The electrofabrication of a Fmoc-protected hydrogel

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Supporting Information

Table of contents

Experimental

1. Materials

Mono-Fmoc 1,3-diaminopropane hydrochloride (Fmoc-DAP.HCl, Fmoc-3, Figure S1a) was purchased from Fluorochem Ltd and stored in a laboratory-grade fridge. 2NapVG (Figure S1b) was synthesized as previously reported[1] and stored at room temperature. Hydrogen peroxide (30 % stabilized) solution was purchased from Avantor and stored in a laboratory-grade fridge.

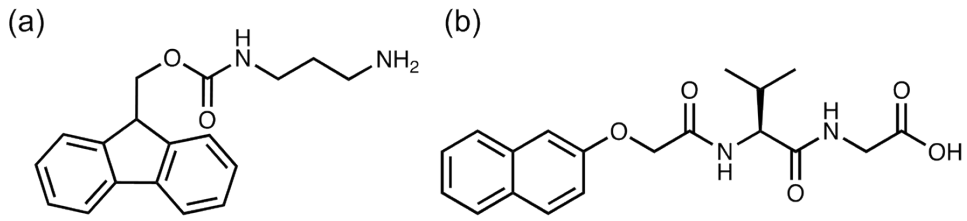

Figure S1. Chemical structure of the low molecular weight gelator molecules (a) Fmoc-3 and (b) 2NapVG. Note Fmoc-3 is available as the hydrochloride salt, but it is the free amine shown that is the gelator at high pH.

2. Preparation of solutions for electrochemical gelation

All solutions of Fmoc-3 and hydrogen peroxide were made fresh at the beginning of every day using deionized Milli-Q water. To prepare Fmoc-3 solutions at a concentration of 5 mg/mL, 35 mg of Fmoc-3 was weighed into a 10.5 mL glass vial. 7 mL of deionized water was added. The solution was then stirred using a magnetic stirring plate and stir bar until all solids had dissolved. Solutions were stored in the fridge during the day prior to use. Immediately before the gelation process, 70 μL of NaCl (0.1 M) and 70 μL of hydrogen peroxide solution (between 1 and 4 M, see main text) were pipetted to make up the final solution.

2NapVG stock solutions (200 mL) were prepared at a concentration of 5 mg/mL and adjusted to pH 8 using NaOH (0.1 M) and HCl (0.1 M). To make the stock solutions, the appropriate mass of 2NapVG was dissolved in water and 1 molar equivalent of NaOH (using a 1 M) in a 250 mL glass jar. The solution was then then left to stir overnight to ensure all solids had dissolved. The following day, the pH was adjusted to pH 8. As hydroquinone oxidizes above pH 10, the pH of the solutions must be adjusted. All 2NapVG solutions were stored at room temperature and made fresh at the beginning of every week using deionized Milli-Q water. To make the gelator solution, hydroquinone (5 mg/mL) was weighed into a glass vial. The appropriate volume of 2NapVG stock solution (5 mg/mL) and NaCl (0.1 M) was then pipetted into the vial until all the hydroquinone had dissolved. This solution was then used immediately.

3. pH measurements

All pH measurements were collected using A FC200 pH probe from HANNA instruments with a 6 mm x 10 mm conical tip. The stated error of each measurement is ±1. To adjust the pH of the 2Nap-VG-OH stock solutions to pH 8, 20 μL aliquots of NaOH (0.1 M)/HCl (0.1 M) were added to the solution. After the addition of each aliquot, the solution was stirred for 30 seconds before taking another measurement. This was repeated until the bulk solution reached the desired pH 8.

4. Fmoc gel forming procedure on FTO slide

To grow the Fmoc-3 hydrogels, the three-electrode set-up consisting of a working electrode (FTO glass slide 12 x 15 mm), reference electrode (Ag/AgCl) and counter electrode (platinum wire) was assembled in a glass chamber (Figure S3a). 7 mL of the Fmoc-3 gelator solution was then pipetted into the chamber immediately prior to the electrochemical experiment. A current density of -0.7 mA/cm^2 was found to be optimal for gel growth and was applied to the working electrode surface (FTO slide) for 900 seconds using chronopotentiometry (Figure S8). After the 900 seconds, the FTO slide with the hydrogel attached was removed and placed within a petri dish (Figure S3b). To prevent the hydrogel from drying out, a wet paper towel was placed around the edges of the petri dish and the lid secured. This procedure was repeated for hydrogels grown in the absence of hydrogen peroxide and all the concentrations of hydrogen peroxide used.

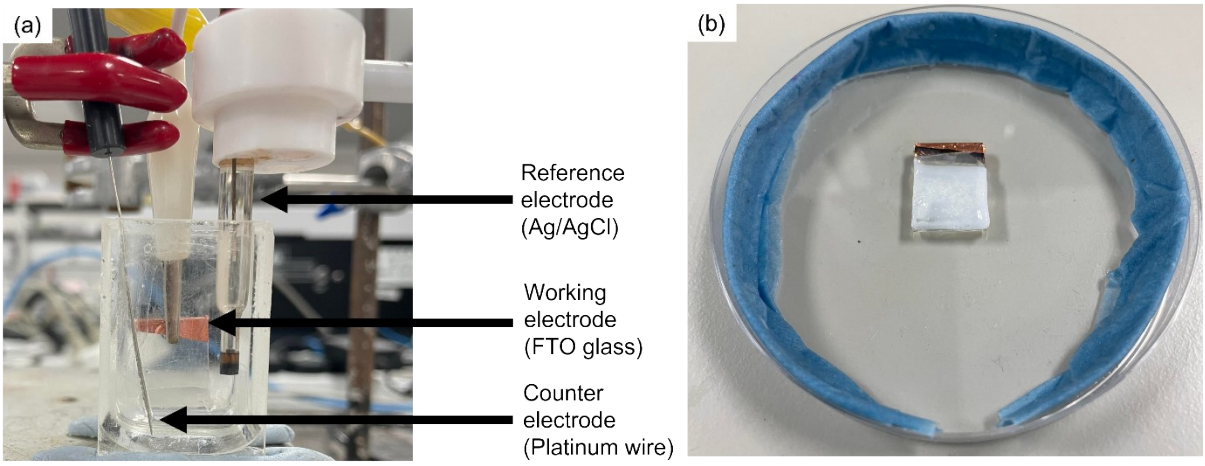

Figure S2. (a) Electrochemical set-up for Fmoc-3 hydrogel formation (b) Fmoc-3 hydrogel on FTO glass slide (12 x 15 mm) in petri dish.

