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Tuning the Antimicrobial Activity of Low Molecular Weight Hydrogels Using Dopamine Autoxidation

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1. Materials

Gelator **1** was prepared as previously reported.[1] All other chemicals were purchased from Sigma Aldrich and were used as received unless otherwise stated. Lysogeny broth, lysogeny agar and phosphate buffered saline (PBS) were prepared aseptically and autoclaved on site. De-ionised water was used throughout as the solvent.

Preparation of gelator solution

Gelator solutions were prepared by weighing out 80 mg of **1** into 14 mL vials then adding deionised H_2O (7.39 mL) and NaOH (aq. 0.1 M, one molar equivalent, 1.61 mL) to a volume of 9 mL. The solution was stirred overnight to ensure all gelator had dissolved. The gelator solutions were then pH adjusted to 6, 7, 8, 9 and 10 respectively with HCl (0.5 M) and de-ionised water, ensuring that the final volume was 10 mL which provides solutions at a final concentration of gelator of 8 mg/mL. Solutions were stored at room temperature.

Preparation of gels

Dopamine hydrochloride (60 mg) was added to 10 mL of gelator solution. The solution was stirred vigorously to ensure the dopamine hydrochloride had dissolved. 2mL of gelator and dopamine solution were pipetted into a 7mL Sterilin vial and left to gel for at least 16 hours [2](time dependent upon specific experiments). Figure S1 shows gels formed at pHs 6, 7, 8, 9 and 10 over a 9 day time period.

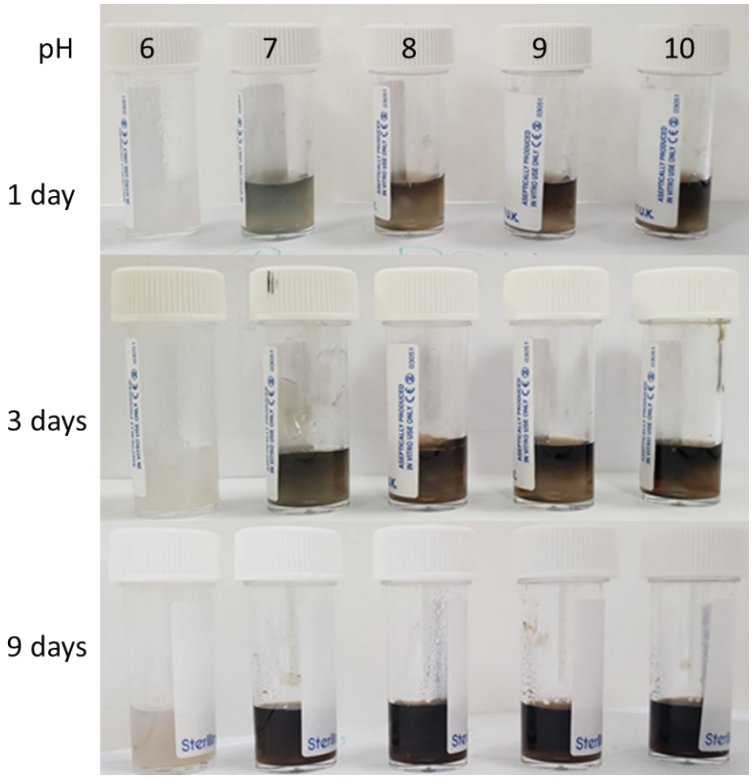

Figure S1. Photographs showing gels formed from gelator **1** at pHs 6, 7, 8, 9 and 10**,** over a 1, 3 and 9 day time point.

2. Methods

pH measurements

pH measurements were recorded using a custom-built pH logger with a Hanna FC500 pH probe with a given error of +/- 0.1. For measuring the pH of gelation over time, 2 mL of gelator and dopamine solution were pipetted into a 7 mL Sterilin vial and the pH probe tip immersed. The pH measurements were recorded with an interval of 0.5 minutes over time (absolute time dependent upon specific experiments).

Determination of the pK_a of **1** has been carried out using different methods previously.[3, 4] The pK_a is that of the aggregate and not that of the free molecule. The absolute charge on the aggregate will depend on the exact pH.[5] Determination of the absolute charge is difficult, especially in the pH window we are examining where the gelation is beginning and the aggregates are evolving. In the current case, this is even more difficult in the presence of the polydopamine.

