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Combining gellan gum with a functional low-molecular-weight gelator to assemble stiff shaped hybrid hydrogels for stem cell growth

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SUPPORTING INFORMATION

S1 General Experimental Methods

All compounds used in synthesis and analysis were purchased from standard commercial suppliers and used as received. The gellan gum employed in all the experiments was bought from Alfa Aesar and used as received. The synthesis of DBS-$CONHNH_2$ was performed in good yields applying previously reported methods.[1, 2] ^{1}H NMR spectra were recorded using a Jeol 400 spectrometer (^{1}H 400 MHz). Samples were prepared in DMSO-d_6 or D_2O and chemical shifts (δ) are reported in parts per million (ppm). IR spectra of xerogels were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer. Optical microscopy images were obtained using a Zeiss Axiocam camera on a Zeiss stereo microscope. SEM images were taken using a JEOL JSM-7600F field emission SEM. TEM images were obtained on a FEI Tecnai 12 G^2 fitted with a CCD camera. Fibre sizes and gel bead diameters were measured using the *ImageJ* software. T_{gel} values were obtained using a high precision thermoregulated oil bath using the tube inversion method and were recorded in triplicate. Rheology was measured on a Malvern Instruments Kinexus Pro+ Rheometer fitted with a 20 mm parallel plate geometry. A high-powered UV lamp (λ = 315-405 nm) was used for activation of the photoacid generator diphenyliodonium nitrate (DPIN). Fluorescence measurements for the cell viability assay were performed using a BMG Labtech Clariostar Plate Reader.

S2 Preparation and characterisation of DBS-$CONHNH_2$, DBS-$CONHNH_2$/gellan gum and gellan gum gels cross-linked with $CaCl_2$

S2.1 Gel preparation

S2.1.1 Preparation of DBS-$CONHNH_2$ gels. DBS-$CONHNH_2$ (0.3 or 0.4% wt/vol) was suspended in water (1 mL). The suspension was sonicated to help the dispersion of the solid particles and then heated until complete dissolution of the compound. The sample was left undisturbed to cool, allowing gel formation in few minutes.

S2.1.2 Preparation of DBS-$CONHNH_2$/gellan gum gel beads. DBS-$CONHNH_2$ (0.3% wt/vol in 1 mL final total volume) was suspended in water (0.5 mL) and sonicated to help the dispersion of the solid particles. An aqueous gellan gum solution (1.0% wt/vol - 0.5 mL) was subsequently added. The resulting suspension was heated until complete dissolution of the DBS-$CONHNH_2$. The hot solution was then added dropwise (20 μL/drop) to paraffin oil (40 mL). The droplets were left undisturbed for 20 mins to allow the formation of the DBS-$CONHNH_2$ network. The gel beads were then transferred to a $CaCl_2$ solution (5.0% wt/vol, 50 mL) and gently mixed for another 20 mins. After this time, to remove residual paraffin oil, the gel beads were immersed in petroleum ether (30 mL, 10 mins), then EtOH (30 mL, 10 mins) and water (30 mL, 10 mins). When necessary, the washings were performed multiple times. The gel beads were then stored in water.

S2.1.3 Preparation of DBS-$CONHNH_2$/gellan gum gels in sample vials (for pH, IR, thermal stability and rheology studies). DBS-$CONHNH_2$ (0.3% wt/vol in 1 mL final total volume) was suspended in water (0.5 mL) and sonicated to help the dispersion of the solid particles. An aqueous gellan gum solution (1.0% wt/vol - 0.5 mL) was then added. The amount of gellan gum and water was adjusted depending on the desired final concentration of the polymer in the different experiments. The resulting suspension was heated until complete dissolution of the DBS-$CONHNH_2$. The sample was left undisturbed for few hours to allow the formation of the DBS-$CONHNH_2$ network. A solution of $CaCl_2$ (5.0 % wt/vol – 1 mL) was then added on top of each gel to crosslink the alginate chains for 30 min. The excess of $CaCl_2$ solution was then removed and the gels were washed with water multiple times.

S2.1.4 Preparation of gellan gum gels in sample vials (for pH, IR, thermal stability and rheology studies). Gellan gum gels were prepared by adding a $CaCl_2$ solution (5.0% wt/vol – 1 mL) to an aqueous gellan gum solution (0.4-1.3% wt/vol). Gelation occurred immediately. The excess of $CaCl_2$ solution was then removed and the gels were washed with water multiple times.

S2.2 NMR assays

S2.2.1 *Self-assembled state of DBS-CONHNH$_2$/gellan gum gel beads.* ^{1}H NMR was employed to validate the efficacy of the gel preparation method and to confirm that the two gelators were in a self-assembled state. The gel beads used for this experiment were prepared combining DBS-$CONHNH_2$ (0.3 % wt/vol) and gellan gum (0.5 % wt/vol) by the emulsion method described in Section S2.1.2. Five gel beads were isolated and transferred into a NMR tube in D_2O (0.7 mL). DMSO (1.4 μL) was added as an internal standard. The gel beads were then analysed by ^{1}H NMR. The lack of DBS-$CONHNH_2$ aromatic signals confirms its self-assembled state.

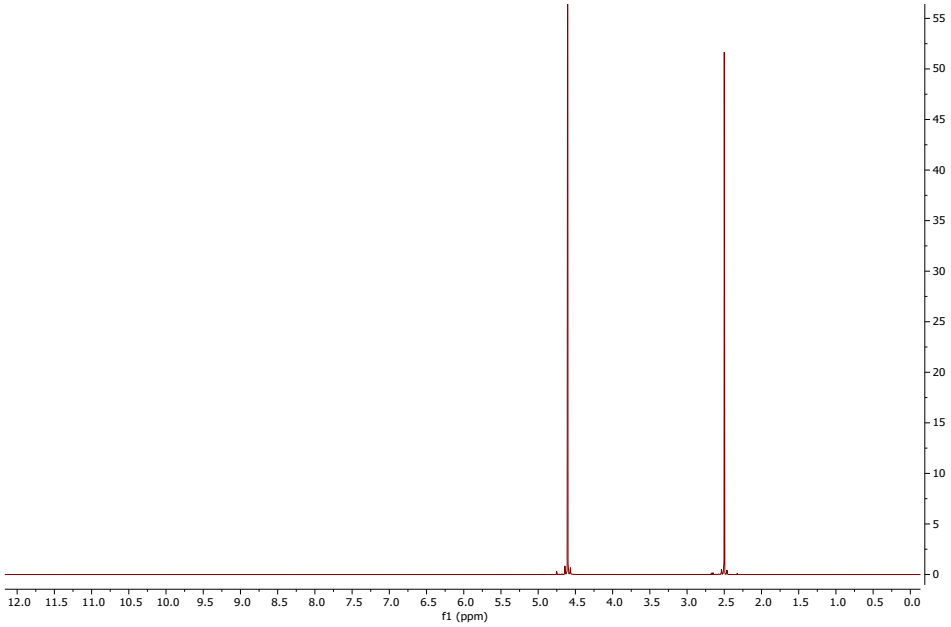

Figure S1. ^{1}H NMR of five DBS-$CONHNH_2$/gellan gum gel beads cross-linked with $CaCl_2$.

S2.2.2 *Quantification of DBS-CONHNH$_2$ incorporated into ten DBS-CONHNH$_2$/gellan gum gel beads.* ^{1}H NMR was employed to calculate the exact amount of DBS-$CONHNH_2$ incorporated into the DBS-$CONHNH_2$/gellan gum gel beads prepared by emulsion. The gel beads used for this experiment were prepared combining DBS-$CONHNH_2$ (0.3 % wt/vol) and gellan gum (0.5 % wt/vol) by the emulsion method described in Section S2.1.2. Ten gel beads were isolated and dried under high vacuum. The resulting solid was dissolved in DMSO-d_6 (0.7 mL), and acetonitrile (1.4 μL) was added as an internal standard. To make sure that all the DBS-$CONHNH_2$ was dissolved, the sample was ground and then sonicated for 30 min. The ^{1}H NMR spectrum was recorded and the concentration of the LMWG calculated by comparison of the integrals of relevant peaks (DBS-$CONHNH_2$ aromatic peaks δ = 7.53 and 7.83 ppm) to that of acetonitrile (δ = 2.09 ppm). To ensure the results were reproducible, this experiment was performed on two different batches of gel beads. It is noted that due to the low solubility of gellan gum in DMSO-d_6, the polymer peaks were not visible.

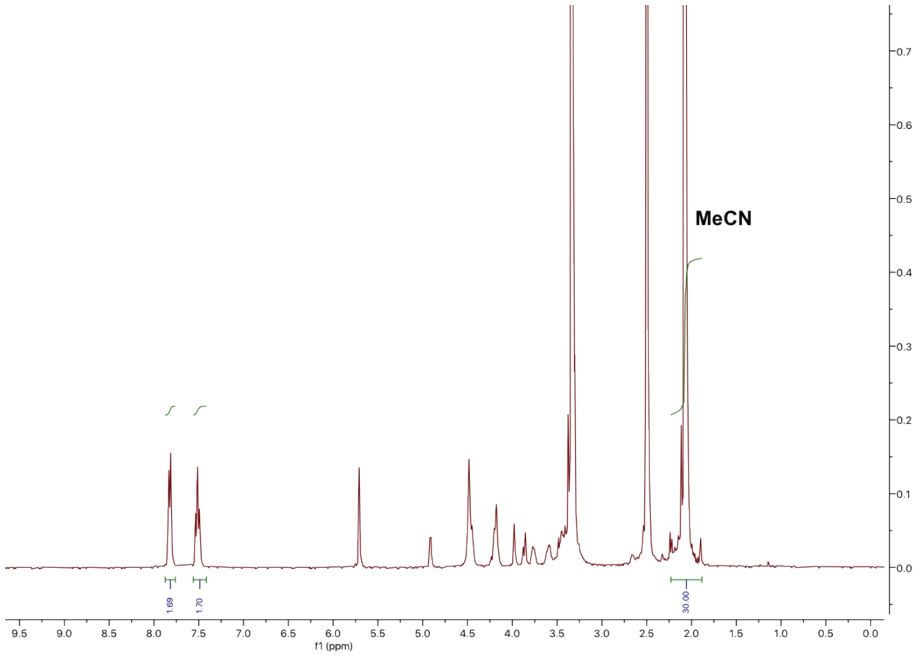

Figure S2. ^{1}H NMR of DBS-CONHNH$_2$ incorporated into 10 DBS-CONHNH$_2$/gellan gum hybrid gel beads prepared using 0.3% wt/vol DBS-CONHNH$_2$ and 0.5% wt/vol gellan gum and cross-linked with $CaCl_2$ (5.0% wt/vol).

S2.3 Infrared (IR) spectroscopy

Xerogel samples for infrared were prepared in sample vials as described in Section S2.1. The solvent was then removed from the gels under high vacuum. A small amount of the resulting powder was placed into the infrared spectrophotometer and the spectra recorded in the range of 450-4000 cm^{-1}.

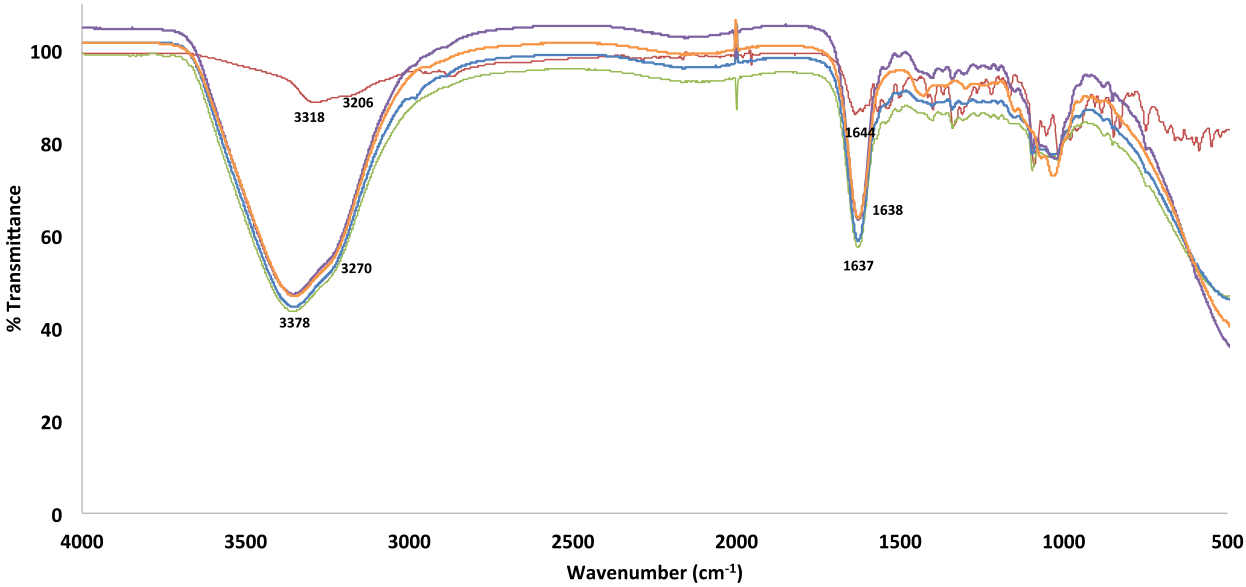

Figure S3. IR spectra of xerogels obtained from DBS-CONHNH$_2$ gel (0.4% wt/vol, red line), gellan gum gel (1.3% wt/vol, orange line) and DBS-CONHNH$_2$/gellan gum two-component gel cross-linked with $CaCl_2$, containing 0.3% wt/vol of DBS-CONHNH$_2$ and 0.3% wt/vol gellan gum (green line), 0.5% wt/vol gellan gum (blue line) and 1.0% wt/vol gellan gum (purple line).

S2.4 Optical microscopy

Optical microscopy images were collected on a Zeiss stereo microscope. The gel beads were dehydrated through an ethanol series, then embedded in LR white resin. Sections were 1 □m thick. Once the section was dried on the slide, it was stained with Toluidine Blue (0.6% with 0.3% Na_2CO_3). All the gel beads were prepared in 20 μL volumes using 0.3% wt/vol of DBS-$CONHNH_2$ and 0.5% wt/vol of gellan gum.

S2.5 Transmission and Scanning Electron Microscopy (TEM and SEM)

S2.5.1 Preparation of samples for TEM. Samples for TEM were obtained by placing a small amount of each sample on a copper grid. The excess of sample was removed with filter paper and allowed to set for 5 min. A negative stain (1% uranyl acetate) was then added and the samples were left to rest for 30 min before taking the images.

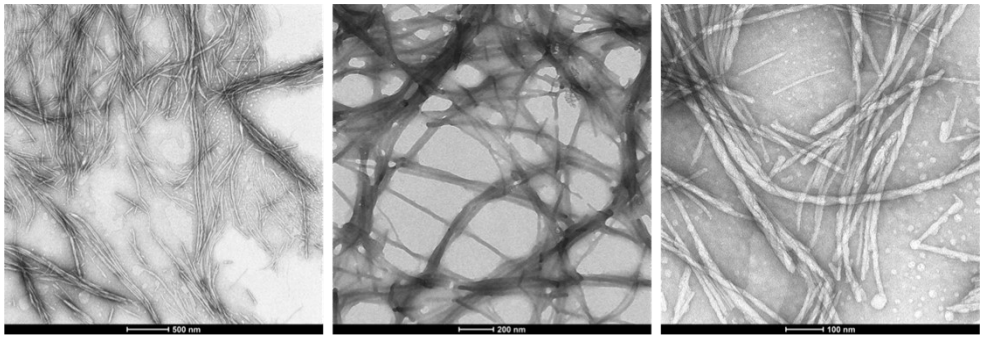

Figure S4. TEM images of DBS-$CONHNH_2$ gel. Scale bars (from left to right): 500, 200 and 100 nm.

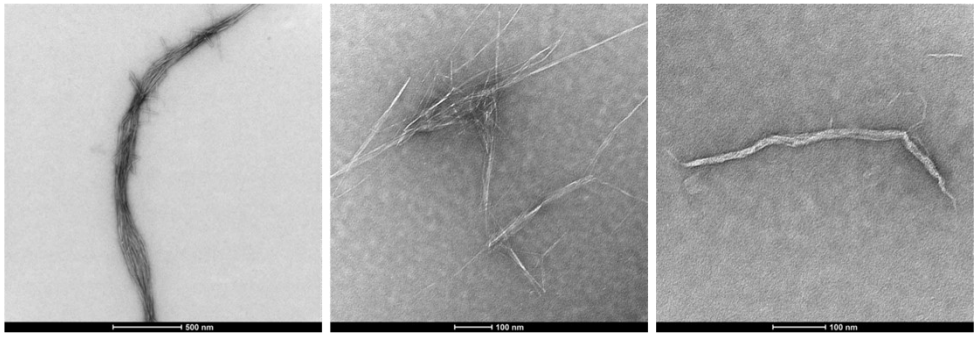

Figure S5. TEM images of DBS-$CONHNH_2$/gellan gum gel cross-linked with $CaCl_2$. Scale bars (from left to right): 500 nm (left) and 100 nm (centre and right).