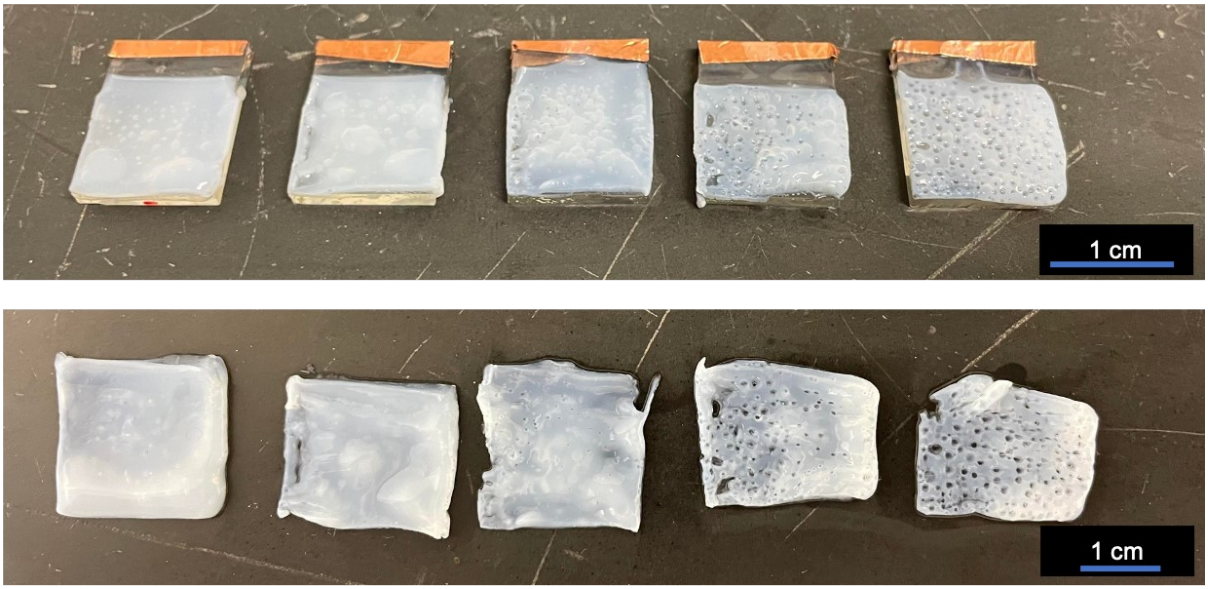

Figure S3. Fmoc-3 hydrogels formed via the electrochemical reduction of hydrogen peroxide. Fmoc-3 hydrogels were grown in the absence of hydrogen peroxide (far right) and 1 M, 2 M, 3 M and 4 M hydrogen peroxide solution (right to left). Scale bar: 1 cm.

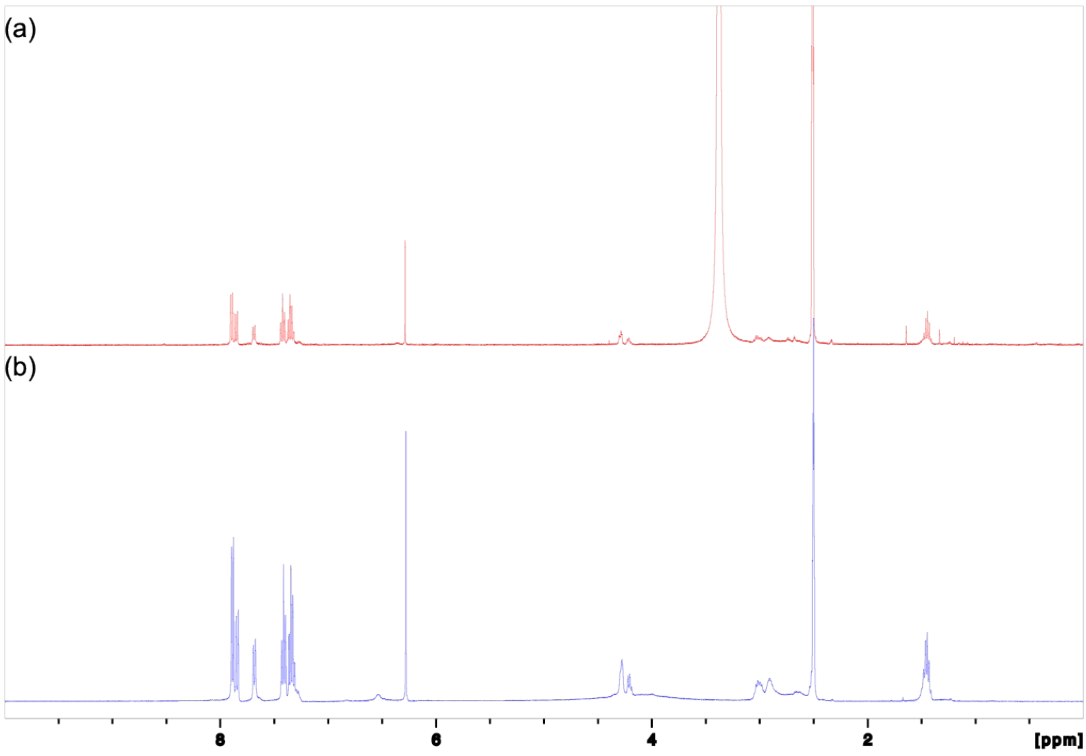

Figure S4. To confirm that the Fmoc group is not cleaved from the gelator molecule during the gelation process, ^{1}H NMR spectra of the freeze-dried Fmoc-3 gels were collected in DMSO-d_6 and compared with the spectrum of the pure Fmoc-3 gelator. (a) Freeze-dried Fmoc-3 gel (b) pure Fmoc-3 gelator.