Rheology

Rheological measurements were carried out using an Anton Paar Physical MCR301 rheometer. A vane (ST10-4V-8.8/97.5) and cup geometry was used to measure the frequency and strain sweeps. Parallel plates (50 mm diameter, sandblasted) were used to measure time sweeps. For measuring the frequency and strain sweeps, 2 mL of the gelator and dopamine solution was added to a Sterilin vial. After complete gelation, the samples in the vials were transferred to the rheometer and the rheological measurements were then recorded at 25 °C. For the time sweeps, 2 mL of gelator and dopamine solution was transferred onto the temperature-controlled plate and the plate lowered on top of the solution with a gap distance of 0.8 mm and trimmed. The sample was then surrounded by mineral oil to prevent the solution/gel from drying whilst gelling.

Frequency sweep: Frequency scans were performed from 1 rad/s to 100 rad/s under a constant strain of 0.5%. Measurements were performed in duplicate and errors were calculated from the standard deviation.

Time sweep: Time sweeps were measured with an angular frequency of 10 rad/s with a strain of 0.5% (Figure S2).

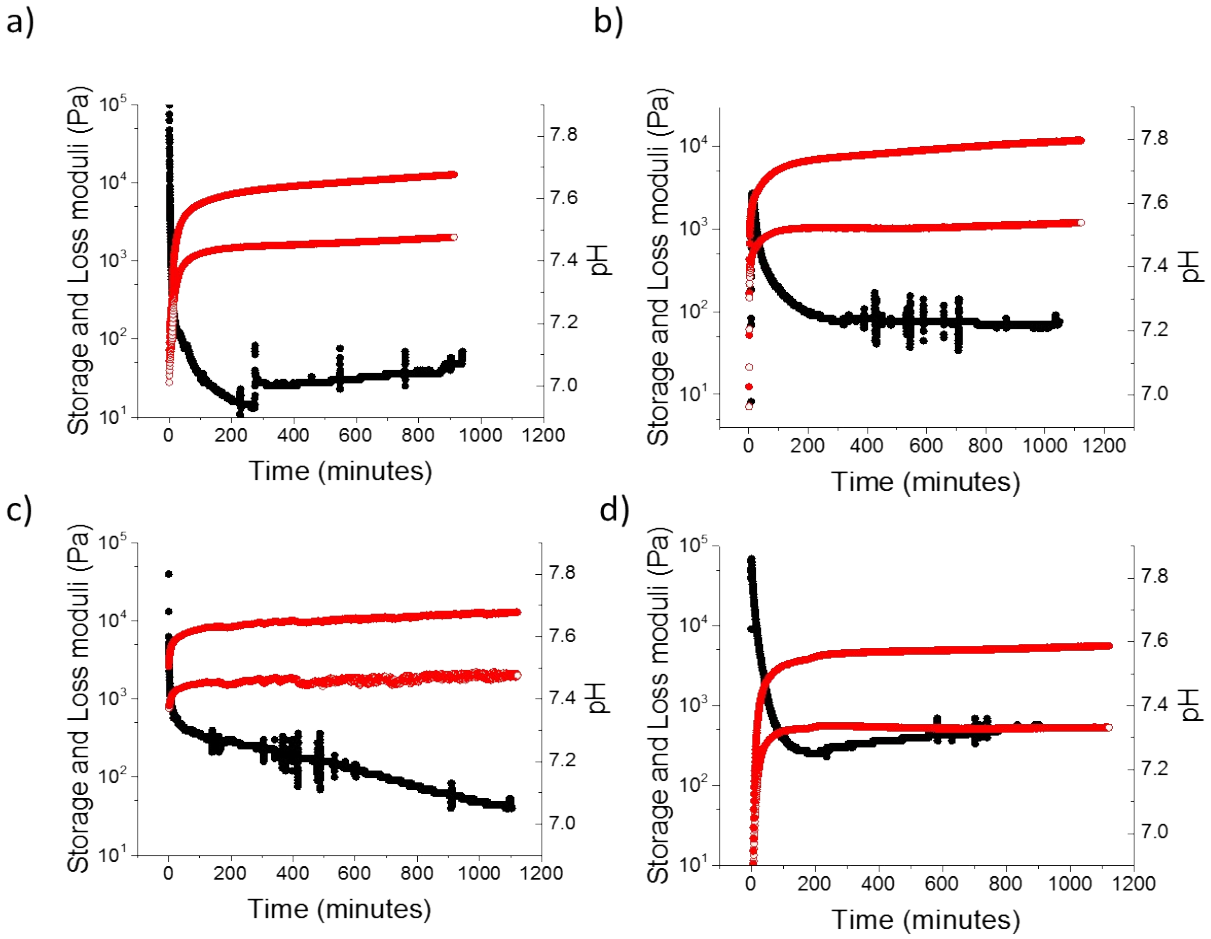

Figure S2. Monitoring gelation over time for solutions of gelator **1** and dopamine starting at a pH of (a) 7; (b) 8: (c) 9; (d) 10. pH changes during gelation (black) are compared with rheological time sweeps. Storage moduli is shown in red full circles and loss moduli are shown in red hollow circles. Rheological time sweeps were performed at a strain of 0.5%, 10 rad/s and at 25 °C.