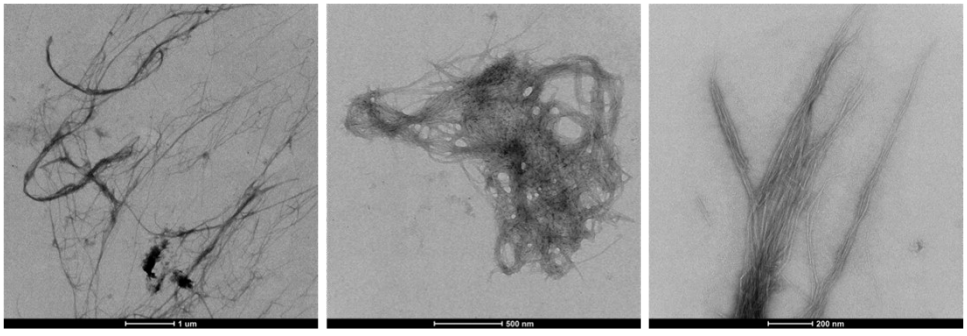

Figure S6. TEM images of gellan gum gel cross-linked with $CaCl_2$. Scale bars (from left to right): 1 μm (left), 200 nm (centre) and 100 nm (right).

S2.5.2 Preparation of samples for SEM. Samples for SEM were obtained by freeze-drying the gels on copper shim pieces. The freeze-dried samples were then mounted on stubs and the images recorded. DBS-$CONHNH_2$/gellan gum gel beads were critical point dried (acetone and liquid CO_2) and mounted on stubs either whole, or halved using a razor blade. Mounted samples were sputter coated with Au/Pd.

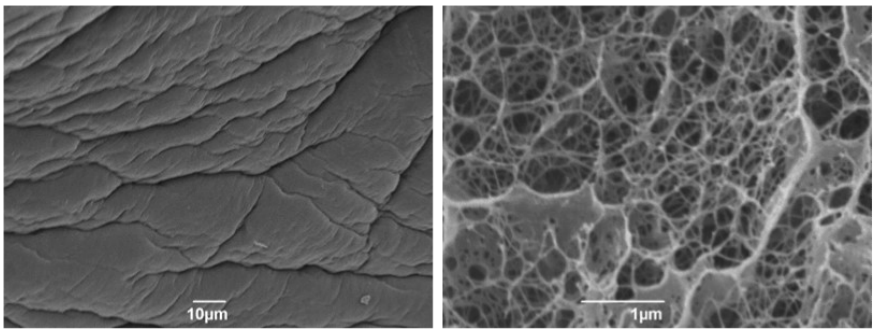

Figure S7. SEM images of DBS-$CONHNH_2$/gellan gum gel bead cross-section (cross-linked with $CaCl_2$). Scale bars: 10 (left) and 1 μm (right).

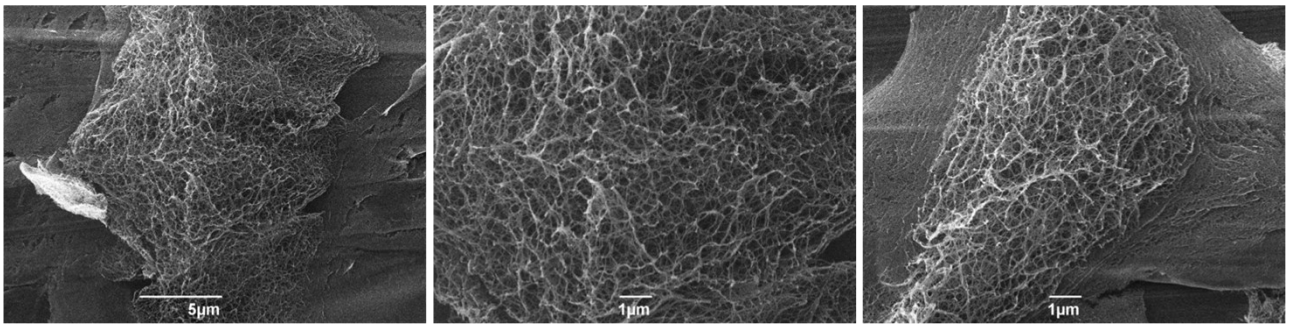

Figure S8. SEM images of gellan gum gel cross-linked with $CaCl_2$. Scale bars (from left to right): 5 (left) and 1 μm (centre and right).

S2.6 Thermal stability studies

All the gels for T_{gel} determination were prepared as described in Section S2 in 2 mL vials (diameter: 1 cm, height: 4 cm). The gels were placed in a high precision thermoregulated oil bath with an initial temperature of 25°C. The temperature was increased by 1°C/ min until 100°C. Every minute the gels were checked by tube inversion method and T_{gel} was considered as the temperature at which the gel began to run down the sides of the vial. These experiments were performed in triplicate to ensure reproducibility and the average is reported. Errors are estimated at ±2°C.

Table S1. T_{gel} values of gels formed by individual gelators and the DBS-$CONHNH_2$/gellan gum gel prepared using $CaCl_2$ as a cross-linker.

Gel (1 mL total volume)	Loading of DBS-$CONHNH_2$ (wt/vol)	Loading of Gellan Gum (wt/vol)	T_{gel}
DBS-$CONHNH_2$	0.4%	-	86 °C
Gellan gum	-	0.4%	>100 °C
Gellan gum	-	0.6%	>100 °C
Gellan gum	-	0.8%	>100 °C
Gellan gum	-	1.3%	>100 °C
DBS-$CONHNH_2$/ Gellan gum	0.3%	0.1%	>100 °C
DBS-$CONHNH_2$/ Gellan gum	0.3%	0.3%	>100 °C

DBS-CONHNH$_2$/ Gellan gum	0.3%	0.5%	>100 °C
DBS-CONHNH$_2$/ Gellan gum	0.3%	1.0%	>100 °C

S2.7 Rheology

Gel samples for rheology were prepared as described in Section S2 using bottomless vials as templates to obtain the intended gel dimensions. All the gellan gum and DBS-CONHNH$_2$/gellan gum hybrid gels were prepared using $CaCl_2$ (5% wt/vol) as a cross-linker. The measurements were carried out at 25°C using a 20 mm parallel plate and a gap of 2 mm. To avoid solvent evaporation and keep the sample hydrated, a solvent trap was used, and the internal atmosphere was kept saturated. Amplitude sweep experiments were performed in the range of 0.05-100% strain at a 1 Hz frequency to identify the linear viscoelastic region. Frequency sweep experiments were performed between 0.1 and 100 Hz using a shear strain of 0.25%. The measurements were repeated three times to ensure reproducibility and the average data are shown.

Table S2. Rheological data as determined using oscillatory rheometry with a parallel plate geometry, for DBS-CONHNH$_2$ gels, calcium alginate gels, and hybrid gels formed by the combination of the two. Loadings are given in wt/vol, and the G'/G" crossover points refer to the % shear strain at which G"=G'.

Gel	Loading of LMWG	Loading of Gellan gum	Total Loading	G' (Pa)	G'/G" Crossover
DBS-CONHNH$_2$	0.4%	-	0.4%	800	25.1%
Gellan Gum	-	0.4%	0.4%	3290	1.0%
Gellan Gum	-	0.6%	0.6%	4560	1.1%
Gellan Gum	-	0.8%	0.8%	10500	2.0%
Gellan Gum	-	1.3%	1.3%	17300	1.0%
Hybrid	0.3%	0.1%	0.4%	3980	6.3%
Hybrid	0.3%	0.3%	0.6%	11200	7.7%
Hybrid	0.3%	0.5%	0.8%	23500	6.9%
Hybrid	0.3%	1.0%	1.3%	46600	2.3%

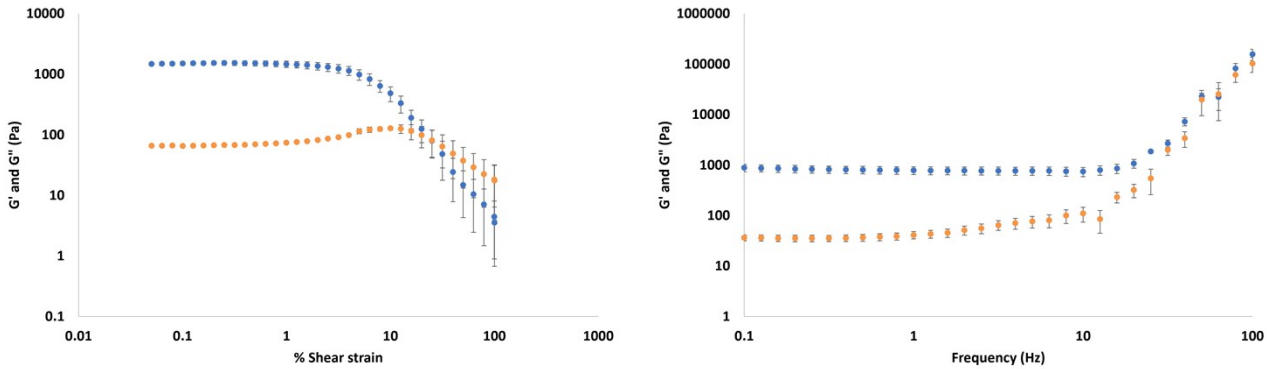

Figure S9. Elastic (G', blue circles) and viscous (G", orange circles) moduli of DBS-CONHNH$_2$ hydrogel (0.4% wt/vol) with increasing shear strain (left) and frequency (right).

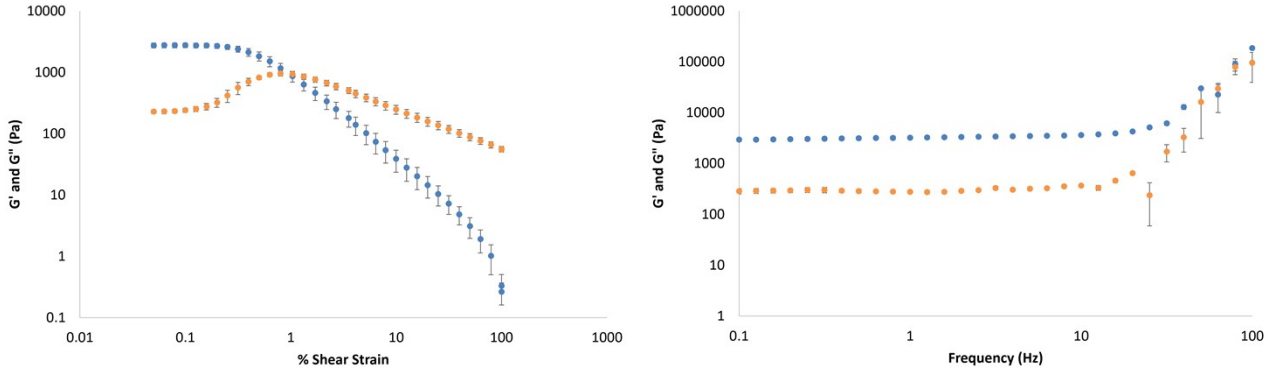

Figure S10. Elastic (G', blue circles) and viscous (G", orange circles) moduli of gellan gum hydrogel (0.4% wt/vol) with increasing shear strain (left) and frequency (right).

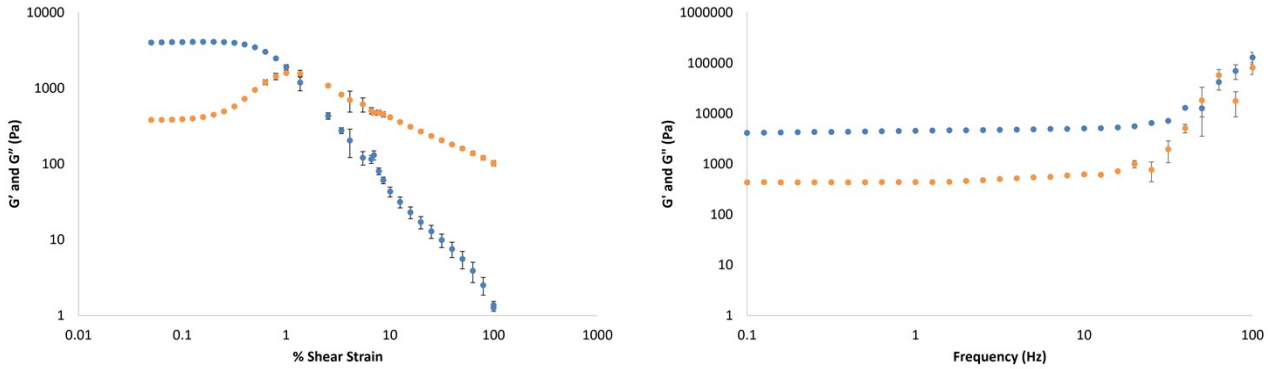

Figure S11. Elastic (G', blue circles) and viscous (G", orange circles) moduli of gellan gum hydrogel (0.6% wt/vol) with increasing shear strain (left) and frequency (right).

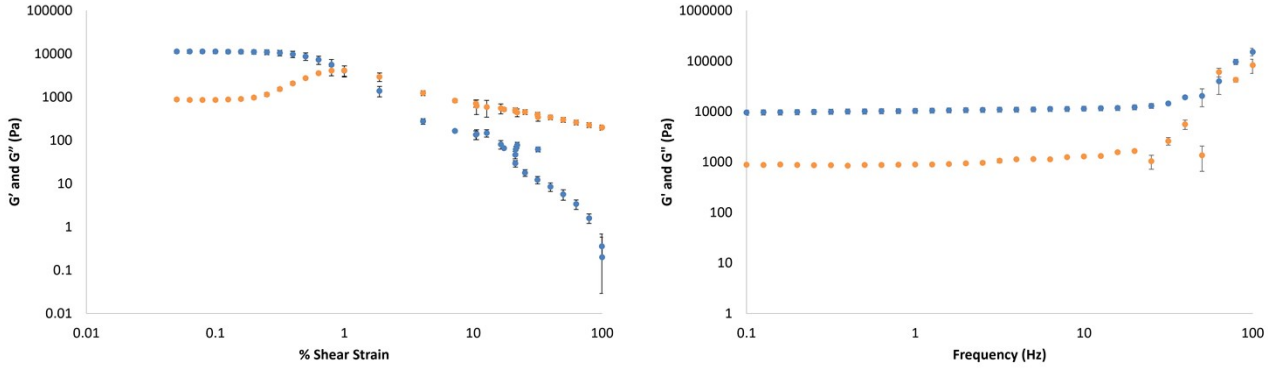

Figure S12. Elastic (G', blue circles) and viscous (G", orange circles) moduli of gellan gum hydrogel (0.8% wt/vol) with increasing shear strain (left) and frequency (right).

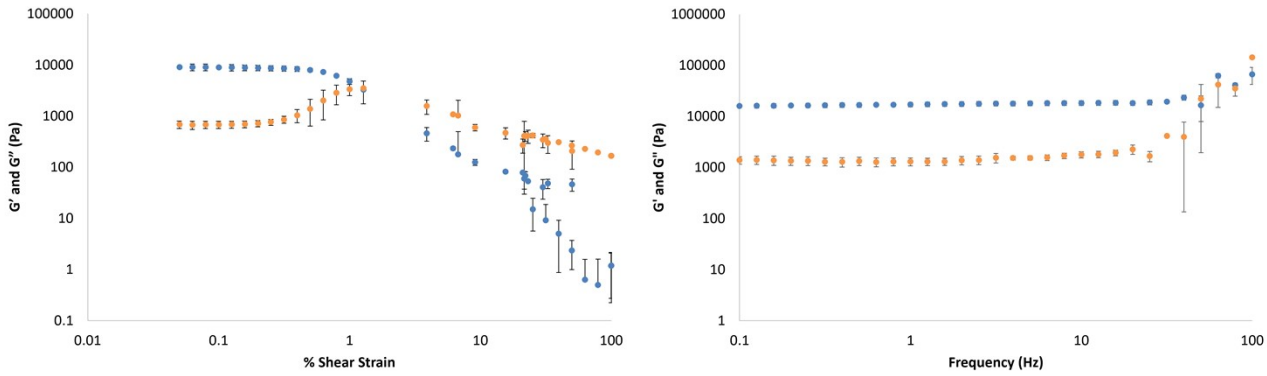

Figure S13. Elastic (G', blue circles) and viscous (G", orange circles) moduli of gellan gum hydrogel (1.3% wt/vol) with increasing shear strain (left) and frequency (right).

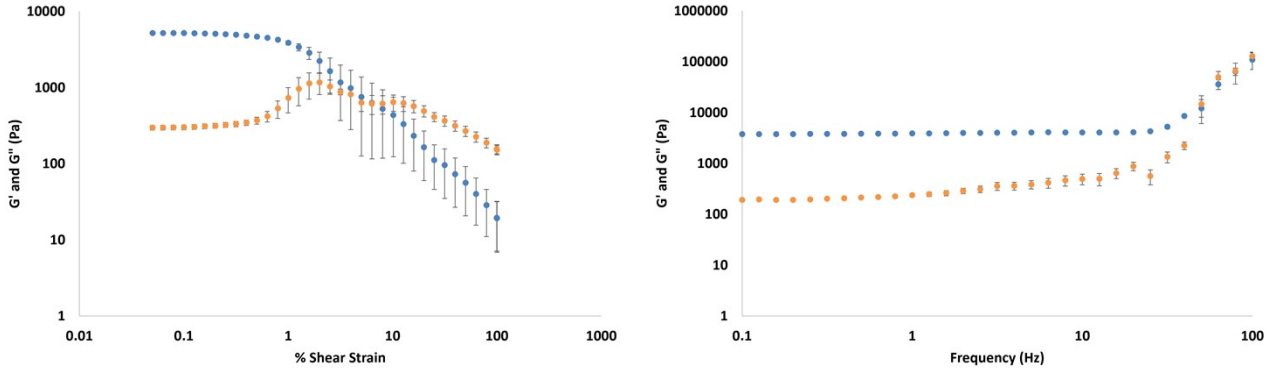

Figure S14. Elastic (G', blue circles) and viscous (G", orange circles) moduli of DBS-$CONHNH_2$/gellan gum two-component hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.1% wt/vol gellan gum) with increasing shear strain (left) and frequency (right).