5. Confocal fluorescence microscopy

A Zeiss LSM510 on a Zeiss Observer Z1 (Zeiss, Jena, Germany) was used for imaging. The gel samples were prepared as previously mentioned and placed within an aqueous Nile blue solution (2 µL/mL of a 0.1 wt% solution) for 30 minutes before imaging. After leaving the gels in the aqueous Nile Blue solution for 30 minutes, the gel was removed, and small sections of the gel were cut using a scalpel and placed on a microscope slide (Thermo scientific, 76 x 26 mm). A cover slip was then placed on top of the gel. Images were then collected by exciting the sample at 633 nm and detected with a Zeiss Meta detector. Data were captured using Zeiss Zen software (Zeiss, Jena, Germany) and analysed using Zeiss LSM image browser (version 4.2.0.121). This was the same procedure for all samples (Figure S5).

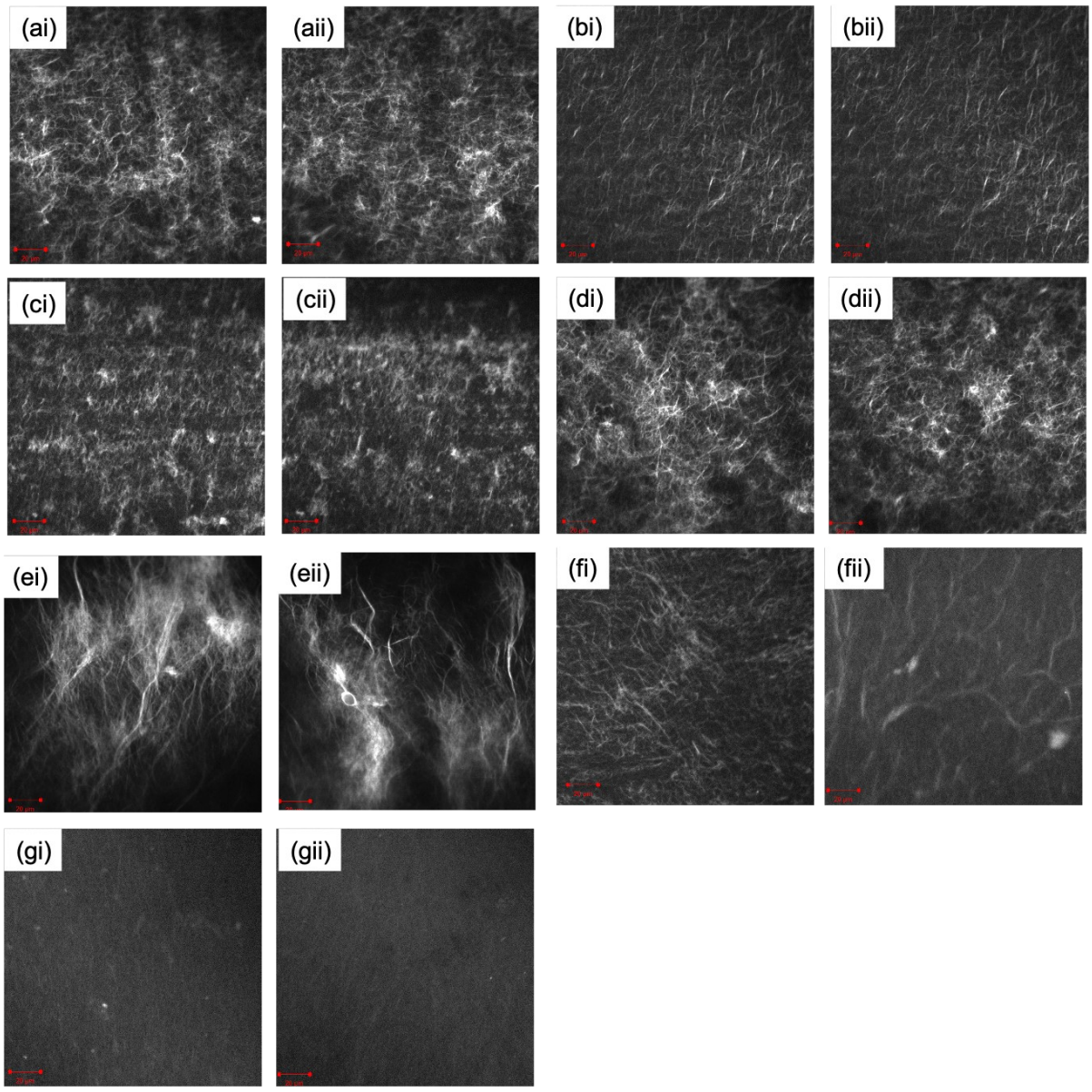

Figure S5. Confocal microscopy images of Fmoc-3 hydrogels fabricated (ai-ii) in the absence of hydrogen peroxide and in the presence of hydrogen peroxide solution (70 uL) of concentration (bi-ii) 1 M (ci-ii) 2M (di-ii) 3M and (ei-ii) 4 M. For the 4 M sample, images were taken of the Fmoc-3 hydrogels at different stages of the deposition process (fi-i) 300 seconds (gi-ii) 600 seconds. Scale bars: 20 μm. In all cases a current density of -0.7 mA/cm^2 was applied. Conditions; Fmoc-3 = [5 mg/mL], NaCl = [0.1 M].