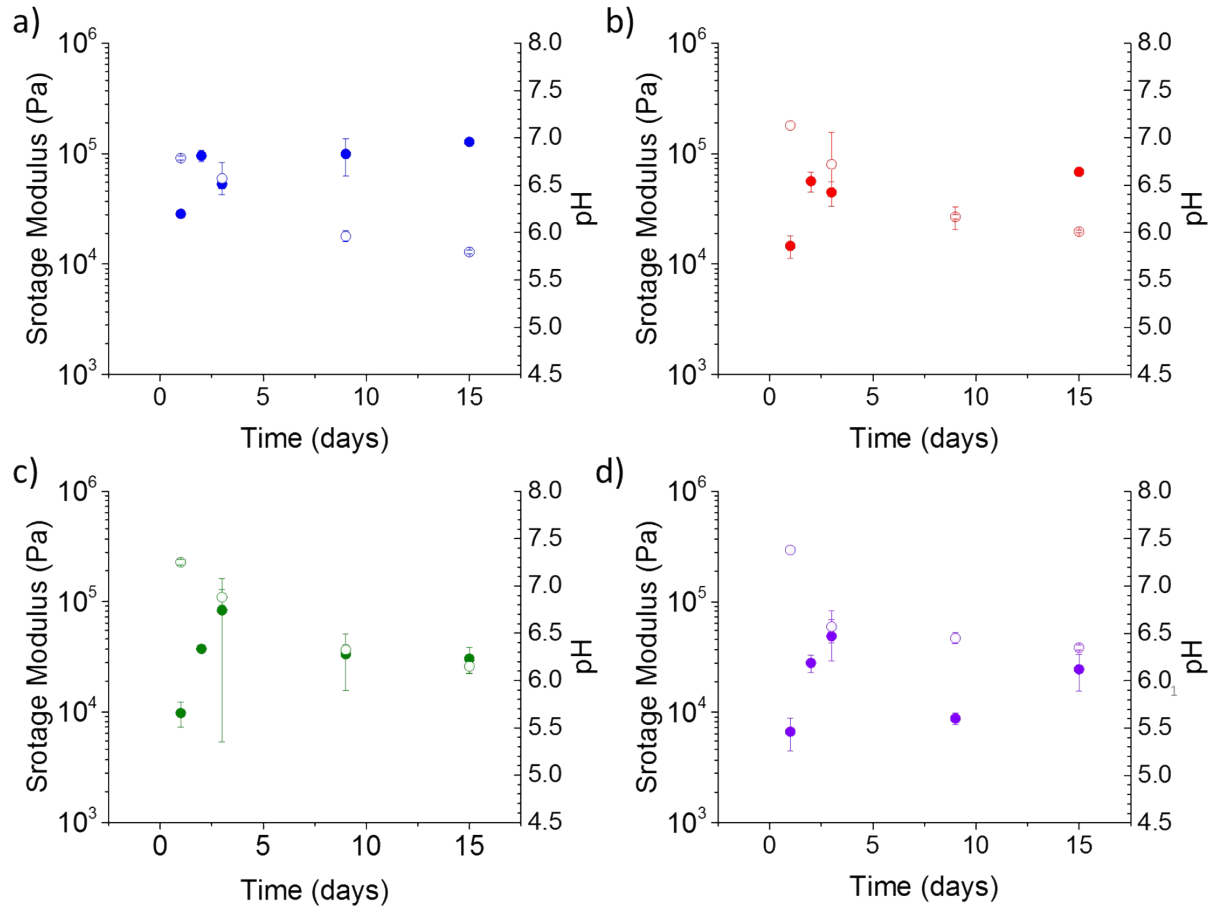

Figure S3. Ageing of the gels with time. In all cases, the full circles are storage modulus (left axis) and the hollow circles show pH (right axis). Data are shown for gels formed from solutions of gelator **1** and dopamine starting at a pH of (a) 7; (b) 8; (c) 9; (d) 10. Error bars are calculated from measurements on three different gels.