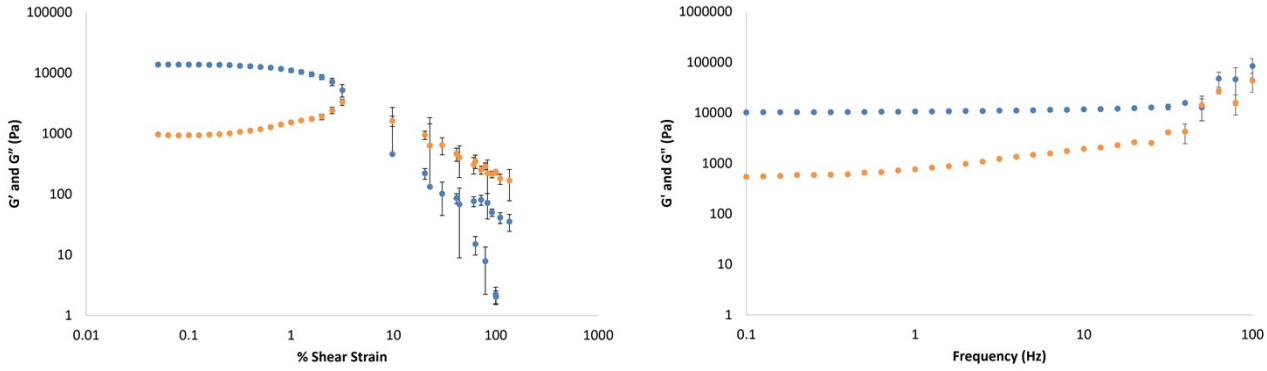

Figure S15. Elastic (G', blue circles) and viscous (G", orange circles) moduli of DBS-$CONHNH_2$/gellan gum multicomponent hydrogel (0.3% wt/vol of both gelators) with increasing shear strain (left) and frequency (right).

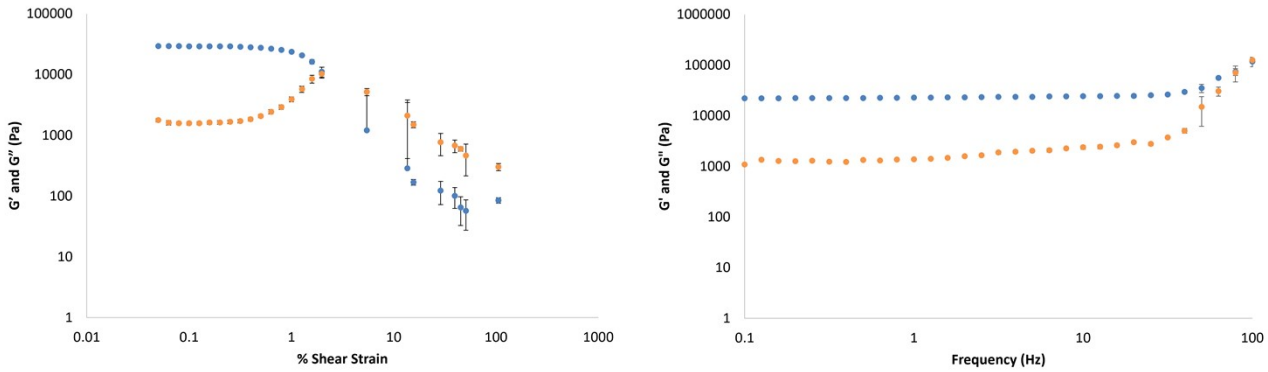

Figure S16. Elastic (G', blue circles) and viscous (G", orange circles) moduli of DBS-CONHNH$_2$/gellan gum multicomponent hydrogel (0.3% wt/vol DBS-CONHNH$_2$ and 0.5% wt/vol gellan gum) with increasing shear strain (left) and frequency (right).

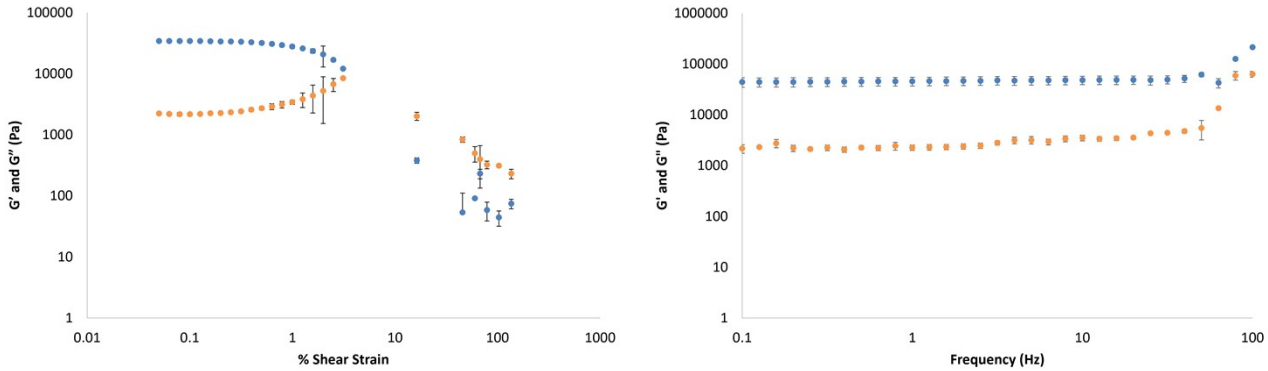

Figure S17. Elastic (G', blue circles) and viscous (G", orange circles) moduli of DBS-CONHNH$_2$/gellan gum multicomponent hydrogel (0.3% wt/vol DBS-CONHNH$_2$ and 1.0% wt/vol gellan gum) with increasing shear strain (left) and frequency (right).

S3 Preparation and characterisation of DBS-CONHNH$_2$, DBS-CONHNH$_2$/gellan gum and gellan gum gels cross-linked with $CaCO_3$ and Glucono-δ-Lactone (GdL)

S3.1 Gel preparation

S3.1.1 Preparation of DBS-CONHNH$_2$/gellan gum multicomponent gel beads. DBS-CONHNH$_2$ (0.3% wt/vol in 1 mL final total volume) and $CaCO_3$ (0.15% wt/vol in 1 mL final total volume) were suspended in water (0.5 mL) and sonicated to help the dispersion of the solid particles. An aqueous gellan gum solution (1.0% wt/vol - 0.5 mL) and GdL (0.8% wt/vol in 1 mL final total volume) were then added. The resulting suspension was heated until complete dissolution of the DBS-CONHNH$_2$. The hot solution was then added dropwise (20 μL/drop) to paraffin oil (c.a. 50 mL). The droplets were left undisturbed overnight to allow gel formation. After this time, the gel beads were collected with a spatula and, to remove residual paraffin oil, they were immersed in petroleum ether (30 mL, 30 mins), then EtOH (30 mL, 30 mins) and, finally, water (30 mL, 30 mins). When necessary, the washings were performed multiple times. The gel beads were then stored in water.

S3.1.2 Preparation of DBS-CONHNH$_2$/gellan gum gels in sample vials using GdL as a pH activator (for pH, IR, thermal stability and rheology studies). DBS-CONHNH$_2$ (0.3% wt/vol in 1 mL final total volume) and $CaCO_3$

(0.15% wt/vol in 1 mL final total volume, unless otherwise specified) were suspended in water (0.5 mL) and sonicated to help the dispersion of the solid particles. An aqueous gellan gum solution (1.0% wt/vol - 0.5 mL) was then added. The amount of gellan gum and water was adjusted depending on the desired final concentration of the polymer in the different experiments. The resulting suspension was heated until complete dissolution of the DBS-$CONHNH_2$ and then transferred to a new sample vial containing GdL (0.8% wt/vol in 1 mL final total volume, unless otherwise specified). The sample was left undisturbed overnight to allow gel formation.

S3.1.3 Preparation of gellan gum gels using GdL as a pH activator. Gellan gum gels were prepared by adding $CaCO_3$ (0.15% wt/vol in 1 mL final total volume, unless otherwise specified) to an aqueous gellan gum solution (0.4-1.3% wt/vol). The suspension was sonicated to help the dispersion of the $CaCO_3$ solid particles and GdL (0.8% wt/vol in 1 mL final total volume, unless otherwise specified) was then added. The sample was left undisturbed overnight to allow gel formation.

S3.2 pH studies

The pH of the DBS-$CONHNH_2$/gellan gum hybrid gels and gellan gum gels was monitored over time at regular time intervals during gel formation. The gels were prepared in sample vials as described in Sections S3.1.2 and S3.1.3 using variable gellan gum concentrations (0.1-0.8% wt/vol) in the presence of $CaCO_3$ (0.15% wt/vol) and GdL (0.8% wt/vol).

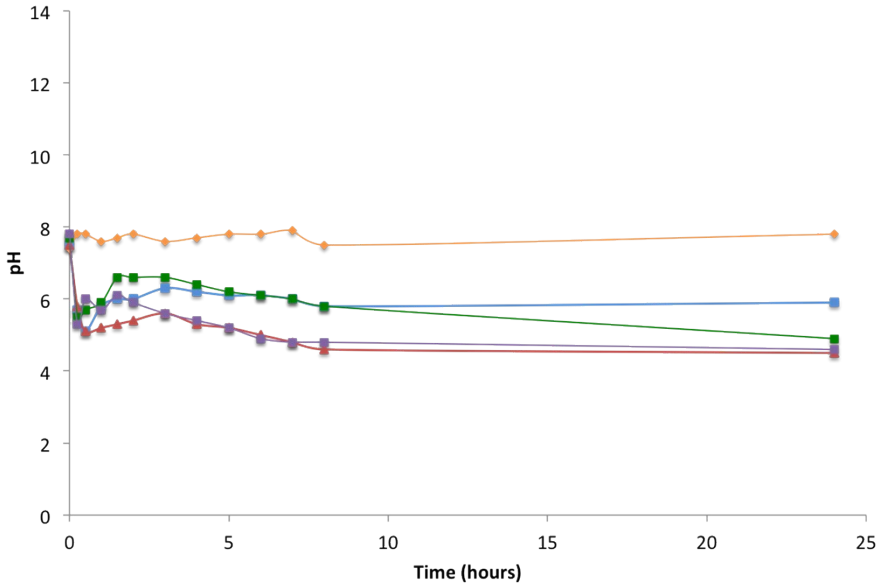

Figure S18. pH changes over time during the formation of the DBS-$CONHNH_2$ gel (0.3% wt/vol, orange line) and DBS-$CONHNH_2$/gellan gum hybrid gels ($CaCO_3$ - 0.15% wt/vol and GdL - 0.8% wt/vol) containing: 0.3% wt/vol of DBS-$CONHNH_2$ and 0.1% wt/vol gellan gum (blue line), 0.3% wt/vol gellan gum (red line), 0.5% wt/vol gellan gum (green line) or 1.0% wt/vol gellan gum (purple line).

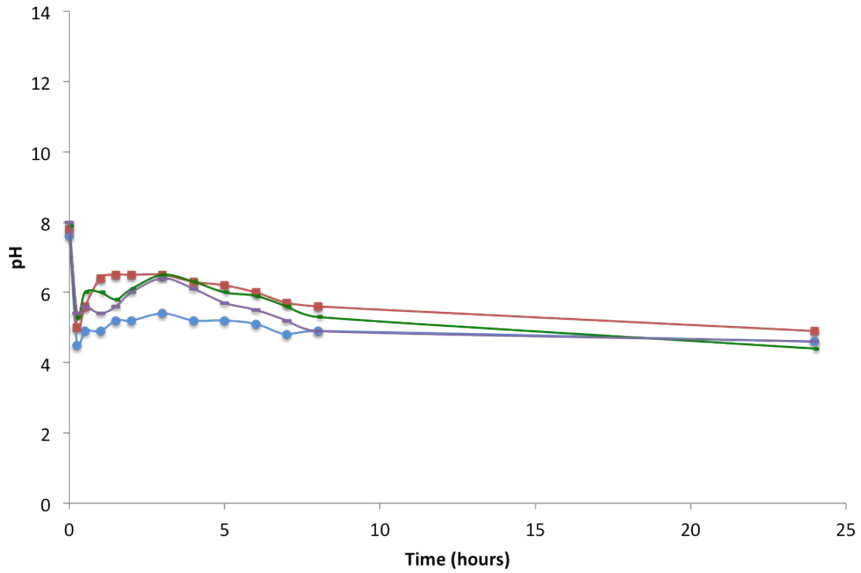

Figure S19. pH changes over time during the formation of gellan gum gels ($CaCO_3$ - 0.15% wt/vol and GdL - 0.8% wt/vol) prepared using a gellan gum concentration of 0.4% wt/vol (blue line), 0.6% wt/vol (red line), 0.8% wt/vol (green line) or 1.3% wt/vol (purple line).

S3.3 NMR assays

S3.3.1 *Self-assembled state of DBS-CONHNH$_2$/gellan gum gel beads.* ^{1}H NMR was employed to validate the efficacy of the gel preparation method and to confirm that the two gelators were in a self-assembled state. The gel beads used for this experiment were prepared combining DBS-CONHNH$_2$ (0.3 % wt/vol) and gellan gum (0.5 % wt/vol) by the emulsion method described in Section S3.1.1. Five gel beads were isolated and transferred into a NMR tube in D_2O (0.7 mL). DMSO (1.4 μL) was added as an internal standard. The gel beads were then analysed by ^{1}H NMR. The lack of DBS-CONHNH$_2$ aromatic signals confirms its self-assembled state.

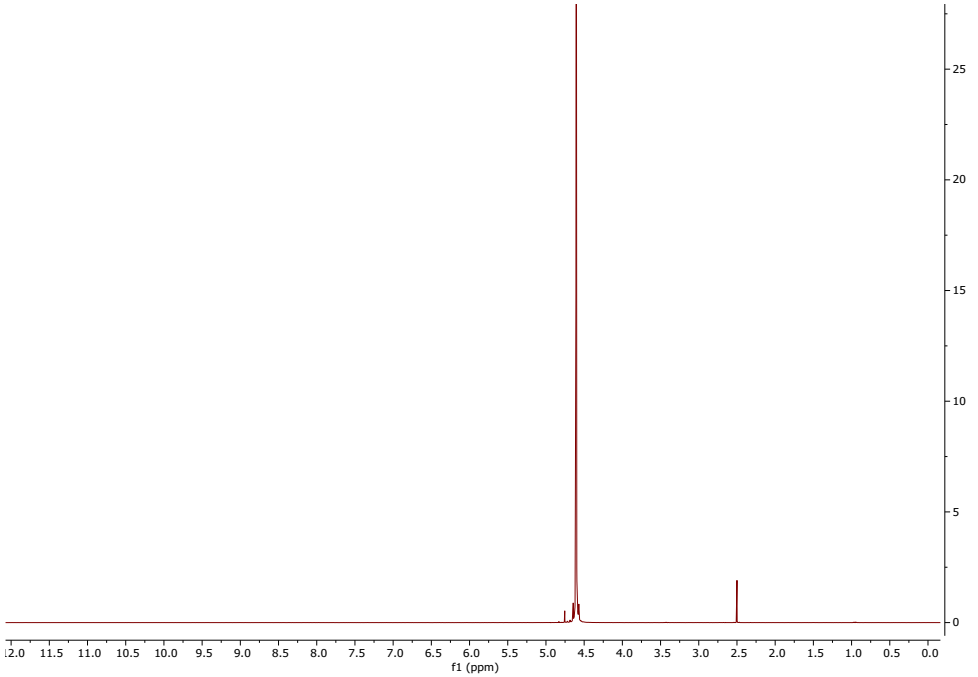

Figure S20. ^{1}H NMR of five DBS-CONHNH$_2$/gellan gum gel beads prepared using $CaCO_3$/GdL as a cross-linker.

S3.3.2 *Quantification of DBS-CONHNH$_2$ incorporated into ten DBS-CONHNH$_2$/gellan gum gel beads.* ^{1}H NMR was employed to calculate the exact amount of DBS-CONHNH$_2$ incorporated into the DBS-CONHNH$_2$/gellan gum gel beads prepared by emulsion using $CaCO_3$/GdL as a cross-linker. The gel beads used for this experiment were prepared combining DBS-CONHNH$_2$ (0.3 % wt/vol) and gellan gum (0.5 % wt/vol) by the emulsion method described in Section S3.1.1. Five gel beads were isolated and dried under high vacuum. The resulting solid was dissolved in DMSO-d_6 (0.7 mL), and acetonitrile (1.4 μL) was added as an internal standard. To make sure that all the DBS-CONHNH$_2$ was dissolved, the sample was ground and then sonicated for 30 min. The ^{1}H NMR spectrum was recorded and the concentration of the LMWG calculated by comparison of the integrals of relevant peaks (DBS-CONHNH$_2$ aromatic peaks δ = 7.53 and 7.83 ppm) to that of acetonitrile (δ = 2.09 ppm). To ensure the results were reproducible, this experiment was performed on two different batches of gel beads.