6. Image analysis

Images of the Fmoc-3 hydrogels growing on the electrode surface were taken at 30 second intervals using an iPhone 12 camera. To analyze these images, the photos were uploaded to the open-source image processing program ImageJ. To calculate the change in gel area with time, the outline of the hydrogel on each image was traced, allowing a gel area vs time graph to be plotted on origin (Figure S6a-b).

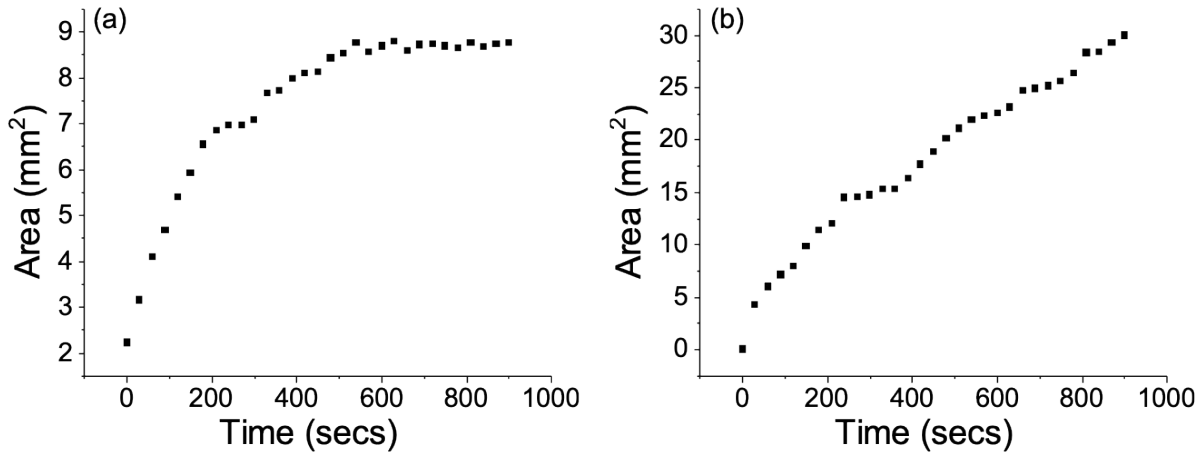

Figure S6. (a) Rate of Fmoc-3 hydrogel growth with time using a starting solution of (a) pH 6.5 (b) pH 8. Starting conditions Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], H_2O_2 = [4 M], deposition time = [900 seconds], current density = [-0.7 mA/cm^2].

7. Quantitative NMR

Using a Bruker Avance III 500 MHz spectrometer, ^{1}H NMR spectra were recorded. To determine the concentration of Fmoc-3 within the hydrogels, the gels were first frozen for two-three hours in a laboratory grade freezer. Once frozen, the gels were placed into the freeze dryer (CHRIST, Alpha 2-4 LSCbasic) for two hours. The freeze-dried gels were then dissolved in in DMSO-d_6 and transferred into an NMR tube. To determine the relative concentration of the Fmoc-3 hydrogels, a lock tube of known concentration (1% PDMS in C_2Cl_4) was used as an external standard. Using quantitative NMR (qNMR), the gelator concentration within the gels could then be calculated (Figure S7).

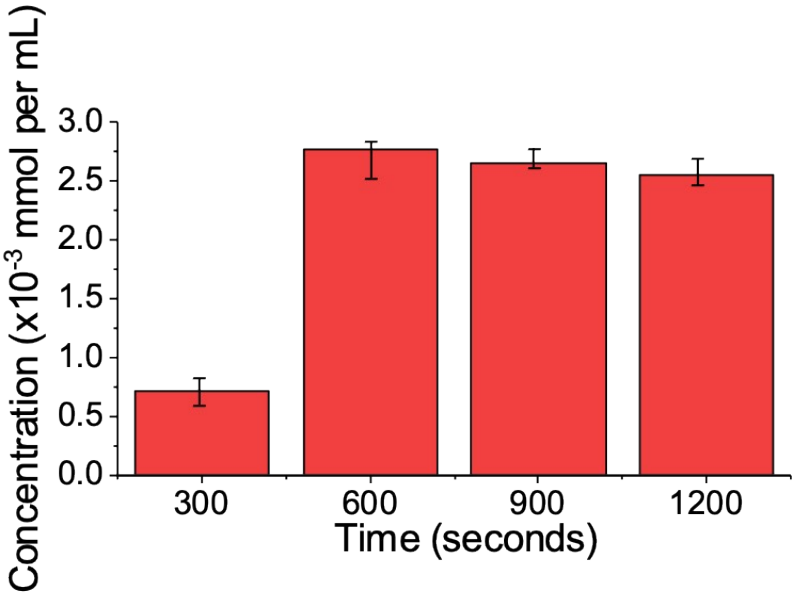

Figure S7. The relative gelator concentration of the Fmoc-3 hydrogels grown using various deposition times. For the 1200 second sample, a second aliquot (70 μL) of hydrogen peroxide was added at 600 seconds. Measurements performed in triplicate; error bars calculated by standard deviation. Conditions Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], H_2O_2 = [4 M], current density = [-0.7 mA/cm^2].

8. Electrochemical measurements

The three-electrode set up (Figure 3a) was used to run chronopotentiometry measurements. All electrochemical measurements and data were collected using a Dropsens potentiostat on the software PSTrace 5.8. To grow the gels, a current density of -0.7 mA/cm^2 was applied to the working electrode using chronopotentiometry for the allocated time (Figure S8).