Small Angle Neutron Scattering

The solutions were prepared as described above in D_2O using NaOD (0.1 M) and DCl (0.1 M) to adjust the pH. SANS measurements were performed using the D11 instrument at the Institut Laue – Langevin, Grenoble, France. A neutron beam with a fixed wavelength of 6 Å and divergence of $\Delta\lambda/\lambda$ = 9% was used to carry out measurements over a Q range [$Q = 4\pi\sin(\theta/2)/\lambda$] of 0.001 to 0.3 $Å^{-1}$ using three sample-detector distances of 1.5 m, 8 m, and 39 m.

Gels were prepared in UV spectrophotometer grade, quartz cuvettes (Hellma) with a 2 mm path length. These were placed in a temperature-controlled sample rack during the measurements.

The data were then reduced to 1D scattering curves of intensity vs. Q using the facility provided software. The electronic background was subtracted, the full detector images for all data were normalized and scattering from the empty cell was subtracted. The scattering from D_2O was also measured and subtracted from the data. The instrument-independent data were then fitted to the models discussed in the text using the SasView 4.2.2 software package version.[2]

The majority of the data were fitted best to a core-shell model combined with a power law to take into account the excess scattering at low Q. Many models were attempted including a

cylinder, a flexible cylinder, elliptical cylinder, flexible elliptical cylinder and hollow cylinders, both with and without a power law. None gave satisfactory fits, with the closest (in terms of chi squared) being the flexible cylinder combined with a power law. However, in this case, the Kuhn Length always tended towards a meaninglessly small value.

To fit to the core-shell and power law model, initally, a core-shell model was used alone, optimised over a Q range of 0.017<Q<0.3, and then the Q range expanded and the core-shell model combined with a power law to take into account the scattering at low Q. Initally, the core scattering length density (SLD) was set to 2.73×10^{-6} Å^{-2} and the SLD for the solvent (D_2O) was 6.3×10^{-6} Å^{-2}. The parameters were then fitted, allowing the SLD of the shell to vary. On the basis of this method, a significantly better fit to the data could be achieved than using any other model. However, the fit does not give always a low value of chi squared, which we suspect is the need for some flexibility, but no flexible core-shell cylinder model is available.

The SLD of the shell optimises to around 6 $\times 10^{-6}$ Å^{-2}, which is higher than expected from the structure of polydopamine. We suggest that this implies that the shell is highly solvated.

For the data for the sample starting at pH 10 however, attempts at fitting to a core-shell model fibres resulted in the radius becoming very small and the thickness becoming the radius of the fibres found by using a combined power law and cylinder model. Hence, a core-shell model does not capture the data for this sample, which gives confidence that the fits to the other data using a core-shell model are correct.

A summary of the fitting parameters from the fits are shown in Table S1. The data and the fits are shown in Figure S3.

	Initially pH 7	**Initially pH 8**	**Initially pH 9**	**Initially pH 10***
Background / cm^{-1}	0.024 ± 4.77x10^{-5}	0.011 ± 4.06x10^{-5}	0.020 ± 5.00x10^{-5}	0.015 ± 6.89x10^{-5}
Scale	0.051±0.002	0.033 ± 0.0001	0.0019 ± 1.19x10^{-6}	0.0034 ± 1.00x10^{-5}
Length / Å	971 ± 15	>1000	294 ± 4	>3000
Radius / Å	38.0 ± 0.03	27.1 ± 0.03	29.9 ± 0.04	26.7 ± 0.03
Thickness / Å	79.7 ± 0.3	44.8 ± 0.1	30.0 ± 0.2	
SLD (shell) / x10^{-6} Å^{-2}	6.21 ± 2	6.00 ± 0.1	5.85 ± 0.1	
Scale	3.44x10^{-5} ± 8.47 x10^{-6}	4.30x10^{-5} ± 8.69x10^{-6}	4.25x10^{-5} ± 1.17x10^{-6}	2.23x10-4 ± 4.64x10-5
Power Law	2.50 ± 0.01	2.45 ± 0.01	2.44 ± 0.01	1.97 ± 0.01
χ^2	14.7	39.7	18.4	6.98

Table S1. Fitting parameters obtained for gels formed from solutions of gelator **1** and dopamine starting at an inital pH of 7, 8, 9, or 10. The data for the gels formed from solutions at an initial pH of 7, 8 and 9 were fitted to a core-shell model combined with a power law. The data for the gels formed from solutions at an initial pH of 10 were fitted to a cylinder model combined with a power law. * Due to the fit not fully capturing the data at low Q, the data were fitted over the range of 0.003<Q<0.3 Å^{-1}.