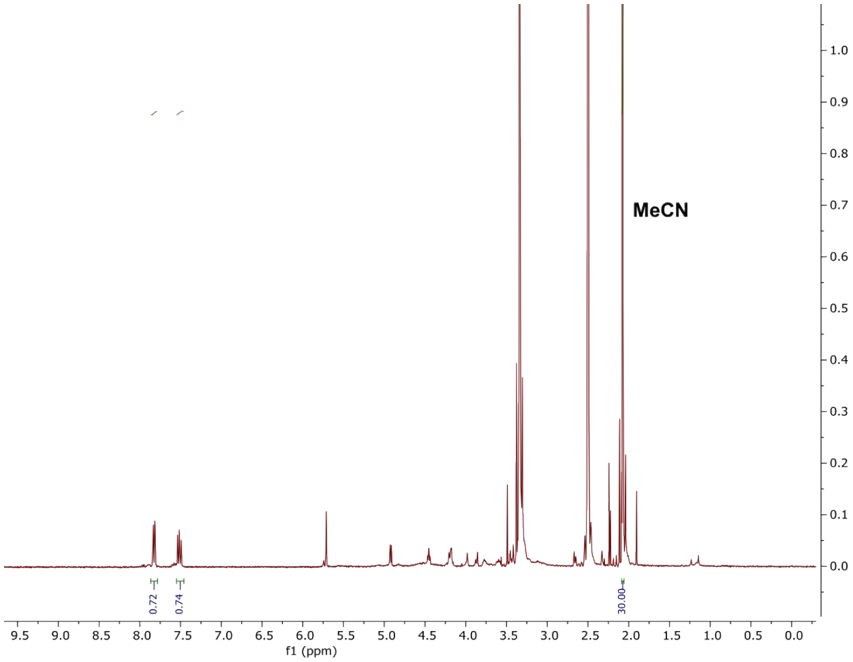

Figure S21. ^{1}H NMR of DBS-CONHNH$_2$ incorporated into 5 DBS-CONHNH$_2$/gellan gum gel beads prepared using $CaCO_3$/GdL as a cross-linker.

S3.3.3 *NMR study over time at 90°C.* ^{1}H NMR was employed to monitor the disruption of the DBS- CONHNH$_2$ network incorporated into the DBS-CONHNH$_2$/gellan gum gel beads prepared by emulsion and cross-linked with $CaCl_2$ and $CaCO_3$/GdL. The gel beads used for this experiment were prepared combining DBS-CONHNH$_2$ (0.3 % wt/vol) and gellan gum (0.5 % wt/vol) by the emulsion method described in Sections S2.1.2 and S3.1.1. Ten gel beads were isolated and transferred into a NMR tube in D_2O (0.5 mL). DMSO (1.4 μL) was added as an internal standard. The samples were then placed in the spectrometer. A ^{1}H NMR was immediately recorded. The sample was then heated to 90 °C. Spectra were recorded at 90 °C every 30 minutes for 14 hours. The concentration of the mobile components was calculated by comparison of the integrals of relevant peaks (DBS-CONHNH$_2$ aromatic peaks δ = 7.55 and 7.62) to that of DMSO (δ = 2.50 ppm).

Table S3. Percentage of unbound DBS-$CONHNH_2$ into DBS-$CONHNH_2$/gellan gum gel beads over time at a constant temperature of 90 °C.

Time (hours)	% of DBS-$CONHNH_2$ released from DBS-$CONHNH_2$/gellan gum gel beads cross-linked with $CaCl_2$	% of DBS-$CONHNH_2$ released from DBS-$CONHNH_2$/gellan gum gel beads cross-linked with $CaCO_3$/GdL
0	0	0
0.25	40	38
0.5	56.4	51.6
1	58.0	57.4
1.5	59.2	60.3
2	59.5	62.8
2.5	59.8	63.4
3	59.9	63.8
3.5	60.3	64.8
4	60.5	67.1
4.5	60.7	67.3
5	60.5	68.9
5.5	60.7	71.0
6	60.8	71.2
6.5	60.4	70.4
7	60.2	69.5
7.5	60.5	69.8
8	60.3	71.4
8.5	59.0	71.0
9	60.2	71.2
9.5	60.4	71.2
10	59.9	70.8
10.5	60.7	71.6
11	60.7	69.7
11.5	60.7	72.6
12	61.0	67.1
12.5	60.7	71.0
13	60.7	71.4
13.5	60.7	71.0
14	60.7	71.2

S3.4 Infrared (IR) spectroscopy

Xerogel samples for infrared were prepared in sample vials as described in Section S3.1.2 and S3.1.2 adding HCl (1M, 15 μL) instead of GdL. The solvent was then removed from the gels under high vacuum. A small amount of the resulting powder was placed into the infrared spectrophotometer and the spectra recorded in the range of 450-4000 cm^{-1}.

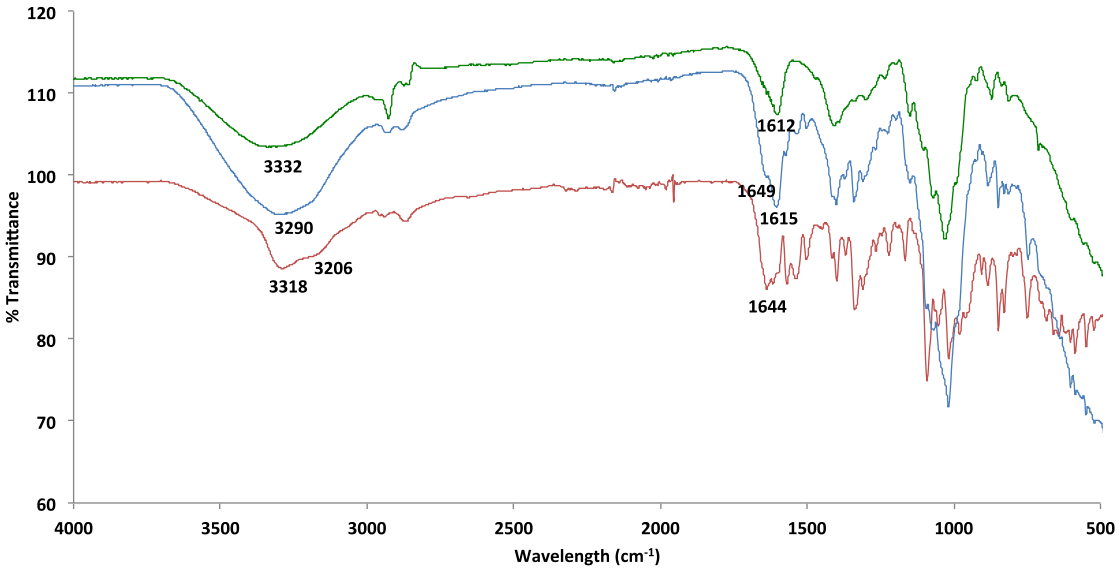

Figure S22. IR spectra of xerogels obtained from DBS-$CONHNH_2$ gel (0.4% wt/vol, red line), gellan gum gel (0.8% wt/vol, green line) and DBS-$CONHNH_2$/gellan gum gel containing 0.3% wt/vol of DBS-$CONHNH_2$ and 0.5% wt/vol gellan gum (green line).

S3.5 Optical microscopy

Optical microscopy images were collected as described in Section xx. All the gel beads were prepared in 20 µL volumes using 0.3% wt/vol of DBS-$CONHNH_2$, 0.5% wt/vol of gellan gum, 0.8% wt.vol GdL and 0.15% wt/vol $CaCO_3$.

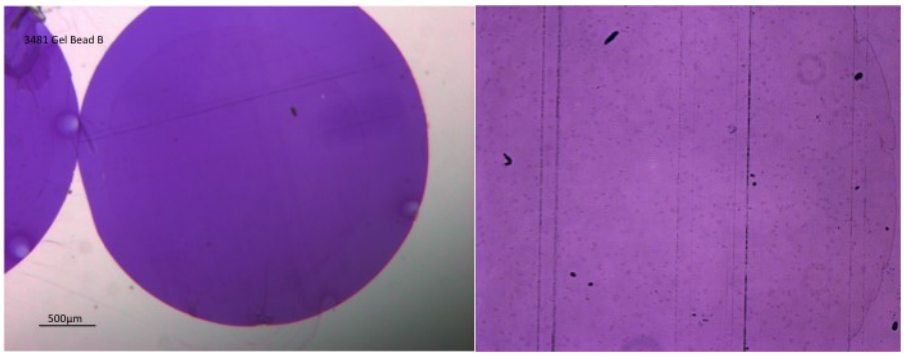

Figure S23. Optical microscopy image of DBS-$CONHNH_2$/gellan gum gel bead embedded in resin and stained with toluidine blue.

S3.6 Transmission and Scanning Electron Microscopy (TEM and SEM)

Samples for TEM and SEM imaging were prepared in as described in Section S3.1 and treated for analysis as described in Section S2.5.

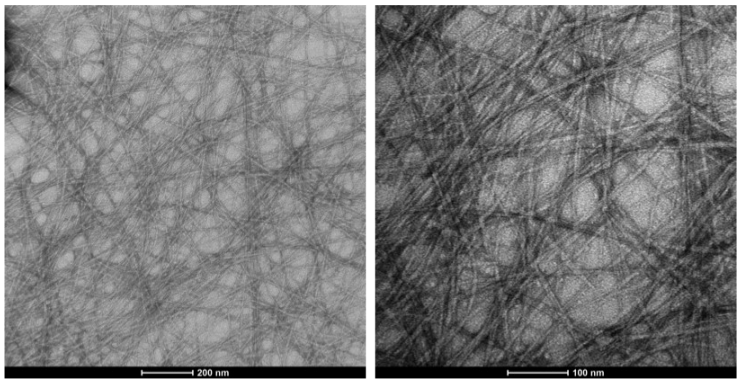

Figure S24. TEM images of DBS-$CONHNH_2$/gellan gum hybrid gel prepared using $CaCO_3$ as a cross-linker and GdL as a pH activator. Scale bars from left to right: 200 and 100 nm.

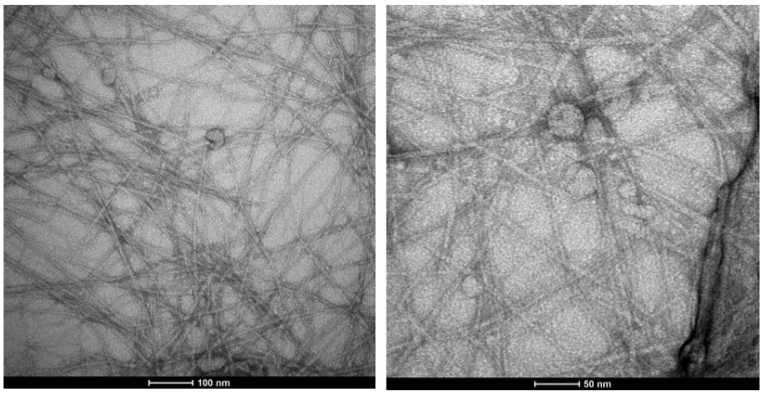

Figure S25. TEM images of gellan gum gel prepared using $CaCO_3$ as a cross-linker and GdL as a pH activator. Scale bars from left to right: 100 and 50 nm.

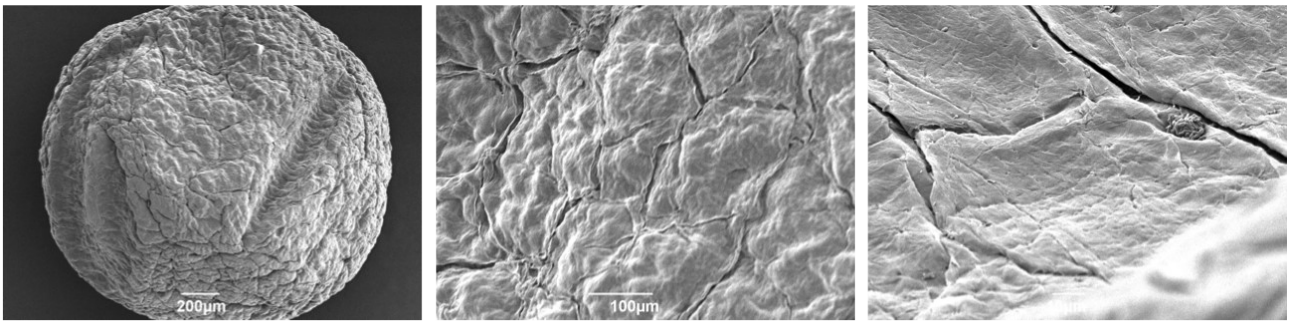

Figure S26. SEM images of DBS-$CONHNH_2$/gellan gum whole gel bead and surface (prepared using $CaCO_3$ as a cross-linker and GdL as a pH activator). Scale bars (from left to right): 200 (left) and 100 μm (centre and right).

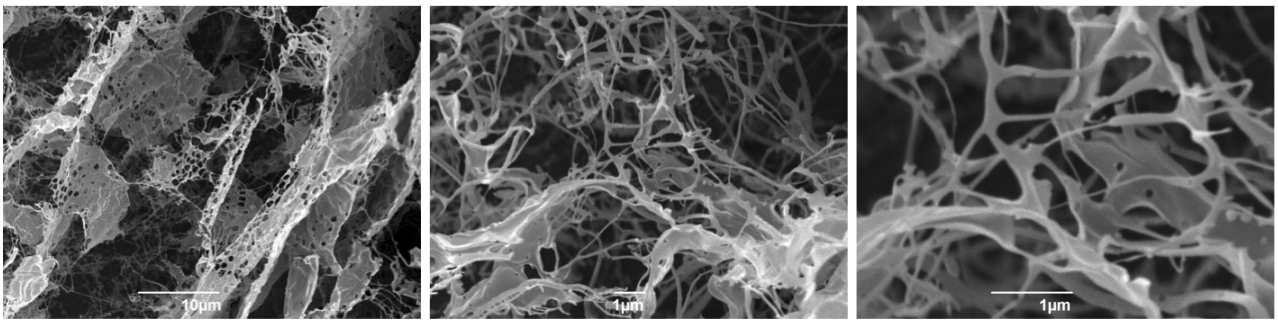

Figure S27. SEM images of DBS-$CONHNH_2$/gellan gum gel bead cross-section (prepared using $CaCO_3$ as a cross-linker and GdL as a pH activator). Scale bars (from left to right): 10 (left) and 1 μm (centre and right).

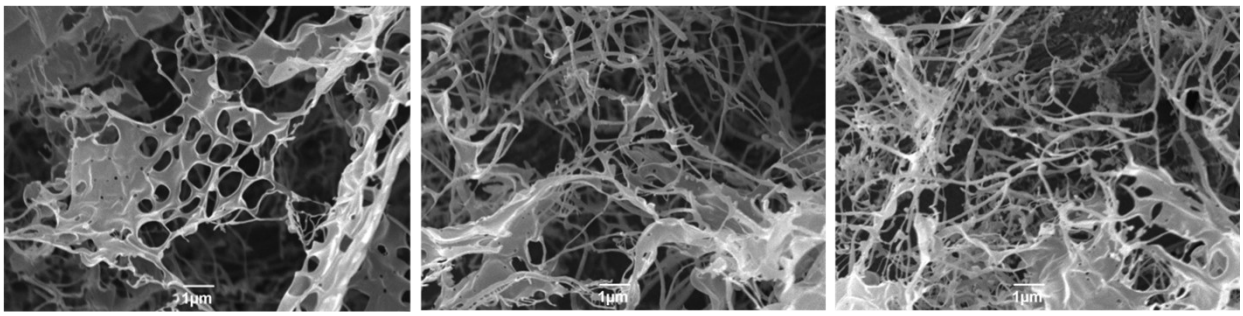

Figure S28. SEM images of DBS-$CONHNH_2$/gellan gum hybrid gel prepared in sample vials using $CaCO_3$ as a cross-linker and GdL as a pH activator. Scale bars: 1 μm.