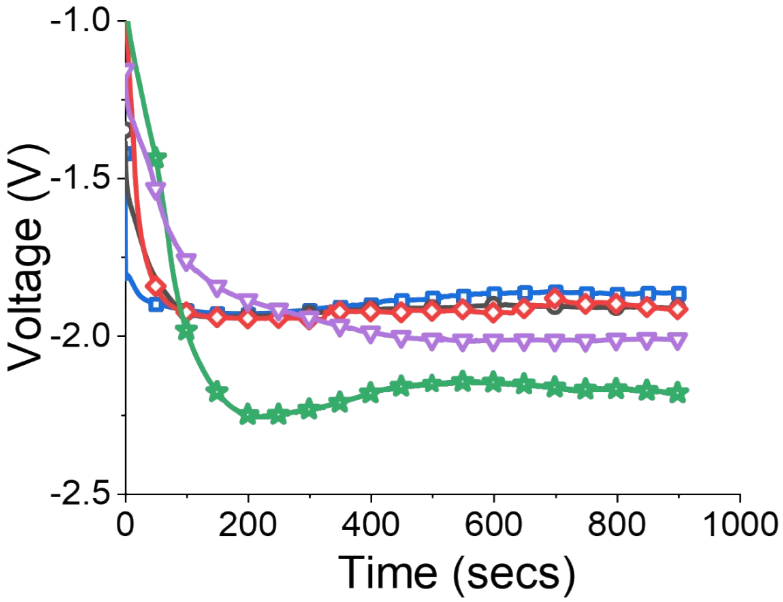

Figure S8. Chronopotentiometry measurements of Fmoc-3 gelator solutions in the absence of hydrogen peroxide (green/star data) and 70 μL of 1 M (pink/triangle data), 2 M (red/diamond data), 3 M (black/circle data) and 4 M (blue/square data) hydrogen peroxide solution. In all cases Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], deposition time = [900 seconds], current density = [-0.7 mA/cm^2].

9. Rheology

Rheological measurements were carried out using an Anton Paar Physica MCR301 rheometer. A parallel plate (12.5 mm diameter, smooth) was used to measure frequency and strain sweeps. For measuring the frequency and strain sweeps, the FTO slide with gel attached was placed onto the rheometer and secured onto the bottom plate using Sellotape to prevent slipping. As the hydrogel was not removed from the FTO surface after gel deposition, no damage occurred. Rheological measurements were recorded at 25 °C. Strain sweeps were measured from 0.01 % to 100 % with a constant frequency of 10 rad/s. Frequency scans were performed from 1 rad/s to 100 rad/s under a constant strain of 0.05%. All measurements were performed in triplicate and errors were calculated from the standard deviation (Figure S9).