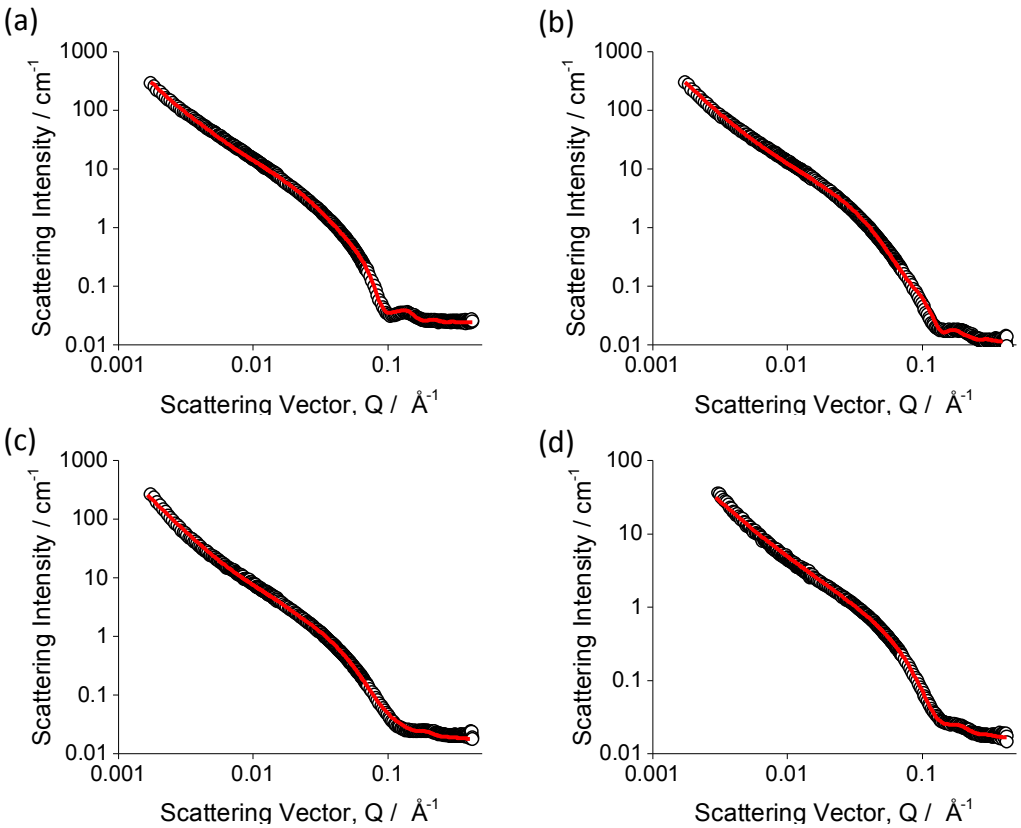

Figure S4. SANS data (black circles) and fits described in Table S1 (red lines) for gels formed over time from solutions of gelator **1** and dopamine starting at a pH of (a) 7; (b) 8: (c) 9; (d) 10.

Bacterial susceptibility assay

Staphylococcus aureus NCTC 10788*, Staphylococcus epidermidis* ATCC 12228*, Pseudomonas aeruginosa* ATCC 15692 *and Escherichia coli* ATCC 15597 were subcultured for 18 hours at 37 ^{o}C in Lysogeny broth and adjusted to an optical density reading of 0.3 at 550 nm in PBS, corresponding to 1x10^{8} CFU/mL, and further diluted (1 in 50) in broth.

100 µL of bacterial suspension was then plated into each well of a microtiter plate containing 100 µL of gelator. Control wells included bacteria in broth as a growth control (100% survival), PBS alone as a negative, sterility control and 2% w/v (hydroxypropyl)methyl cellulose (HPMC) as an inert hydrogel to examine the effect of gelation on bacterial viability.

Inoculated microtiter plates were incubated for 24 hours at 37 ^{o}C and 20 µL samples were taken from each well, serially diluted in PBS (10^{-1} to 10^{-8}) and transferred to Lysogeny agar plates for colony counting via the Miles and Misra technique.[3] Each experiment was performed in triplicate and results were displayed as the mean (Log_{10} CFU/mL) of nine replicates. Results of these experiments are shown in Figure S5.