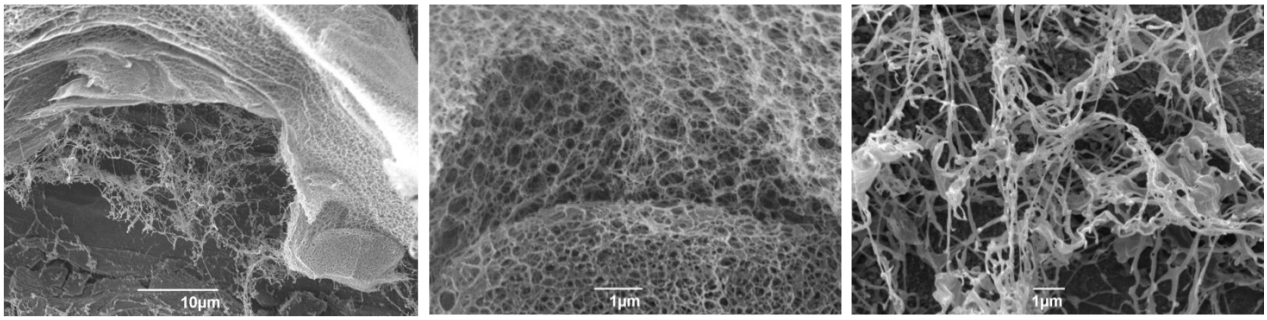

Figure S29. SEM images of gellan gum gel prepared using $CaCO_3$ as a cross-linker and GdL as a pH activator. Scale bars: 10 μm (left) and 1 μm (centre and right).

S3.7 Thermal stability studies

All the gels for T_{gel} determination were prepared as described in Section S3.1 in 2 mL vials (diameter: 1 cm, height: 4 cm). Tgel measurements were conducted as described in Section 2.6.

Table S4. T_{gel} values of gels formed by individual gelators and the gellan gum and DBS-$CONHNH_2$/gellan gum gels prepared using $CaCO_3$ as a cross-linker (0.15% wt/vol) and GdL (1.0% wt/vol) as a pH activator.

Gel (1 mL total volume)	Loading of DBS-$CONHNH_2$ (wt/vol)	Loading of Gellan gum (wt/vol)	T_{gel}
DBS-$CONHNH_2$	0.4%	-	86 °C
Gellan gum	-	0.8%	>100 °C
DBS-$CONHNH_2$/gellan gum gel	0.3%	0.5%	>100 °C

S3.8 Rheology

Rheology measurements were performed as described in Section S2.7. All the gellan gum and DBS-$CONHNH_2$/gellan gum hybrid gels were prepared using $CaCO_3$ (0.15% wt/vol) as a cross-linker and GdL (1.0% wt/vol) as a pH activator, unless otherwise specified.

Table S5. Rheological data as determined using oscillatory rheometry with a parallel plate geometry, for DBS-$CONHNH_2$ gels, calcium gellan gum gels, and hybrid gels formed by the combination of the two. Loadings are given in wt/vol, and the G'/G" crossover points refer to the % shear strain at which G"=G'.

Gel	Loading of LMWG	Loading of Gellan gum	Total Loading	Loading of $CaCO_3$	Loading of GdL	G' (Pa)	G'/G" Crossover
Gellan gum	-	0.4%	0.4%	0.15%	1.0%	1760	0.8%
Gellan gum	-	0.6%	0.6%	0.15%	1.0%	4630	0.5%
Gellan gum	-	0.8%	0.8%	0.15%	1.0%	8950	1.27%
Hybrid	0.3%	0.1%	0.4%	0.15%	1.0%	2580	15.9%
Hybrid	0.3%	0.3%	0.6%	0.15%	1.0%	11300	7.4%
Hybrid	0.3%	0.3%	0.6%	0.075%	1.0%	5720	3.2%
Hybrid	0.3%	0.3%	0.6%	0.3%	1.0%	17200	5.2%
Hybrid	0.3%	0.3%	0.6%	0.15%	0.8%	10900	2.5%
Hybrid	0.3%	0.3%	0.6%	0.15%	1.2%	14300	8.0%
Hybrid	0.3%	0.5%	0.8%	0.15%	1.0%	19300	5.4%

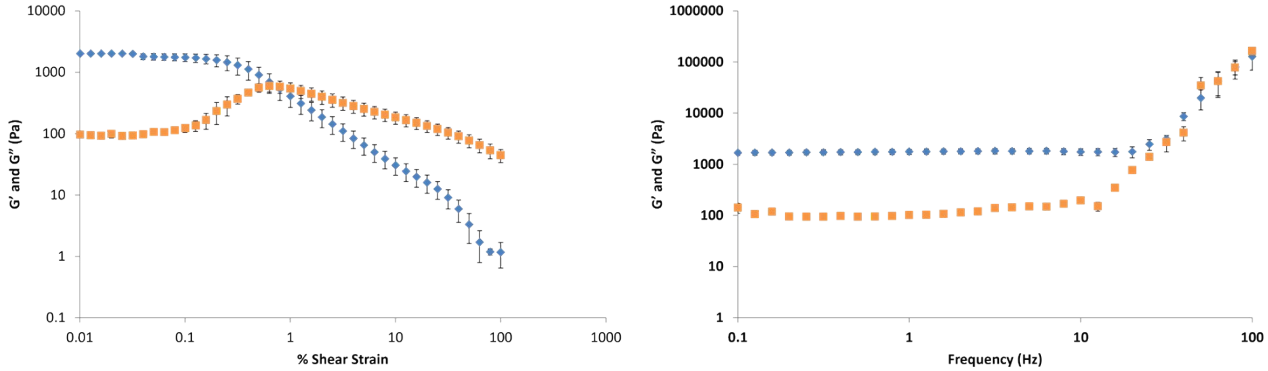

Figure S30. Elastic (G', blue circles) and viscous (G", red circles) moduli of gellan gum hydrogel (0.4% wt/vol) prepared with 0.15% wt/vol $CaCO_3$ and 0.8% wt/vol GdL with increasing shear strain (left) and frequency (right).

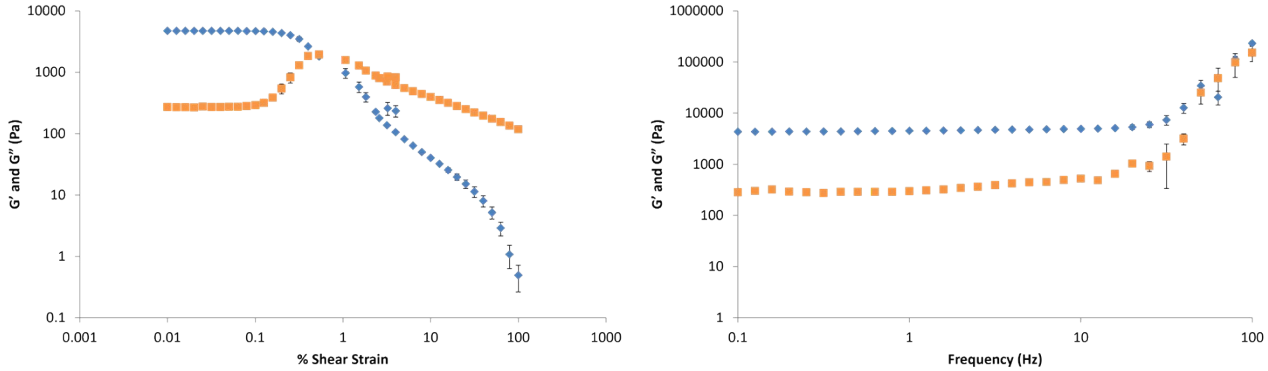

Figure S31. Elastic (G', blue circles) and viscous (G", red circles) moduli of gellan gum hydrogel (0.6% wt/vol) prepared with 0.15% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

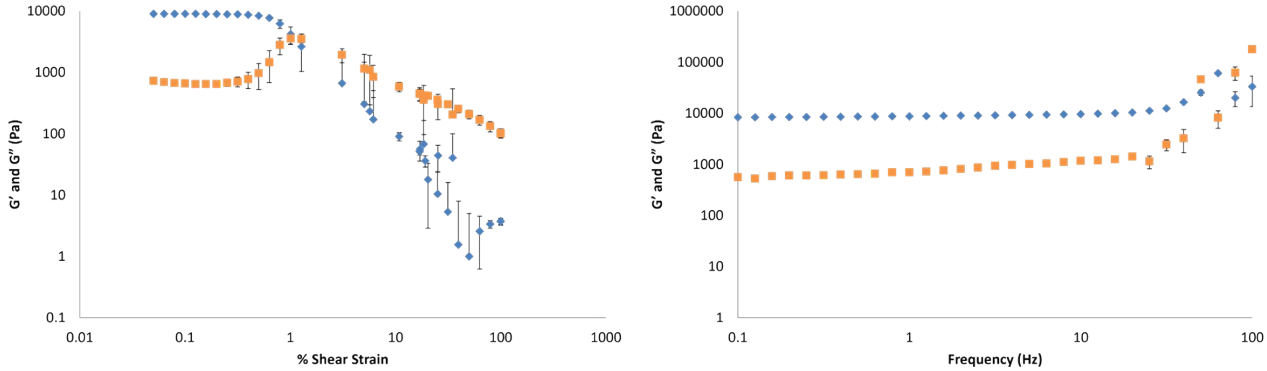

Figure S32. Elastic (G', blue circles) and viscous (G", red circles) moduli of gellan gum hydrogel (0.8% wt/vol) prepared with 0.15% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

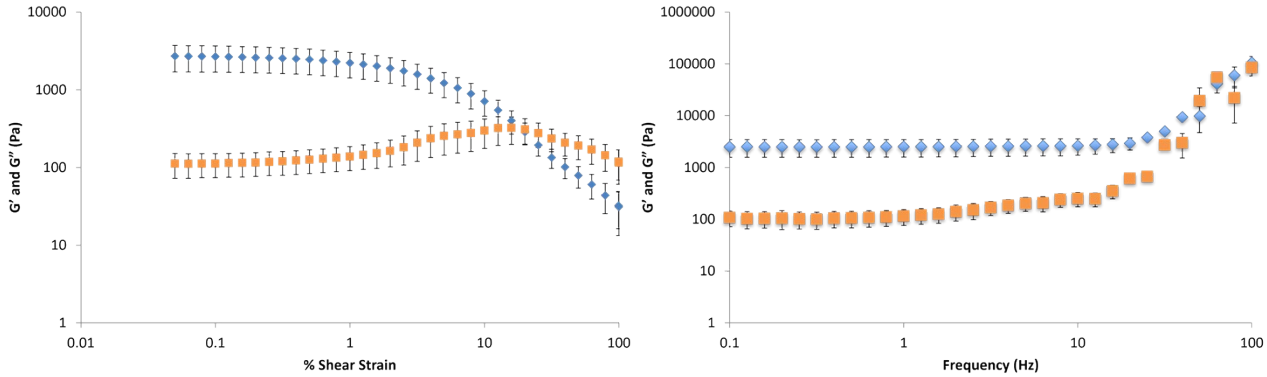

Figure S33. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.1% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

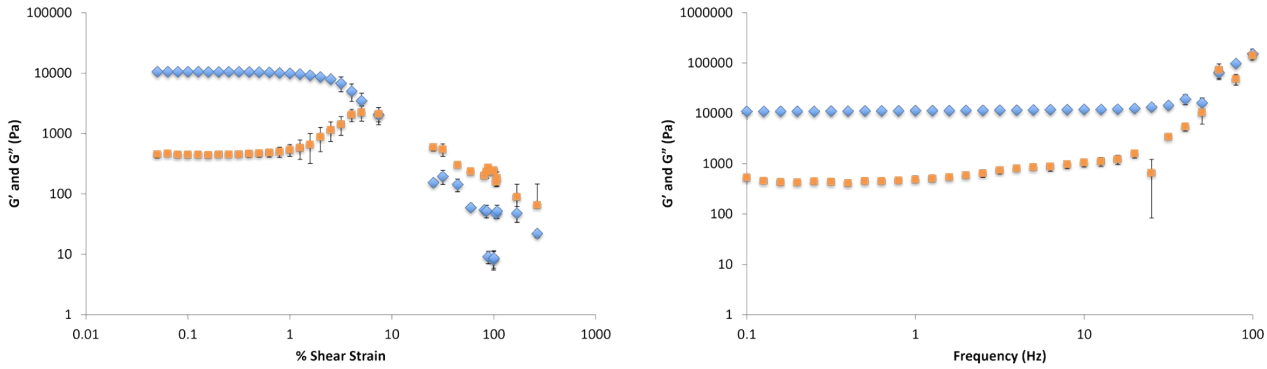

Figure S34. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

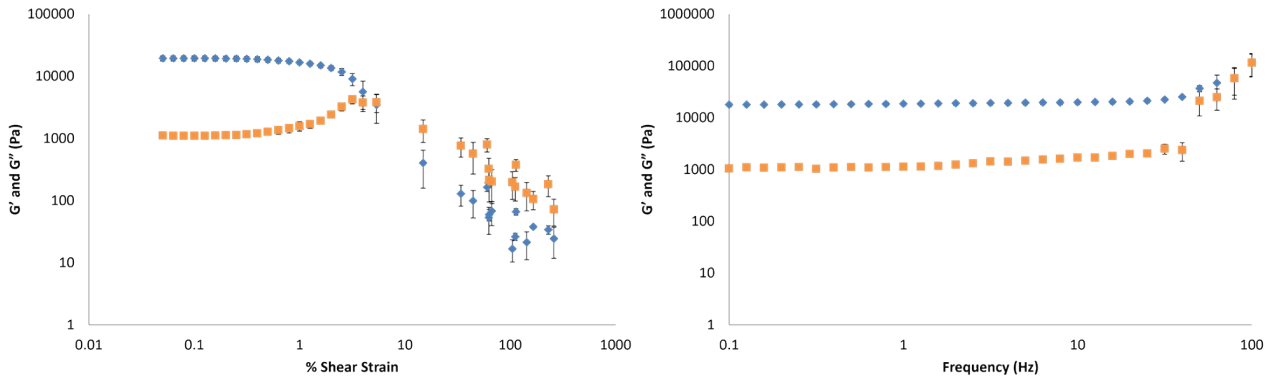

Figure S35. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.5% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

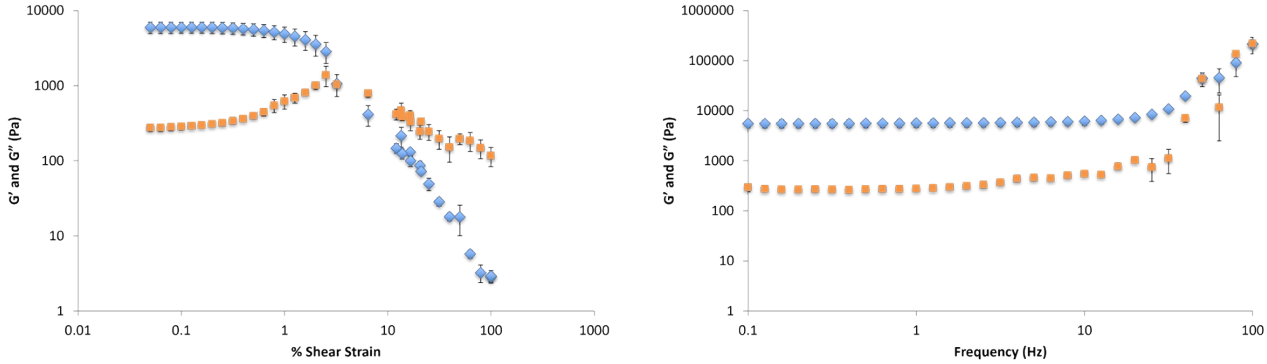

Figure S36. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.0075% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

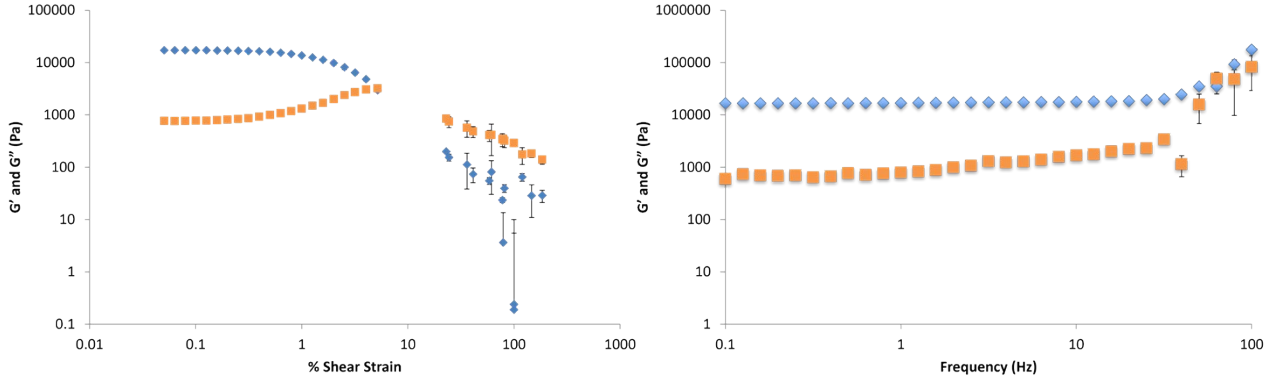

Figure S37. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.3% wt/vol $CaCO_3$ and 1.0% wt/vol GdL with increasing shear strain (left) and frequency (right).