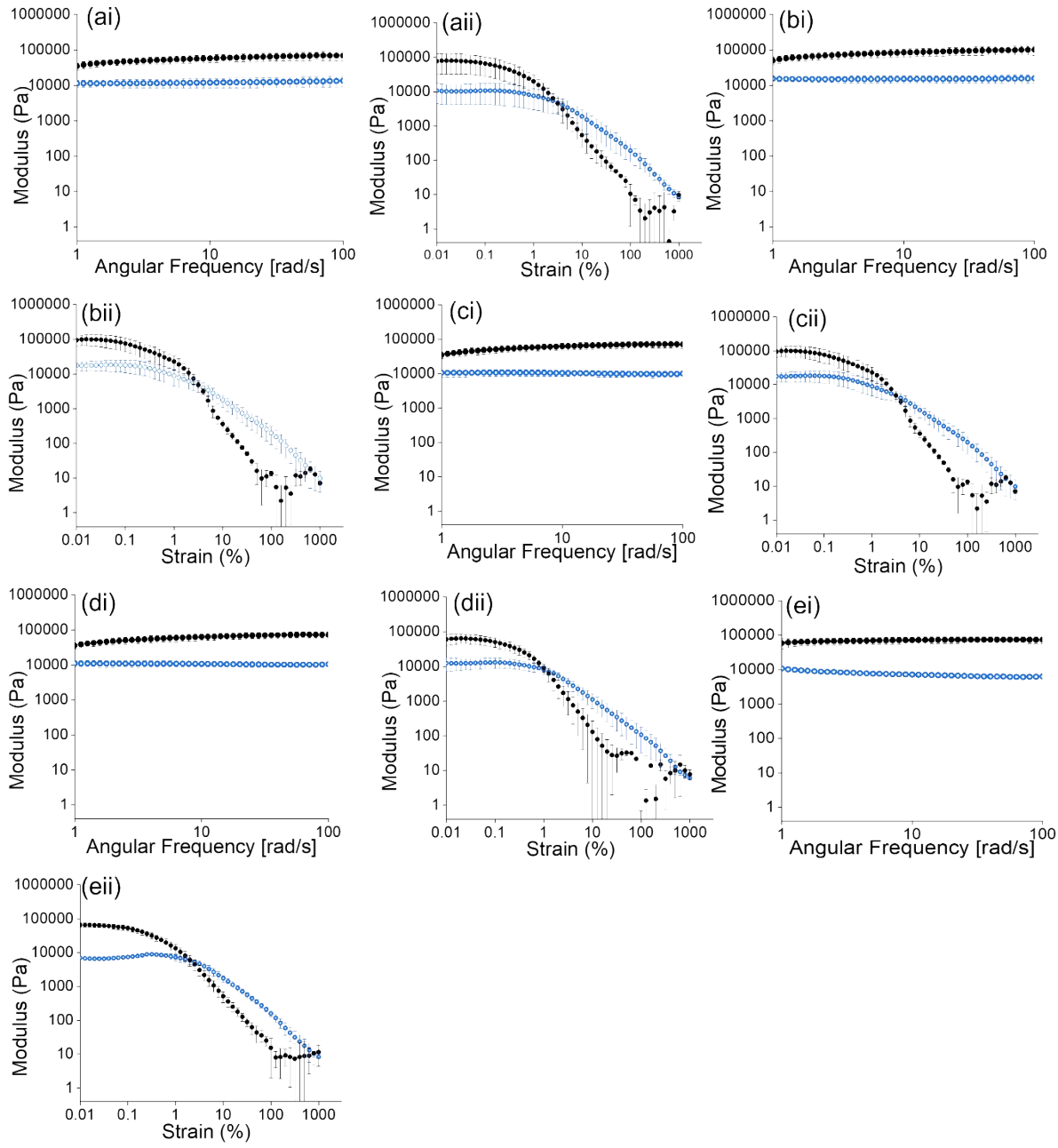

Figure S9. Frequency and strain sweeps showing the storage (G′, black/full circle data) and loss modulus (G″, blue/hollow circle data) of Fmoc-3 hydrogels (ai-aii) in the absence of hydrogen peroxide and in the presence of hydrogen peroxide solution (70 µL) at various concentrations (bi-ii) 1 M (ci-cii) 2 M (di-dii) 3 M (ei-eii) 4 M. Measurements performed in triplicate; error bars calculated by standard deviation. In all cases Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], deposition time = [900 seconds], current density = [-0.7 mA/cm^2]. For all gels, strain sweeps were measured from 0.01 % to 100 % with a constant frequency of 10 rad/s. As the linear elastic region deviated at values >0.05% strain, frequency sweeps were performed from 1 rad/s to 100 rad/s under a constant strain of 0.05%.

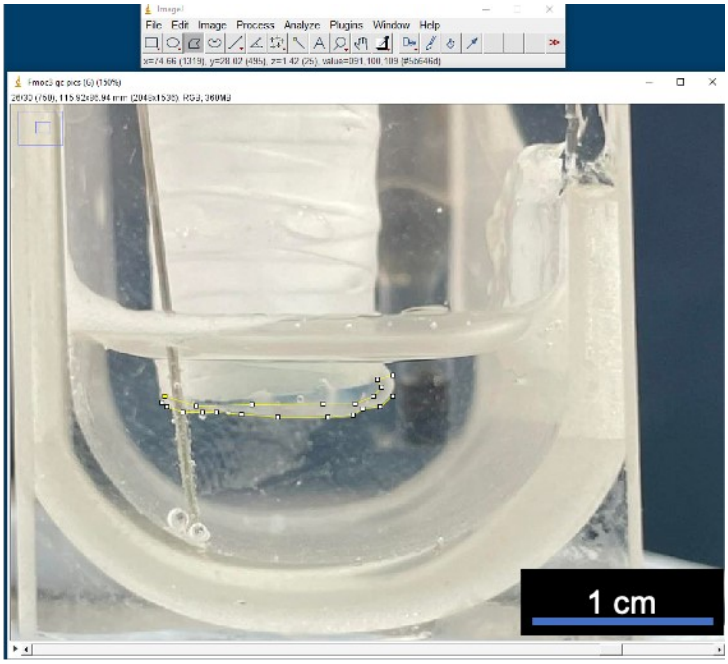

Figure 10. The open-source software ImageJ was used to trace around images of the gel growing with time on a circular glassy carbon electrode (diameter: 1.2 cm). This allowed gel growth to be followed with deposition time.

10. Small angle X-ray scattering

Small-angle x-ray scattering (SAXS) measurements were performed using Anton Paar SAXS Point 2.0 (Cu, K_{α}= 1.54 Å) at the University of Bath. This beamline operates at a fixed energy of 8.04 keV and SAXS patterns were collected at a sample-detector distance of 572mm, resulting in a Q range of 0.07– 3.3 nm^{-1}. For each sample, 1 frame was acquired with an acquisition time of 30 mins. An Anton Paar Multiple-Solid Sample Holder was used to load the samples. It comprises two metal plates with 5*4 grids (11*11 mm for each grid) sandwiching a Kapton sheet. Appropriate amounts (ca. 10 mg each) of the electrochemically processed gels were transferred into different cells of the sample holder, followed by topping with 10µl of DI water. All 2D patterns were acquired on a Dectris Eiger detector and reduced by azimuthal integration into 1D I vs Q plots using the Anton Paar SAXS Analysis software.

The scattering length density of Fmoc-3 was calculated using the National Institute of Standards and Technology Neutron[3] activation and scattering calculator to give a value of 14.069 x 10^{-6}/Å^2. A scattering length density of 9.469 x 10^{-6} Å^{-2} was used for the solvent.

Small sections of gel (2 mm thickness) grown for 900 seconds were cut and sealed in paste cells with Kapton windows. 10 µL of deionised water was added to prevent the hydrogel drying out or the formation of air pockets.

For all the samples, the collected water background resulted in an over-subtraction of the data. To account for this, the water background for each sample was normalized to the intensity of the Kapton peak (~4 nm^{-1}). For all the 1D I vs Q plots, the scaled water background was subtracted in excel before loading the subtracted data in the SasView software package (Version 5.0.5).[4] In all cases, the subtracted data were fitted to a flexible elliptical cylinder model with polydispersity of the radius to give low X^2 values as is shown in the table below. Fitting errors are provided as ±. It is important to note that the error is obtained from the fitting software and does not consider any other sources of error.

