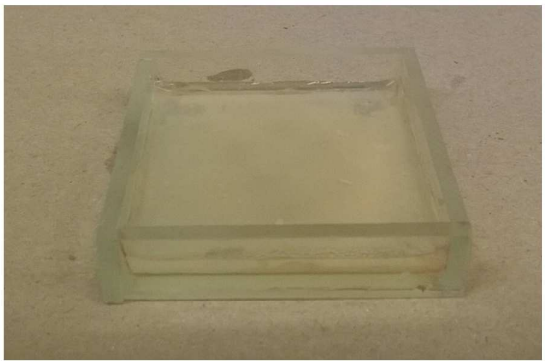


Figure S14. Weak gel of DBS-Gly using DPIN as acidifying agent, formed in a glass mould.

12. Preparation of Multi-Component Gels Combining GdL and Photo-Activation

Preparation of multi-component gels incorporating DPIN for NMR: 32 mg of DPIN was dissolved in 2 mL D_2O (with 2 μL mL^{-1} DMSO as an internal standard) to make a stock solution. 3.15 mg of both DBS-Gly and DBS-CO_2H were suspended by sonication in 0.3 mL D_2O (with 2 μL mL^{-1} DMSO as an internal standard) in 2.5 mL vials, followed by addition of 51 μL of $NaOH_{(aq)}$ (0.5 M) to dissolve. 350 μL of DPIN stock solution was then added, followed by 4 mg GdL. The solutions were immediately transferred to NMR tubes and placed in the spectrometer to record an initial spectrum. The tubes were then allowed to stand overnight, after which a second spectrum was recorded. The tubes were then placed under high-intensity UV light for 1 hour, and then a final NMR spectrum was recorded.

13. SEM Images of Multi-Component Gels Prepared with GdL and DPIN

Preparation of samples for SEM: as described above.

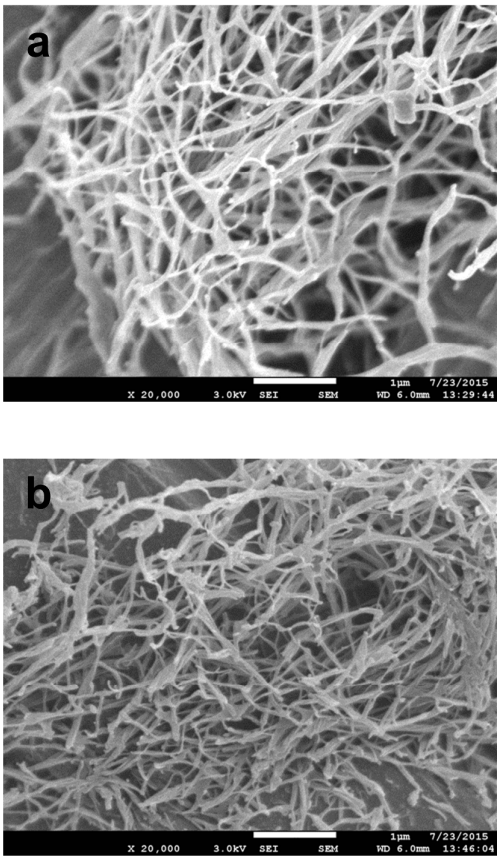


Figure S15. SEM images of multi-component gels of DBS-CO_2H and DBS-Gly (0.45% wt/vol each), a) after GdL hydrolysis, and b) after both GdL hydrolysis and activation of DPIN. Scale bars = 1 µm.

14. Preparation of Photopatterned Multi-Component Gels

To prepare gel: 48 mg of DPIN was dissolved in 3 ml H_2O to make a stock solution. 22.5 mg of both DBS-Gly and DBS-CO_2H were suspended in 2.5 mL H_2O by sonication, followed by addition of 255 µl $NaOH_{(aq)}$ (0.5 M) to dissolve. 2.5 mL of DPIN stock was then added, followed by 20 mg of GdL. The solution was then transferred to a 50 mm x 50 mm x 10mm square glass dish, and left overnight for a translucent gel to form. The next day, a cardboard mask was placed over the top so that only part of the gel was exposed. The dish was then placed under a long-wave UV light and cured for 1 hour, after which the exposed region of the solution had become visibly opaque.

For gels with pH indicator: The gel was prepared as above, using a mask which covered half of gel. A 0.1% wt/vol solution of Congo Red was prepared, and injected into the gel in small volumes (*ca.* 50 µL) using a syringe. In the photopatterned regions, the indicator became red-purple in colour, indicating a pH of *ca*. 4, whilst in the non-patterned regions it remained bright red, indicating a pH of >5.

15. References

S1 Cornwell, D. J.; Okesola B. O.; Smith, D. K. *Soft Matter* **2013**, *9*, 8730-8736.

S2 (a) Avrami, M. *J. Chem. Phys.* **1939**, *7*, 103-112; (b) Avrami, M. *J. Chem. Phys.* **1940**, *8*, 212; (c) Avrami, M. *J. Chem. Phys*. **1941**, *9*, 177-184.

S3 Huang, X.; Terech, P.; Raghavan, S. R.; Weiss, R. G. *J. Am. Chem. Soc.* **2005**, *127*, 4336-4344.

S4 Raeburn, J.; Pont, G.; Chen, L.; Cesbron, Y.; Lévy, R.; Adams, D. J. *Soft Matter* **2012**, *8*, 1168–1174.