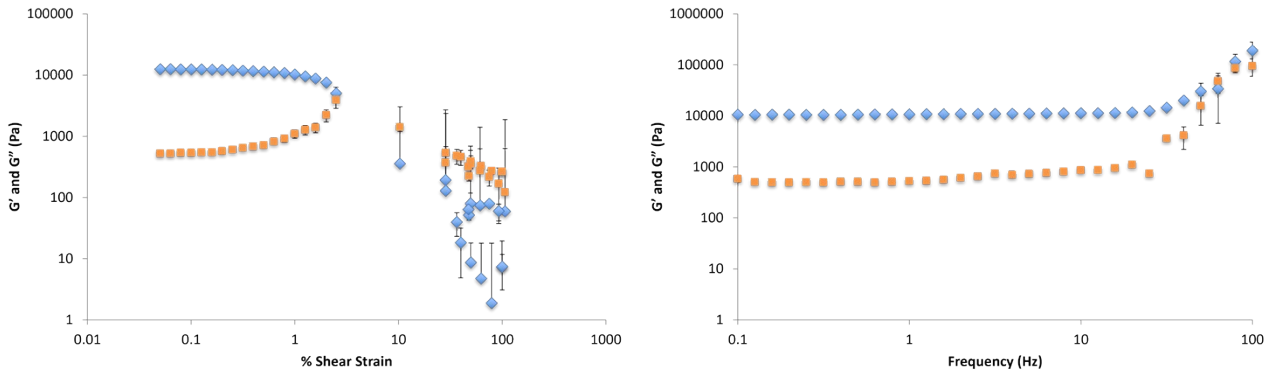

Figure S38. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 0.8% wt/vol GdL with increasing shear strain (left) and frequency (right).

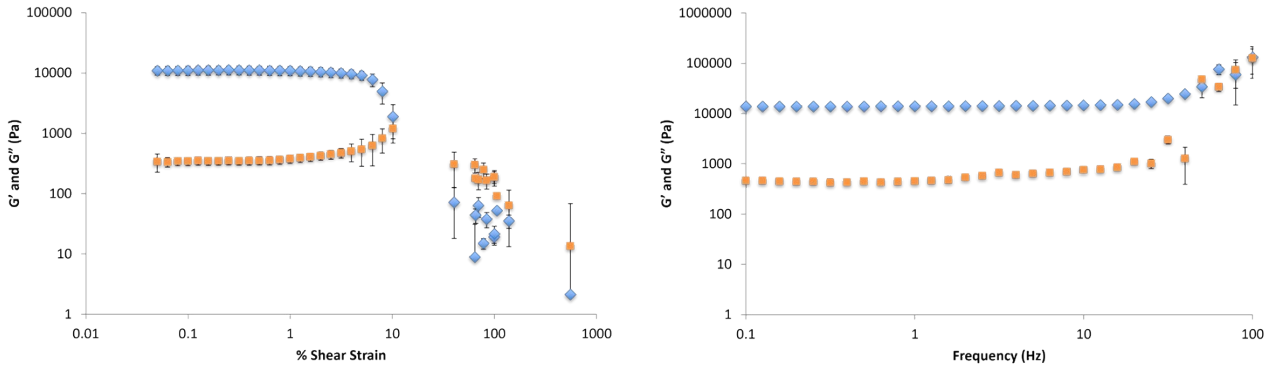

Figure S39. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 1.2% wt/vol GdL with increasing shear strain (left) and frequency (right).

S4 Preparation and characterisation of UV responsive DBS-$CONHNH_2$/gellan gum and gellan gum gels cross-linked with $CaCO_3$ and Diphenyliodonium Nitrate (DPIN)

S4.1 Gel preparation and photopatterning

S4.1.1 Preparation of DBS-$CONHNH_2$/gellan gum UV responsive gels using DPIN as a pH activator. DBS-$CONHNH_2$ (0.3% wt/vol in 1 mL final total volume) and $CaCO_3$ (0.15% wt/vol in 1 mL final total volume) were suspended in water (0.5 mL). The suspension was sonicated to help the dispersion of the solid particles and subsequently mixed with a DPIN aqueous solution (0.8% wt/vol in 1 mL final total volume), which was acidified by addition of a 1 M HCl solution (2.5 μL). An aqueous gellan gum solution (1.0% wt/vol - 0.5 mL) was then added. The amount of gellan gum and water was adjusted depending on the desired final concentration of the polymer in the different experiments. The resulting suspension was heated until complete dissolution of the DBS-$CONHNH_2$ and then placed in ice and exposed to UV light for 2 hours to allow gel formation.

S4.1.2 Preparation of UV responsive gellan gum gels using diphenyliodonium nitrate (DPIN) as a pH activator. Gellan gum gels were prepared by adding $CaCO_3$ (0.15% wt/vol in 1 mL final total volume) to an aqueous

gellan gum solution (0.4-1.3% wt/vol). The suspension was sonicated to help the dispersion of the $CaCO_3$ solid particles and subsequently mixed with a DPIN aqueous solution (0.8% wt/vol in 1 mL final total volume), which was acidified by addition of a 1 M HCl solution (2.5 μL). The amount of gellan gum and water was adjusted depending on the desired final concentration of the polymer in the different experiments. The sample was subsequently placed in ice and exposed to UV light for 2 hours to allow gel formation.

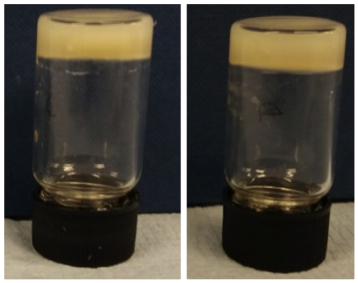

Figure S40. Photographic images of DBS-$CONHNH_2$/gellan gum (left) and gellan gum gels (right) prepared by photo-activation using $CaCO_3$ (0.15% wt/vol) and DPIN (0.8% wt/vol).

S4.1.3 Preparation of photopatterned DBS-$CONHNH_2$/gellan gum gels in trays. DBS-$CONHNH_2$ (0.3% wt/vol in 5 mL final total volume) and $CaCO_3$ (0.15% wt/vol in 5 mL final total volume) were suspended in 3.5 mL of a DPIN aqueous solution (0.8% wt/vol in 5 mL final total volume), which was acidified by addition of a 1 M HCl solution (12.5 μL). The suspension was sonicated to help the dispersion of the solid particles and subsequently mixed with an aqueous gellan gum solution (1.0% wt/vol - 1.5 mL). The resulting suspension was heated until complete dissolution of the DBS-$CONHNH_2$. The hot solution was then quickly transferred into a 5 x 5 cm glass tray. The sample was left undisturbed for 15 minutes to allow the formation of the DBS-$CONHNH_2$ network. A laser printed mask was then placed on top of the glass tray and the gel was exposed to UV light for two hours. To avoid the disruption of gelation due to heating effects, ice was placed below the glass tray.

S4.2 Transmission and Scanning Electron Microscopy (TEM and SEM)

Samples for TEM and SEM imaging were prepared in sample vials as described in Section S4.1 and treated for analysis as described in Section S2.5.

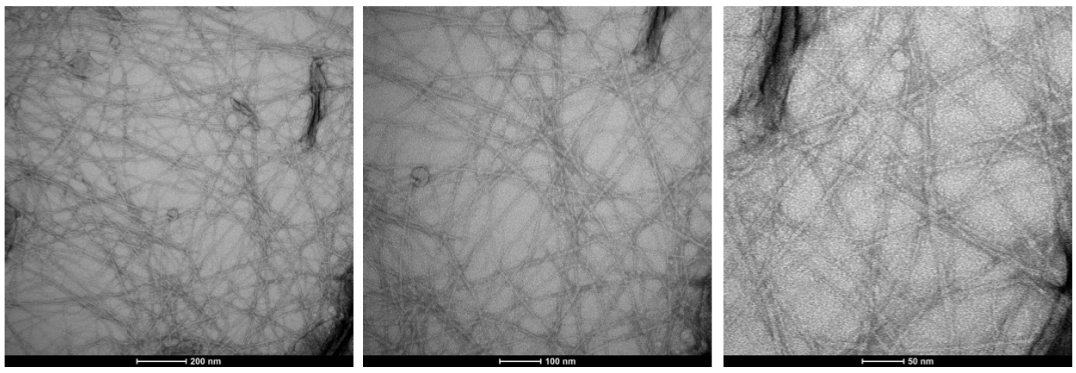

Figure S41. TEM image of photoactivated gellan gum gel prepared using $CaCO_3$ as a cross-linker and DPIN as a pH activator. Scale bars (from left to right): 200 nm, 100 nm and 50 nm.

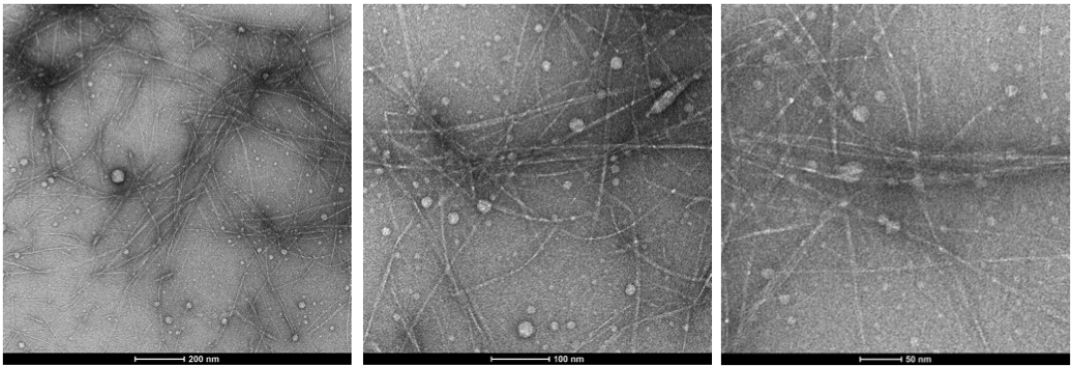

Figure S42. TEM image of photoactivated DBS-$CONHNH_2$/gellan gum hybrid gel prepared using $CaCO_3$ as a cross-linker and DPIN as a pH activator. Scale bars (from left to right): 200 nm, 100 nm and 50 nm.

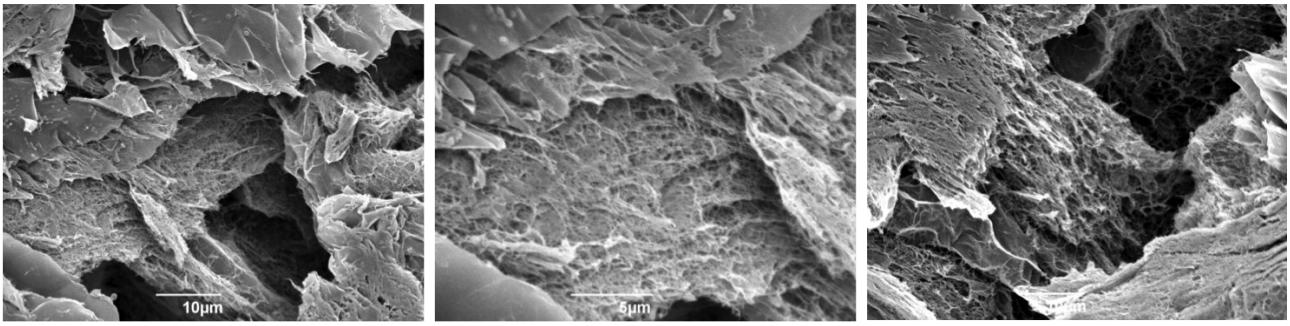

Figure S43. SEM images of photoactivated gellan gum prepared using $CaCO_3$ as a cross-linker and DPIN as a pH activator. Scale bars (from left to right): 10, 5 and 1 μm.

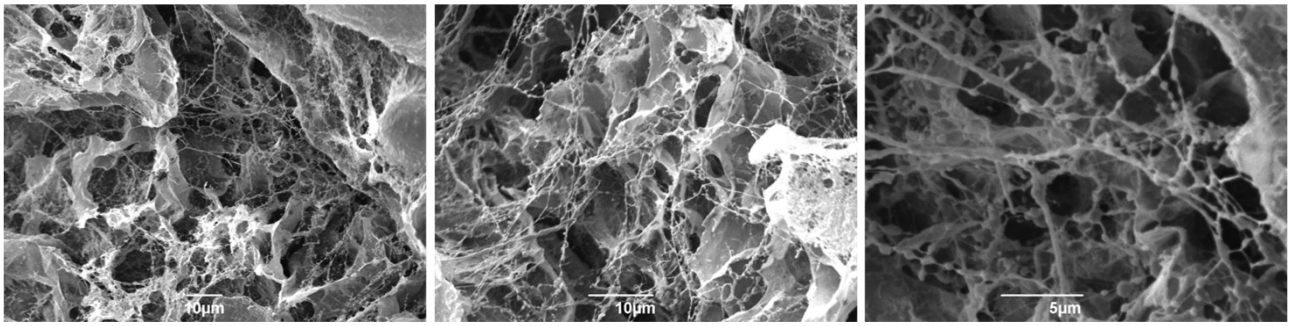

Figure S44. SEM images of photoactivated DBS-$CONHNH_2$/gellan gum prepared using $CaCO_3$ as a cross-linker and DPIN as a pH activator. Scale bars (from left to right): 10 μm (left and centre) and 5 μm (right).

S4.3 Thermal stability studies

All the gels for T_{gel} determination were prepared as described in Section S4.1 and T_{gel} values were obtained as descried in Section 2.6. These experiments were performed in triplicate to ensure reproducibility and the average is reported. Errors are estimated at ±2°C.

Table S6. T_{gel} values of photoactivated DBS-$CONHNH_2$/gellan gum and gellan gum gels prepared using $CaCO_3$ as a cross-linker (0.15% wt/vol) and DPIN (0.8% wt/vol) as a pH activator.

Gel (1 mL total volume)	**Loading of DBS-$CONHNH_2$ (wt/vol)**	**Loading of Gellan gum (wt/vol)**	**T_{gel}**
Gellan gum	-	0.8%	>100 °C
DBS-$CONHNH_2$/gellan gum hybrid gel	0.3%	0.5%	>100 °C

S4.4 Rheology

Gel samples for rheology were prepared as described in Section S4.1 using bottomless vials and the experiments were carried out as described in Section S2.7.

Table S7. Rheological data as determined using oscillatory rheometry with a parallel plate geometry, for photoactivated DBS-$CONHNH_2$/gellan gum and gellan gum gels. Loadings are given in wt/vol, and the G'/G" crossover points refer to the % shear strain at which G"=G'.

Gel	Loading of LMWG	Loading of Gellan gum	Total Loading	Loading of $CaCO_3$	Loading of DPIN	G' (Pa)	G'/G" Crossover
Hybrid	0.3%	0.3%	0.6%	0.15%	0.8%	12486	8.0%
Gellan gum	-	0.6%	0.6%	0.15%	0.8%	8670	6.4%

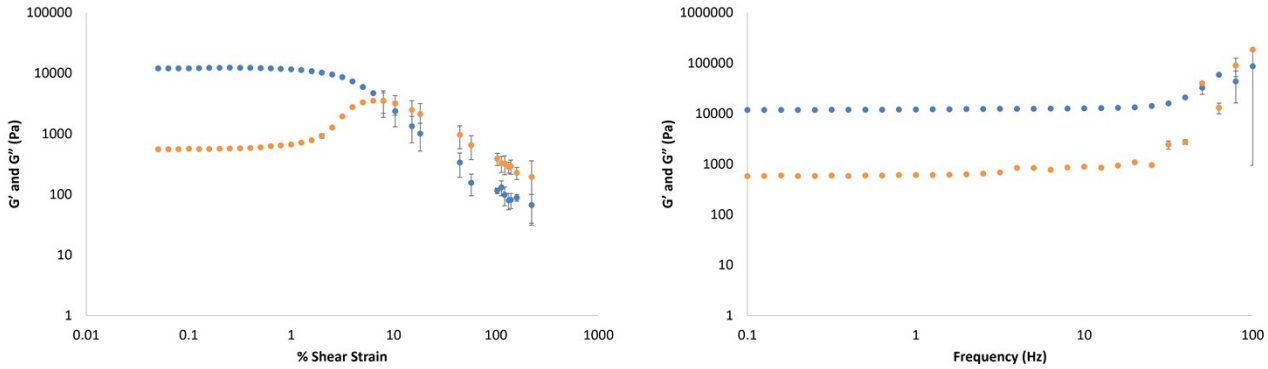

Figure S45. Elastic (G', blue circles) and viscous (G", red circles) moduli of photoactivated DBS-$CONHNH_2$/gellan gumhydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) prepared with 0.15% wt/vol $CaCO_3$ and 0.8% wt/vol DPIN with increasing shear strain (left) and frequency (right).

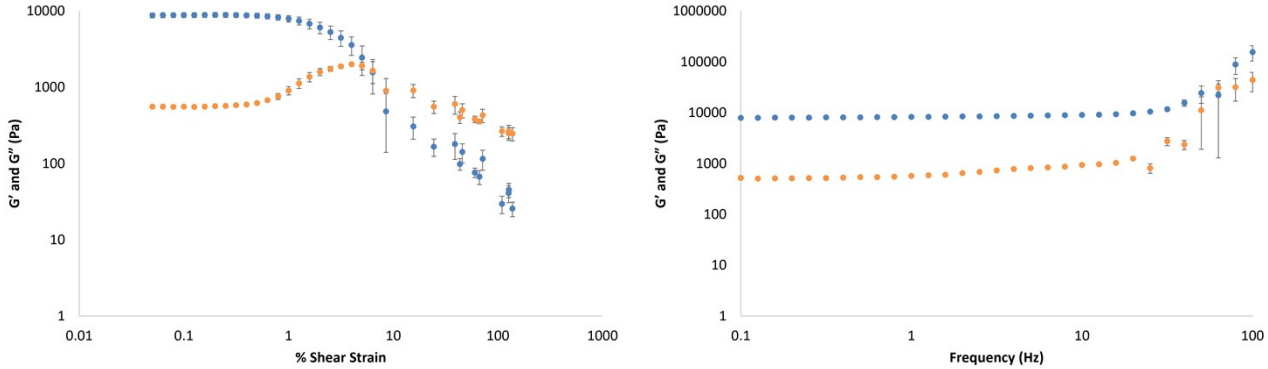

Figure S46. Elastic (G', blue circles) and viscous (G", red circles) moduli of photoactivated gellan gum hydrogel (0.6% wt/vol) prepared with 0.15% wt/vol $CaCO_3$ and 0.8% wt/vol DPIN with increasing shear strain (left) and frequency (right).