Sample	Conditions
1	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [4 M], deposition time = [300 secs]
2	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [4 M], deposition time = [600 secs]
3	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [4 M], deposition time = [900 secs]
4	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [3 M], deposition time = [900 secs]
5	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [2 M], deposition time = [900 secs]
6	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], H_2O_2 = [1 M], deposition time = [900 secs]
7	Fmoc-3 = [5 mg/mL], NaCl = [aq. 0.1 M], deposition time = [900 secs]

Table S1. Sample list of electrochemically fabricated Fmoc-3 hydrogels

	1	**2**	**3**	**4**	**5**	**6**	**7**
Model	Flexible elliptical cylinder	Flexible elliptical cylinder	Flexible elliptical cylinder	Flexible elliptical cylinder	Flexible elliptical cylinder	Flexible elliptical cylinder	Flexible elliptical cylinder
Scale	0.02 ± 3.08 x 10^{-5}	0.007 ± 3.22 x 10^{-5}	0.012 ± 2.75 x 10^{-5}	0.013 ± 2.85 x 10^{-5}	0.016 ± 3.23 x 10^{-5}	0.02 ± 3.37 x 10^{-5}	0.016 ± 3.50 x 10^{-5}
Background	1.54 ± 0.04	1.60 ± 0.04	2.35 ± 0.04	2.24 ± 0.04	2.45 ± 0.04	2.56 ± 0.04	1.65 ± 0.04
Polydispersity of the radius *	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Length*	1000	1000	1000	1000	1000	1000	1000
Kuhn Length	63.5 ± 0.3	68.4 ± 0.9	48.4 ± 0.2	45.4 ± 0.4	50.0 ± 0.3	67.8 ± 1.3	70.5 ± 0.1
Radius	34.1 ± 0.1	35.6 ± 0.2	34.7 ± 0.1	35.1 ± 0.1	37.7 ± 0.1	42.2 ± 0.1	43.2 ± 0.1
Axis ratio	7.50 ± 0.03	7.17 ± 0.07	7.36 ± 0.04	7.28 ± 0.05	6.33 ± 0.03	6.41 ± 0.04	5.95 ± 0.06
χ^2	2.92	1.31	1.79	1.56	2.18	2.48	1.64

Table S2. Fitting parameters for the electrochemically fabricated Fmoc-3 hydrogels **1-7** in H_2O. As the length is outside of the Q range of the experiment, an arbitrarily high value of 1000 Å was fixed and not allowed to refine. * indicates parameter was not allowed to fit.

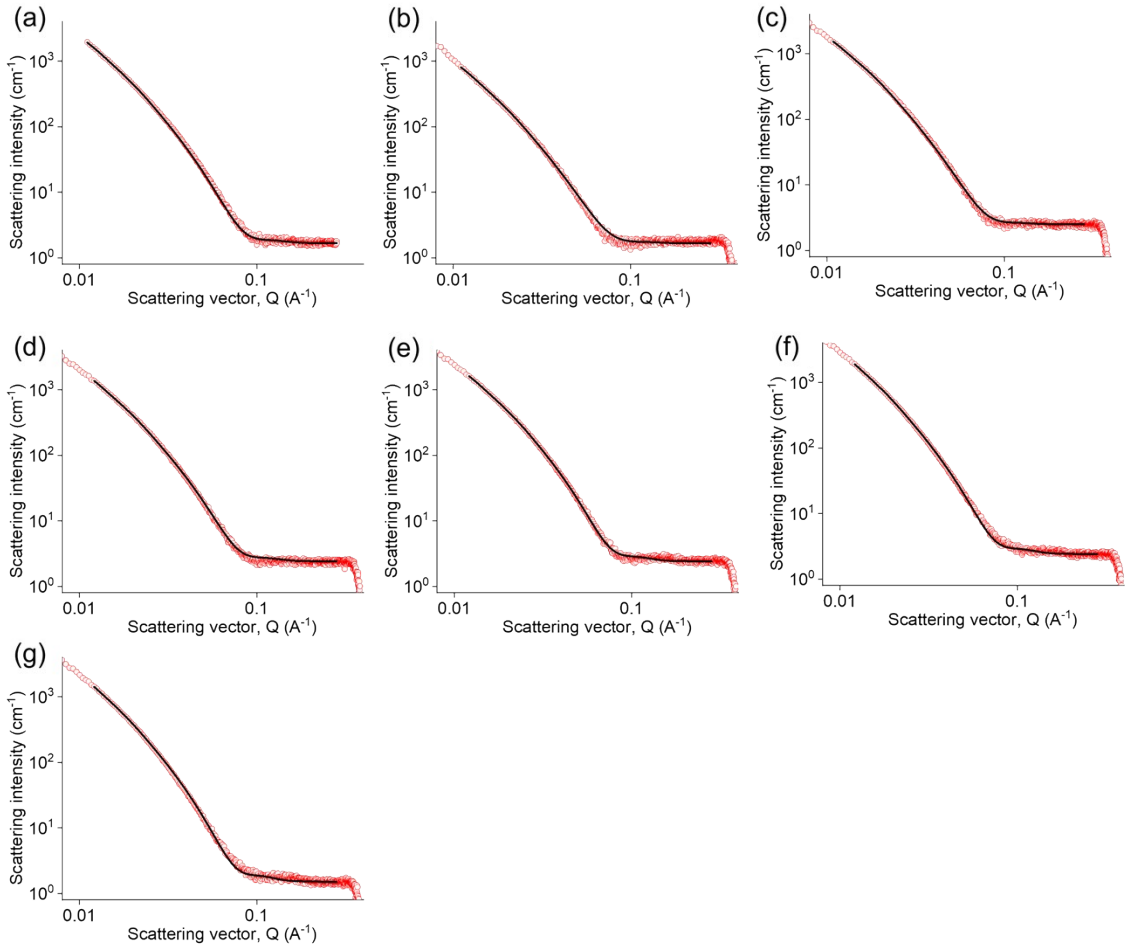

Figure S11. SAXS 1D I vs Q plots for hydrogels shown as open red circles and fits to the data (fitting parameters shown in Table S1) shown as black lines (a) **1**; (b) **2**; (c) **3**; (d) **4**; (e) **5**; (f) **6**; (g) **7**.