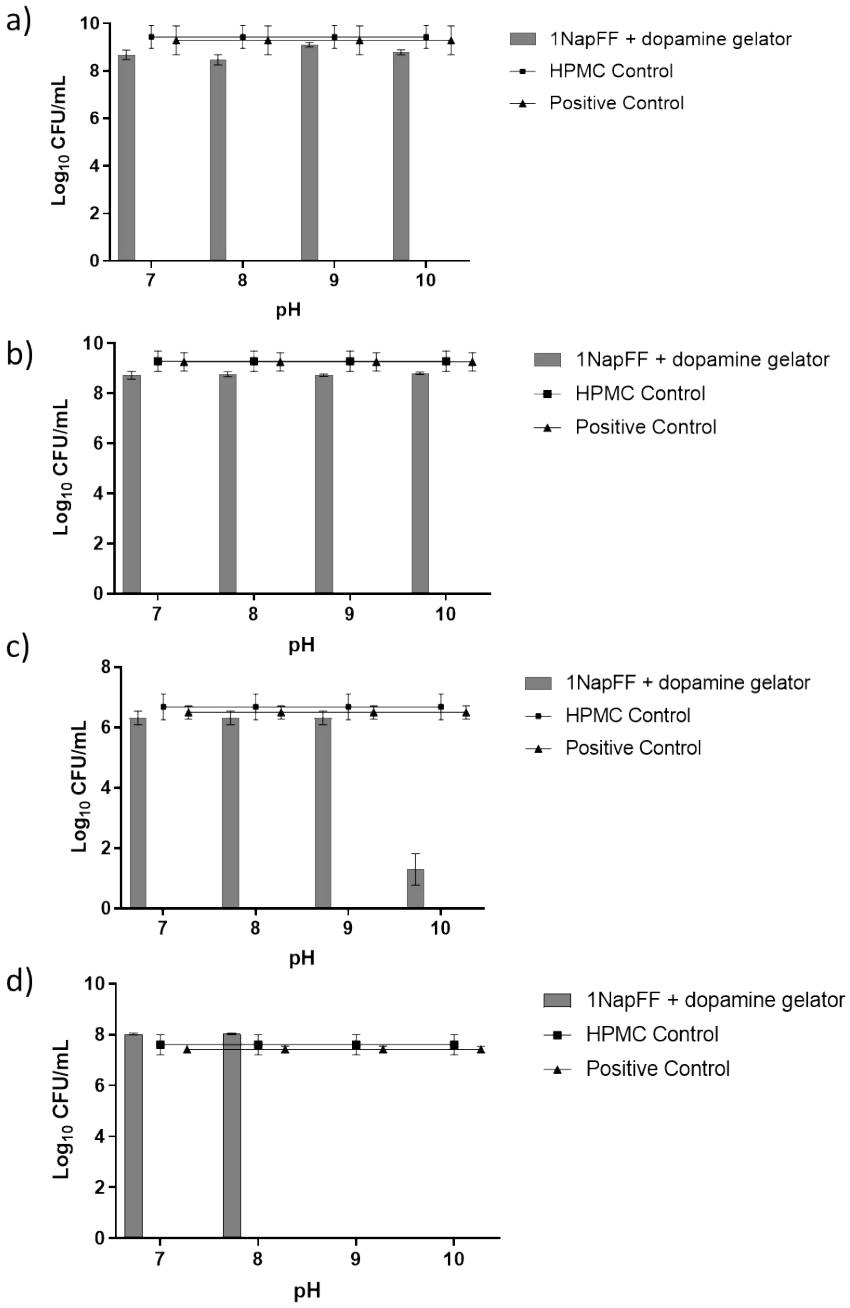

Figure S5. Bacterial susceptibility assay data for gel **1** at pH 7, 8, 9 and 10. a) *P.aeruginosa* ATCC 15692 b) *E. coli* ATCC 15597 c) *S. aureus* NCTC 10788 and d) *S. epidermidis* ATCC 12228. Control wells included bacteria in broth as a growth control (100% survival), PBS alone as a negative PBS control, sterility control and 2% w/v (hydroxypropyl)methyl cellulose (HPMC) as an inert hydrogel to examine the effect of gelation on bacterial viability.

References

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5. L. Chen, G. Pont, K. Morris, G. Lotze, A. Squires, L. C. Serpell and D. J. Adams, *Chem. Commun.*, 2011, **47**, 12071-12073.