S5 Preparation and characterisation of DBS-$CONHNH_2$, DBS-$CONHNH_2$/gellan gum and gellan gum $CaCO_3$/GdL gels loaded with silver nanoparticles (AgNPs)

S5.1 *In situ* formation of Ag NPs

To induce the *in situ* formation of Ag NPs, each gel was thoroughly washed with water multiple times and immersed in 1 or 3 mL of $AgNO_3$ solution (10 mM) for 3 days. After 3 days, the supernatant was gently removed with a pipette and the gels were washed with water multiple times. A colour change was observed in the samples in which the Ag was reduced from Ag(I) to Ag(0).

S5.2 Uptake of Ag(I)

S5.2.1 *Uptake of Ag (I) into DBS-$CONHNH_2$ and gellan gum gels (prepared in sample vials).* The gels used to estimate the uptake of Ag (I) were prepared in water (1 mL) as described in section S3.1. Each of these gels was thoroughly washed with water multiple times and immersed in 1 or 3 mL of a 10 mM $AgNO_3$ solution (containing respectively 0.01 or 0.03 mmoles of Ag (I)) for 3 days. After 3 days, the supernatant was transferred into a vial and used to titrate 0.0005 mmol of NaCl (1 mL) in the presence of K_2CrO_4 (5% - 1 mL) as an indicator. To evaluate precisely the volume of titrant used, the titration was performed by slowly adding the titrant to the NaCl solution in 10 µL drops under stirring. The volume of supernatant added as a titrant, was used to calculate the mmoles of residual Ag (I) in the supernatant (*i.e.* the Ag (I) that was not incorporated into the gel). This was subtracted from the initial mmoles of Ag (I) added, to give the mmoles of Ag (I) incorporated into the gel. To ensure data reproducibility, this experiment was performed in triplicate for each gel and average values are reported.

S5.2.2 *Uptake of Ag (I) DBS-$CONHNH_2$/gellan gum gel beads.* The gel beads used to estimate the uptake of Ag (I) were prepared as described in Section S3.1. Each gel bead was prepared in a 20 µL volume. Since ten gel beads per type of gel were prepared, a 200 µL total volume of gel (*i.e.* 1/5 of the volume used for the gels described in Section S5.2.1) was considered for the subsequent addition of $AgNO_3$ in the same proportion used for the other gels. Once ready, the gel beads were thoroughly washed with water multiple times and immersed in 0.2 or 0.6 mL of a 10 mM $AgNO_3$ solution (containing respectively 0.002 or 0.006 mmoles of Ag(I)) for 3 days. After 3 days, the supernatant was transferred into a vial and used to titrate a 0.0005 mmol of NaCl (1 mL) in the presence of K_2CrO_4 (5% - 1 mL) as an indicator. To evaluate precisely the volume of titrant used, the titration was performed by slowly adding the titrant to the NaCl solution in 10 µL drops under stirring. The volume of supernatant added as a titrant, was used to calculate the mmoles of residual Ag (I) in the supernatant (*i.e.* the Ag (I) that was not incorporated into the gel). This was subtracted to the initial mmoles of Ag (I) added, to give the mmoles of Ag (I) incorporated into the gel. To ensure data reproducibility, this experiment was performed in triplicate for each gel and average values are reported.

Table S8. Evaluation of Ag (I) uptake into DBS-$CONHNH_2$, gellan gum gels and DBS-$CONHNH_2$/gellan gum hybrid gel beads by precipitation titration.

Gel	Loading of DBS-$CONHNH_2$ (wt/vol)	Loading of Gellan gum (wt/vol)	Mmoles of $AgNO_3$ loaded onto gel	Mmoles of Ag (I) incorporated into gel	Mmoles of Ag (I) incorporated / mL of gel	% of Ag (I) incorporated
DBS-$CONHNH_2$	0.3 %	-	0.03	0.0167	0.0167	55.5 %
DBS-$CONHNH_2$	0.3 %	-	0.01	0.0068	0.0068	68.5 %
Gellan gum	-	0.6 %	0.03	0.0100	0.0100	34.5 %
Gellan gum	-	0.6 %	0.01	0.0050	0.0050	53.3 %
Hybrid gel	0.3 %	0.3 %	0.006	0.0026	0.0130	44.0 %

(10B)						
Hybrid gel (10B)	0.3 %	0.3 %	0.002	0.00094	0.0047	46.9 %

S5.3 Release of Ag(I) from DBS-$CONHNH_2$/gellan gum gel beads loaded with Ag NPs

S5.3.1 Preparation of gel beads. The DBS-$CONHNH_2$/gellan gum gel beads (40 beads/sample) were prepared as described in Section S3.1 using 20 µL volume/gel bead and washed with water multiple times. The *in situ* formation of Ag NPs was induced by immersing the gels in 3 ml of a 10 mM solution of $AgNO_3$ for 24 hours. After 24 hours, the supernatant was removed and used to calculate the exact amount of Ag (I) incorporated by precipitation titration as described in Section S5.2.2. To ensure reproducibility, three samples of 40 beads each were prepared for each time point and the exact amount of Ag (I) incorporated in each sample was calculated and reported in Table S9.

Table S9. Amount of Ag(I) incorporated in each sample of 40 DBS-$CONHNH_2$/gellan gum gel beads used for the release study.

Sample name	Mmoles of Ag (I) incorporated into 40 gel beads	% of Ag (I) incorporated
A	0.011	37.5
B	0.015	50.0
C	0.016	54.5
D	0.016	54.5
E	0.013	44.4
F	0.013	44.4
G	0.015	50.0
H	0.011	37.5
I	0.013	44.4
J	0.015	50.0
K	0.011	37.5
L	0.011	37.5
M	0.013	44.4
N	0.017	58.3
O	0.013	44.4
P	0.015	50.0
Q	0.013	44.4
R	0.016	54.5
S	0.011	37.5
T	0.015	50.0
U	0.011	37.5
V	0.011	37.5
W	0.013	44.4
X	0.018	61.5

S5.3.2 Release of Ag(I) from DBS-$CONHNH_2$/gellan gum gel beads. Each sample (40 gel beads/sample) was immersed in 2 ml of water. At the specified time intervals, the release medium was removed and used to titrate 0.0005 mmol of NaCl (1 mL) in the presence of K_2CrO_4 (5% - 1 mL) as an indicator. To evaluate precisely the volume of titrant used, the titration was performed by slowly adding the titrant to the NaCl solution in

10 µL drops under stirring. The volume of supernatant added as a titrant, was used to calculate the mmoles of released Ag (I) (Table S10). To ensure data reproducibility, this experiment was performed in triplicate for each gel and average values were reported in the final graph.

Table S10. Ag(I) released from each sample of 40 DBS-CONHNH$_2$/gellan gum gel beads loaded with Ag NPs.

Sample name	Time point (hours)	Mmoles of Ag (I) released from gel beads	% of Ag (I) released	Average %	Standard error
A	0.5	0.0025	22.2	18.4	3.04
B	0.5	0.0026	17.5		
C	0.5	0.0027	16.5		
D	1	0.0025	15.7	17.4	1.66
E	1	0.0025	18.7		
F	1	0.0024	18.3		
G	1.5	0.0026	17.5	19.1	1.81
H	1.5	0.0024	21.2		
I	1.5	0.0025	19.2		
J	2	0.0026	17.5	20.5	3.00
K	2	0.0024	21.7		
L	2	0.0026	23.4		
M	4	0.0024	18.3	15.8	2.55
N	4	0.0023	13.3		
O	4	0.0022	16.7		
P	6	0.0026	17.5	16.9	2.37
Q	6	0.0025	19.2		
R	6	0.0024	14.5		
S	8	0.0025	22.8	20.0	3.26
T	8	0.0025	16.7		
U	8	0.0024	21.7		
V	24	0.0024	21.2	17.9	3.12
W	24	0.0026	19.2		
X	24	0.0027	15.0		

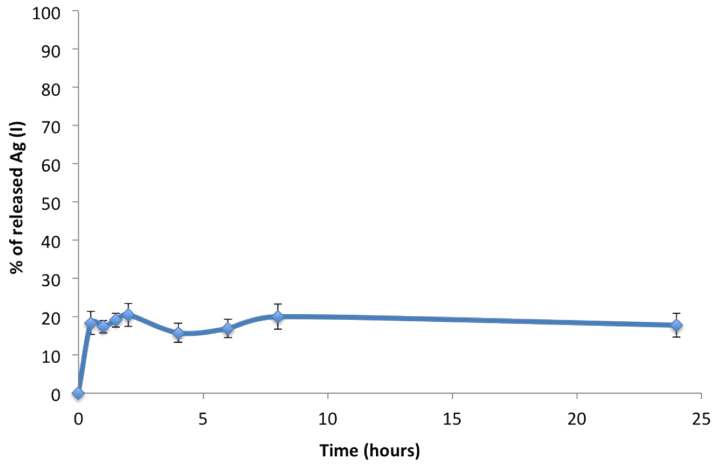

Figure S47. Release over time of Ag(I) ions from DBS-CONHNH$_2$/gellan gum hybrid gel beads loaded with Ag NPs.

S5.4 Transmission Electron Microscopy (TEM)

Samples for TEM imaging were prepared as described in Section S3.1, loaded with Ag NPs (Section S5.1) and then treated for TEM analysis as described in Section S2.5.

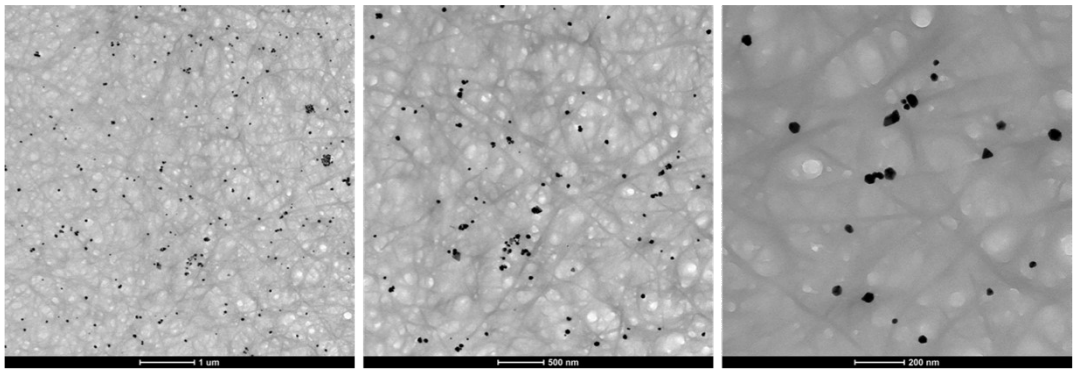

Figure S48. TEM images of DBS-$CONHNH_2$ gel incorporating AgNPs. Scale bars (from left to right): 1 µm, 500 nm and 200 nm.

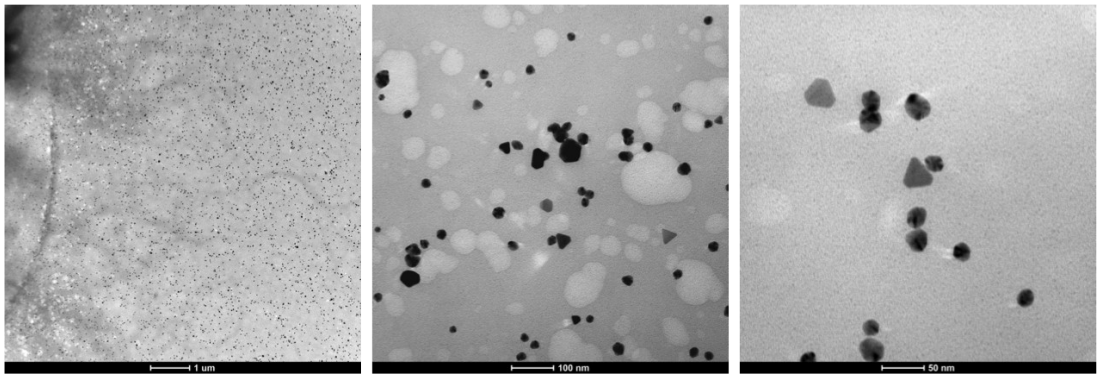

Figure S49. TEM images of DBS-$CONHNH_2$/gellan gum gel beads incorporating AgNPs. Scale bars (from left to right): 1 µm, 100 nm and 50 nm.

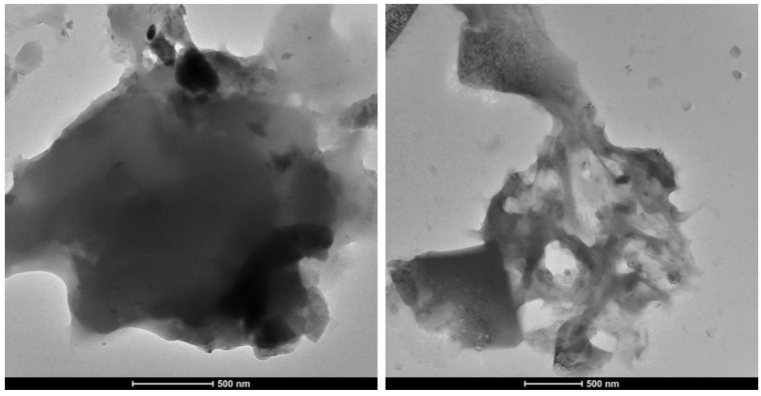

Figure S50. TEM images of gellan gum gel incorporating AgNPs. Scale bars: 500 nm.

S5.5 Rheology

Gel samples for rheology were prepared in bottomless vials, using a 0.15% wt/vol $CaCO_3$ concentration and a 1.0% wt/vol GdL concentration, as described in Section S3.1. Once ready, the gels were washed with water and then treated with 1 or 3 mL of a 10 mM $AgNO_3$ solution (containing respectively 0.01 or 0.03 mmoles of

Ag (I)) for 3 days. After 3 days, the supernatant was removed and the mechanical properties of the gels were analysed as described in Section S2.7.

Table S11. Rheological data as determined using oscillatory rheometry with a parallel plate geometry, for DBS-$CONHNH_2$, DBS-$CONHNH_2$/gellan gum and gellan gum gels with Ag NPs. Loadings are given in wt/vol, and the G'/G" crossover points refer to the % shear strain at which G"= G'.