11. Dual hydrogel formation in H-Cell

To grow Fmoc-3 and 2NapVG hydrogels simultaneously, a custom-built H-cell consisting of two 25 mL cell chambers separated by a Nafion membrane was used to connect the two gelator solutions (Figure S10). The left-hand side chamber used to grow the 2Nap-VG-OH hydrogel consisted of the working electrode (1.2mm glassy carbon) and the reference electrode (Ag/AgCl). The right-hand side chamber used to grow the Fmoc-3 hydrogel consisted of the counter electrode (platinum wire).

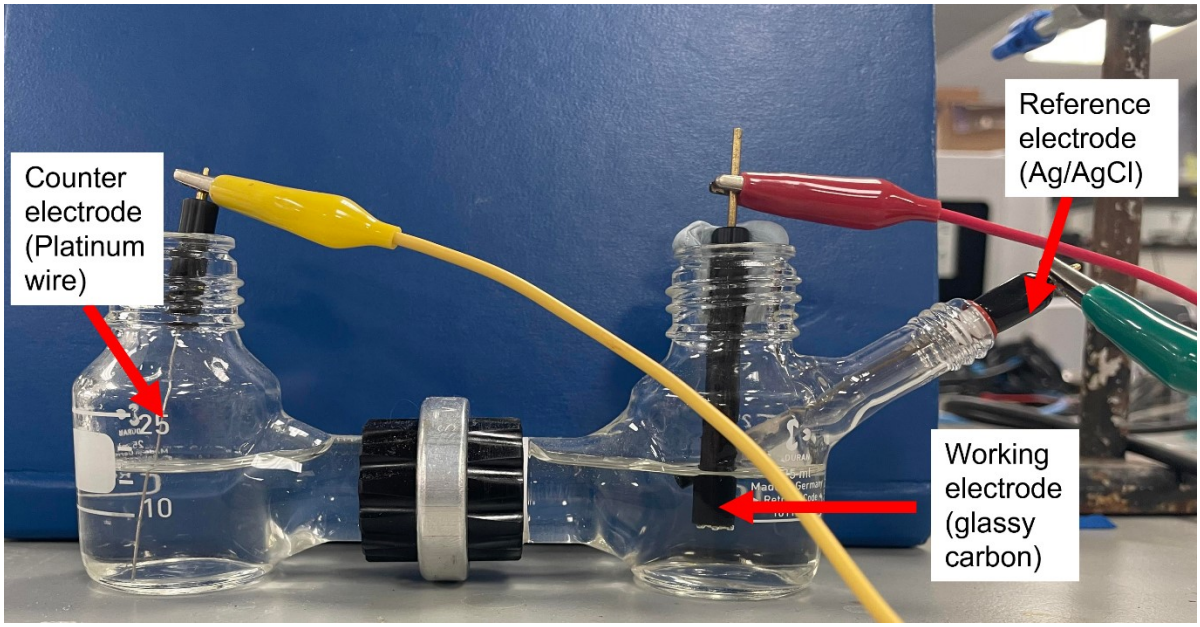

Figure S12. Electrochemical set-up for dual hydrogel formation. In the RHS chamber, initial reaction conditions; Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], H_2O_2 = [200 µL, 4M]. In LHS chamber, initial reaction conditions; 2Nap-VG-OH = [5 mg/mL], hydroquinone [5 mg/mL], NaCl = [0.1 M], pH = [8].

Prior to gel deposition, 20 mL of 2NapVG/hydroquinone solution (pH 8) was pipetted into the right-hand side chamber followed by pipetting 20 mL of Fmoc-3/hydrogen peroxide solution into the left-hand side chamber. To grow the hydrogels simultaneously current density of 22 mA/cm^2 was applied to the working electrode (Figure S11b). This seen the formation of hydrogels on both the working and counter electrode surface.

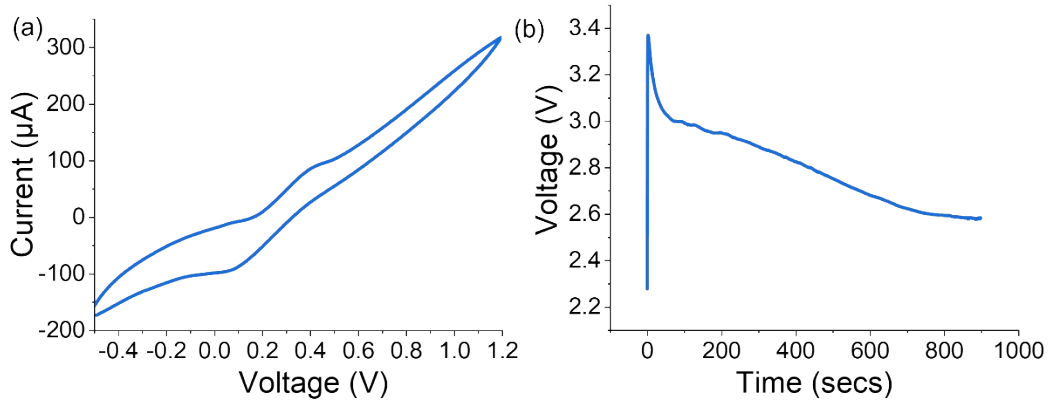

Figure S13. (a) Cyclic voltammetry and (b) chronopotentiometry measurements of the dual hydrogel system. Initial conditions; Fmoc-3 = [5 mg/mL], NaCl = [0.1 M], H_2O_2 = [200 µL, 4M], 2NapVG = [5 mg/mL], hydroquinone [5 mg/mL], NaCl = [0.1 M], pH = [8]. Current density = [22 mA/cm^2], deposition time = [900 seconds].

References

1. L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466-13471.

2. S. Panja and D. J. Adams, *Chemical Communications*, 2019, **55**, 47-50.

3. NIST, https://www.ncnr.nist.gov/ resources/activation/ (accessed: May 2022)

4. SasView, http://www.sasview.org/ (accessed: May 2022).