Gel	Loading of LMWG	Loading of Gellan gum	Total Loading	Volume of $AgNO_3$ (10 mM) added	G' (Pa)	G'/G" Crossover
DBS-$CONHNH_2$	0.4%	-	0.4%	1 mL	9.72	39.7%
DBS-$CONHNH_2$	0.4%	-	0.4%	3 mL	7.79	31.5%
Hybrid	0.3%	0.3%	0.6%	1 mL	11700	8.8%
Hybrid	0.3%	0.3%	0.6%	3 mL	4950	10.3%
Gellan gum	-	0.6%	0.6%	1 mL	4150	2.2%
Gellan gum	-	0.6%	0.6%	3 mL	2870	2.6%

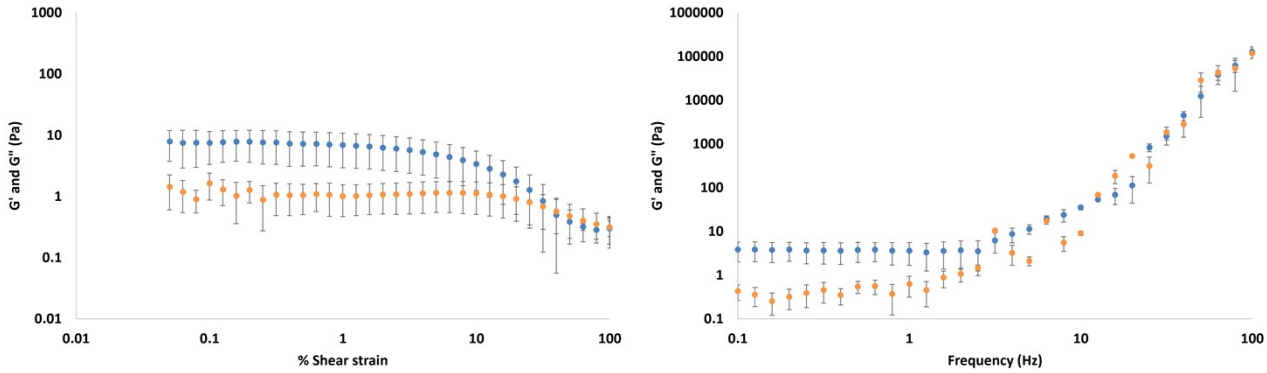

Figure S51. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$ hydrogel (0.4% wt/vol - loaded with 1 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

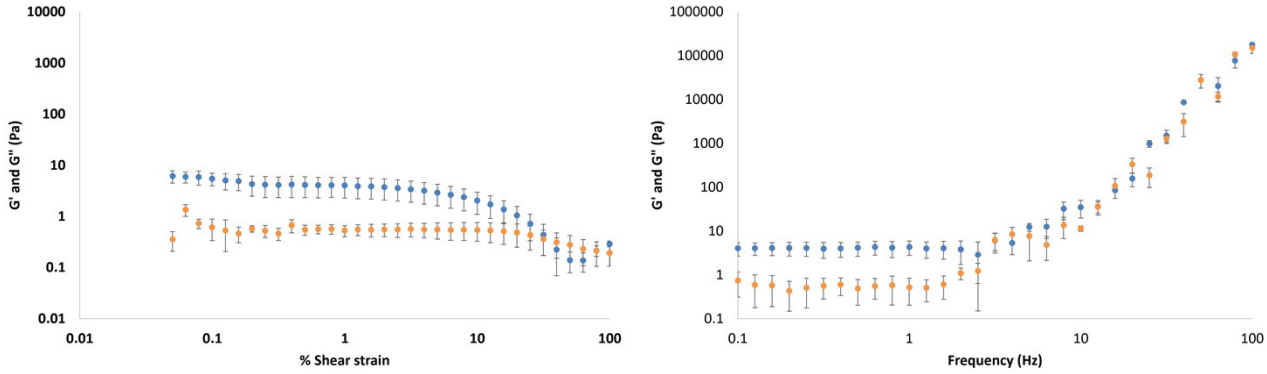

Figure S52. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$ hydrogel (0.4% wt/vol - loaded with 3 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

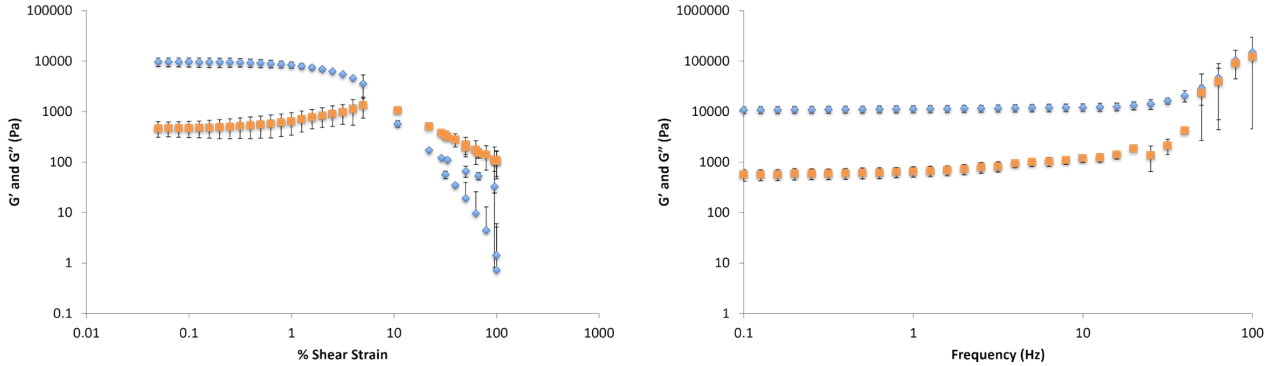

Figure S53. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum – loaded with 1 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

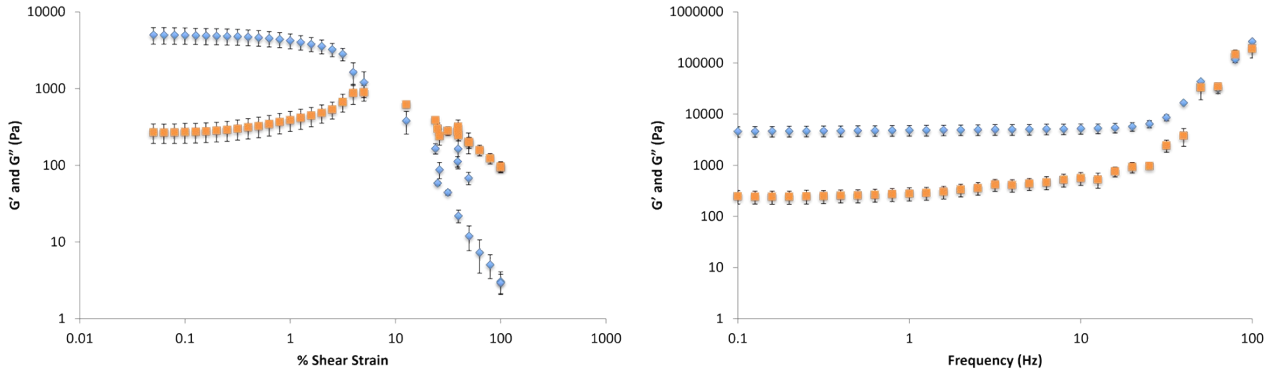

Figure S54. Elastic (G', blue circles) and viscous (G", red circles) moduli of DBS-$CONHNH_2$/gellan gum hydrogel (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum – loaded with 3 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

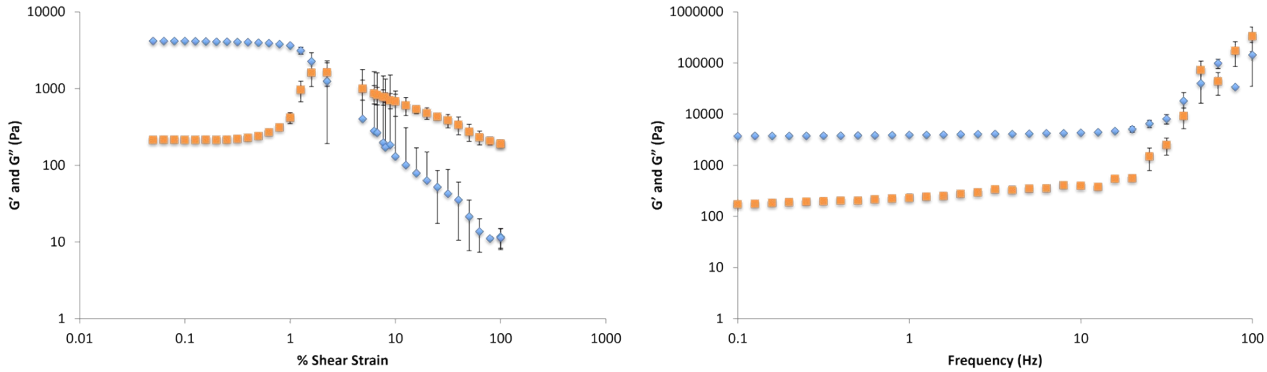

Figure S55. Elastic (G', blue circles) and viscous (G", red circles) moduli of gellan gum hydrogel (0.6% wt/vol– loaded with 1 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

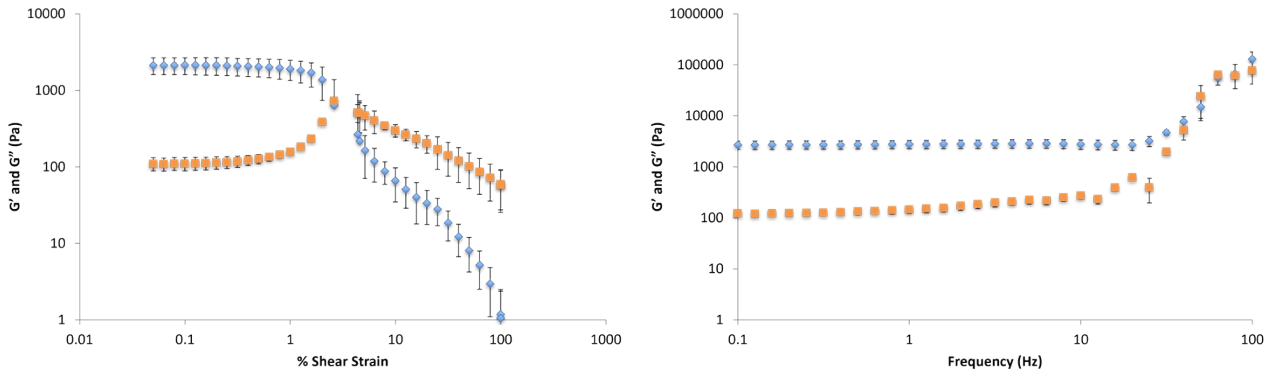

Figure S56. Elastic (G', blue circles) and viscous (G", red circles) moduli of gellan gum hydrogel (0.6% wt/vol– loaded with 3 mL $AgNO_3$ 10 mM) with increasing shear strain (left) and frequency (right).

S6 Biological studies

S6.1 Cell line (Y201 immortalized human mesenchymal stem cells – MSCs).[3]

Y201 MSCs were grown in a T175 flask with Dulbecco's Modified Eagle's Medium (DMEM) with fetal bovin serum (FBS - 10%) and penicillin/streptomycin (P/S - 1%). To obtain the cells, the medium was removed from the flask and the cells washed with Dulbecco's phosphate buffer saline solution (11 mL). Trypsin/EDTA (2 mL) was then added and the cells were incubated at 37°C for approximately five mins. When cell detachment was observed by optical microscopy, trypsin was neutralised with 9 mL DMEM (10% FBS, 1% P/S). The cells were then transferred in a tube and isolated by centrifugation. After centrifugation, the supernatant was removed and the cell pellet was dispersed in 5 mL DMEM (10% FBS, 1% P/S). Cell count was performed using a Countess Automated Cell Counter (Thermo Fisher) on a 10 □L aliquot of a stock solution obtained by mixing 20 μL of cell suspension with 20 μL of trypan blue.

S6.2 Cytotoxicity assay

S6.2.1 Gel preparation. DBS-$CONHNH_2$/gellan gum hybrid gels (0.3% wt/vol DBS-$CONHNH_2$ and 0.3% wt/vol gellan gum) and gellan gum gels (0.6% wt/vol) for cytotoxicity assays were prepared in triplicate in a 48-well plate (300 μL volume), in the presence of $CaCO_3$ (0.15% wt/vol) and GdL (1.0% wt/vol), as described in Section S3.1. The *in situ* formation of Ag NPs was induced by addition of an $AgNO_3$ solution (300 μL – 0.0125 mM or 10 mM) on top of the gels. The gels were left undisturbed for 72 hours, subsequently washed with Dulbecco's Modified Eagle's Medium (DMEM) multiple times (400 μL) and then transferred in the middle of a 6-well plate.

DBS-$CONHNH_2$ gels were directly prepared in triplicate in a 6-well plate (300 μL volume; 0.3% wt/vol), using small bottomless vials (c.a. 1 cm diameter). Once the gels were formed, the vials were removed, leaving self-supporting gels in the middle of each well.

S6.2.2 Plate seeding. The cells (100000/well) were seeded on the bottom of the wells around the gels in the 6-well plates and covered with DMEM (10% FBS, 1% P/S - 2 □L).

S6.2.3 Crystal violet staining. After 48 hours, the DMEM was removed and each well was washed with PBS (1 mL). A crystal violet methanol solution (1 mL) was added to each well and the plates were left undisturbed for 20 mins. After 20 mins, the stain was collected and the plates were washed multiple times in a distilled water bath and then left to dry. Plates were imaged with an Epson PhotoScanner.

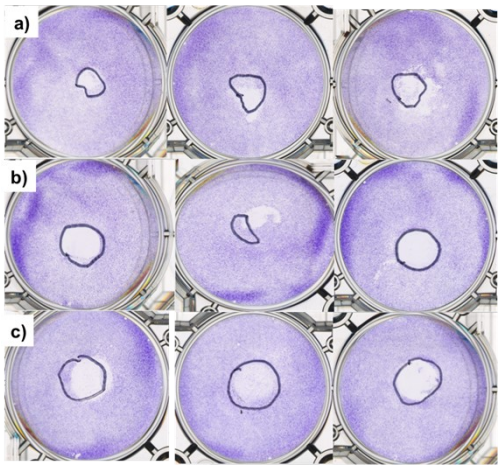

Figure S57. Scanned images of the cytotoxicity assay. Control gels without AgNPs. (a) DBS-$CONHNH_2$/gellan gum gel, (b) gellan gum gel, (c) DBS-$CONHNH_2$ gel.

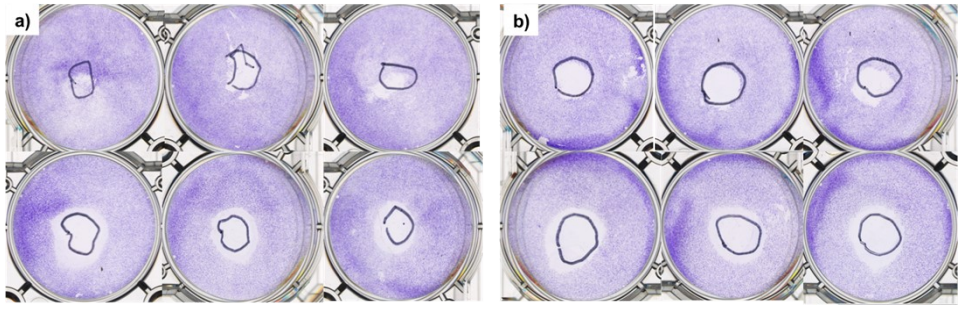

Figure S58. Scanned images of the cytotoxicity assay. Gels loaded with AgNPs (0.0125 mM top and 10 mM bottom), the white rings around the gels indicate the zone of cell growth inhibition. (a) DBS-$CONHNH_2$/gellan gum gel, (b) gellan gum gel.

S6.3 Viability assay

S6.3.1 Gel preparation in 96-well plates and plate seeding. Gels were prepared as described in Section S3.1 in 96-well plates in 75 μL volume. Since the gel beads were prepared using 20 μL volume per bead, four gel beads were placed in well. The *in situ* formation of Ag NPs was induced by addition of an $AgNO_3$ solution (75 μL – 0.00625, 0.0125, 0.05, 0.1, 1.0 or 10 mM) on top of the gels. The gels were left undisturbed for 72 hours. After this time, the supernatant was removed and the gels were washed multiple times with DMEM (10% FBS, 1% P/S - 200 μL). After the last wash, the gels were soaked with DMEM (10% FBS, 1% P/S - 100 μL) and the cells (25000/well) were seeded and covered with further DMEM (10% FBS, 1% P/S - 100 μL).

S6.3.2 Alamar Blue viability assay. Cell viability was measured at different time points using the Alamar Blue viability assay (*Thermo Fisher Scientific*). The cell culture medium was removed from each well and a 10% solution of Alamar Blue in DMEM (100 μL) was added. The plates were incubated at 37°C for 4 hours. After this time, 20 μL aliquots were taken from each well and diluted with DMEM (180 μL) in a new 96 well plate. Fluorescence was then measured with a fluorimeter (excitation 530-560 nm and emission 590 nm). This experiment was performed in sixuplicates and average values are reported with the error bars representing standard error. Control experiments with no cells were performed for each gel type.

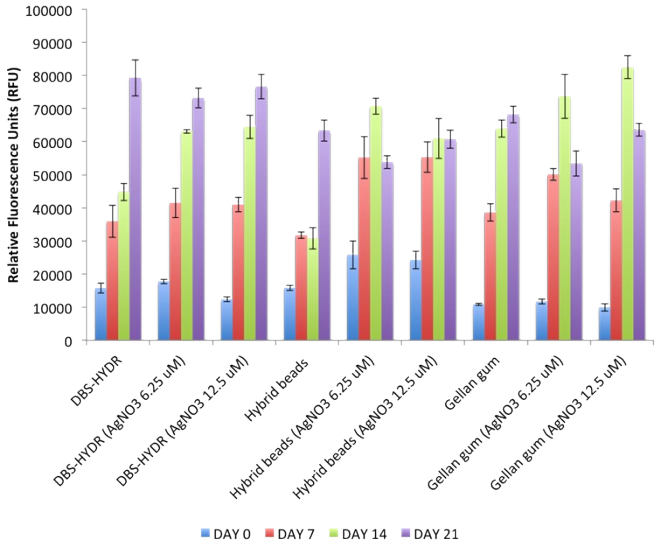

Figure S59. Alamar blue assay results at day 0, 7, 14 and 21 for gels loaded with different $AgNO_3$ concentrations (N=6, mean reported, error bars represent standard error, DBS-HYDR = DBS-$CONHNH_2$).

S7 References

[1] B. O. Okesola and D. K. Smith, *Chem. Commun*., 2013, **49**, 11164-11166.

[2] D. J. Cornwell, B. O. Okesola and D. K. Smith, *Soft Matter*, 2013, **9**, 8730-8736.

[3] S. James, J. Fox, F. Afsari, J. Lee, S. Clough, C. Knight, J. Ashmore, P. Ashton, O. Preham, M. Hoogduijn, R. D. R. Ponzoni, Y. Hancock, M. Coles and P. Genever, *Stem Cell Reports*, 2015, **4**, 1004-1015.