Supporting Information

3D-extrusion printing of stable constructs composed of photoresponsive polypeptide hydrogels

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1. Experimental

1.1 Preparation of S-(*o*-nitrobenzyl)-L-cysteine (NBC) NCA

Briefly, L-Cysteine (6.10 g, 50.35 mmol) was stirred in 80 mL of deionised water at 0 °C. Once solubilised, triethylamine (4.85 mL, 47.82 mmol) was added and the solution was allowed to stir for 30 mins. A solution of *o*-nitrobenzyl bromide (10.33 g, 47.93 mmol) in 130 mL of methanol was then added dropwise over 20 mins. The reaction solution was stirred for 18 hours in darkness and allowed to come to room temperature. The pale-yellow precipitate was first washed with ethyl acetate (3 × 80 mL) and then deionized water (3 × 80 mL). The product was then recrystallised in a mixture of the water/acetone (NBC/water/acetone 1:20:5) to afford long shiny yellow needles (9.0 g, 70 %). S-(*o*-nitrobenzyl)-L-cysteine (4.50 g, 17.56 mmol) and activated charcoal (2.50 g) were suspended in 100 mL of anhydrous THF and heated to reflux in darkness. A solution of triphosgene (2.35 g, 7.91 mmol) dissolved in 30 mL anhydrous THF was then added drop-wise to the suspension and it was allowed to stir for 5 hours. The solution was allowed to cool and it was then bubbled with nitrogen for 30 mins before being filtered and reduced to 1/3 of its volume in vacuo. It was then precipitated by addition of 120 mL hexane and stored overnight at -18 °C. The NCA solid was thoroughly dried, re-dissolved in anhydrous ethyl acetate and re-precipitated in excess hexane and allowed crystallize at -18 °C. The crude NCA crystals were then recrystallized twice in a mixture of ethyl acetate/hexane (1:4 v/v) followed by drying in vacuo to afford shiny yellow crystals (3.80 g, yield 77%). ^{1}H-NMR (Figure S1).

1.2 Preparation of γ-tert-butyl-L-glutamate (TBLG) NCA

γ-tert-butyl-L-glutamate (10.0 g, 48.37 mmol) and α-pinene (13.37 g, 94.86 mmol) were suspended in anhydrous ethyl acetate (140 mL) and stirred under reflux. A solution of triphosgene (7.25 g, 24.18 mmol) in dry ethyl acetate (40 mL) was added dropwise t the suspension, which was stirred until the solution became clear and all solids disappeared (3 hours). The reaction was allowed to cooled and filtered, and the solvent was reduced to approximately 1/3 of its original volume in vacuo. It was then precipitated by addition of 150 mL hexane and stored overnight at -18 °C. The crude NCA was recrystallized twice in a mixture of ethyl acetate/hexane (1:4 v/v) and thoroughly dried under vacuum to afford a white powder (8.30 g, yield 78 %). ^{1}H-NMR (Figure S2).

1.3 Preparation of L-isoleucine (LI) NCA

L-isoleucine (5.00 g, 38.12 mmol) and α-pinene (5.19 g, 91.48 mmol) were suspended in 100 mL of anhydrous THF and heated under reflux. A solution of triphosgene (4.76 g, 16.01 mmol) in 40 mL anhydrous THF was then added drop-wise to the suspension until all solids disappeared and the solution became clear (3 hours). The solution was allowed to cool, filtered and then reduced to 1/3 of its volume in vacuo. It was then precipitated by addition of 120 mL hexane and stored overnight at -18 °C. The crude NCA solid was dried, re-dissolved in dry ethyl acetate and re-precipitated in excess hexane twice to afford off white crystals. The NCA was then recrystallized in a mixture of ethyl acetate/hexane (1:3 v/v) followed drying in vacuo to afford long white crystals (4.52 g, yield 75 %). ^{1}H-NMR (Figure S3).

1.4 Preparation of 4-arm PEG-propiolate

4-arm PEG-OH (9.0 g, 5.50 mmol) was dissolved in a mixture of toluene (90 mL) and mesitylene (50 mL), followed by the addition of 2 drops of concentrated H_2SO_4. The solution was heated to 80 °C with stirring until clear. Propiolic acid (2.5 g, 35.69 mmol) was added to the reaction solution, which was then heated and allowed to reflux using a Dean–Stark apparatus. The reaction was stopped after 24 hours after no more water was collected in the condenser, and the resultant brown solution was allowed to cool. The solvents were then removed under reduced pressure to yield a brown oil, which was dissolved in CH_2Cl_2 (100 mL) and washed with brine solution (20 mL) and deionised water. The organic phase was dried ($MgSO_4$), charcoal was added (0.1 g) and the suspension was stirred for 30 min at 40 °C, followed by filtration through Celite 545. The solvent was removed in vacuo and the resultant oil was redissolved in deionised water (20 mL) and made pH neutral (7.5) with $NaHCO_3$. It was then dialysed exhaustively against deionised water for two days and freeze-dried to afford a light yellow-orange oil (5.5g, yield 55 %). ^{1}H-NMR (Figure S4).

2. Additional Tables

Table S1. Synthesis of photoresponsive random and block copolymers

	[NCA]/[initiator] (mol/mol)[a]*	[NCA]/[initiator] (mol/mol)[b]*	M_n (^{1}H-NMR)	M_n, (SEC)	M_w/M_n (SEC)
PECl-1	45:25:25/1	54:34:24/1	20,700	13,500	1.35
PECl-2	45:25:25/1	50:28:27/1	18,900	11,400	1.29

[a] Theoretical degree of polymerisation value based on feed ratio.
[b] Experimental degree of polymerisation value based on ^{1}H-NMR integration.
*based on TBLG, NBC and LI in that order.

3. Additional Figures

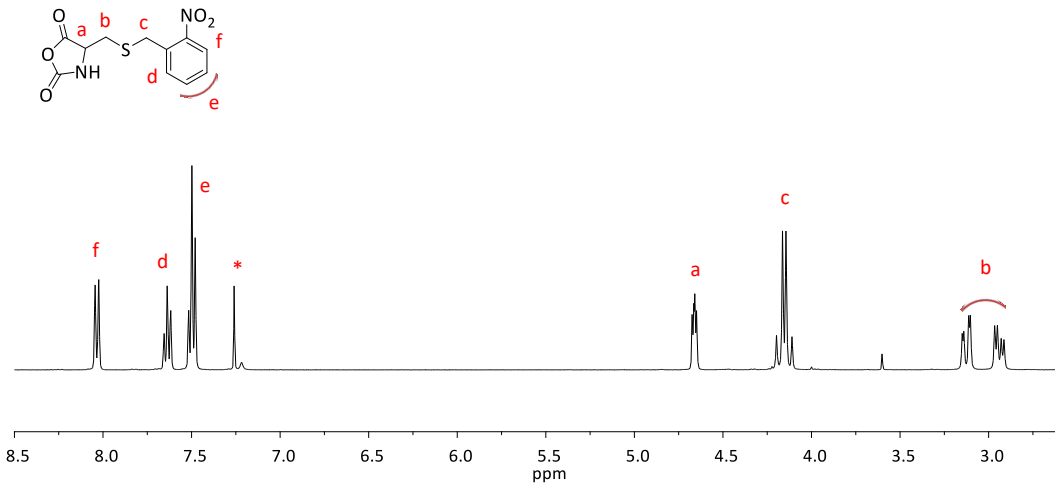

Fig. S1 ^{1}H-NMR spectra of S-(o-nitrobenzyl)-L-cysteine (NBC) NCA.

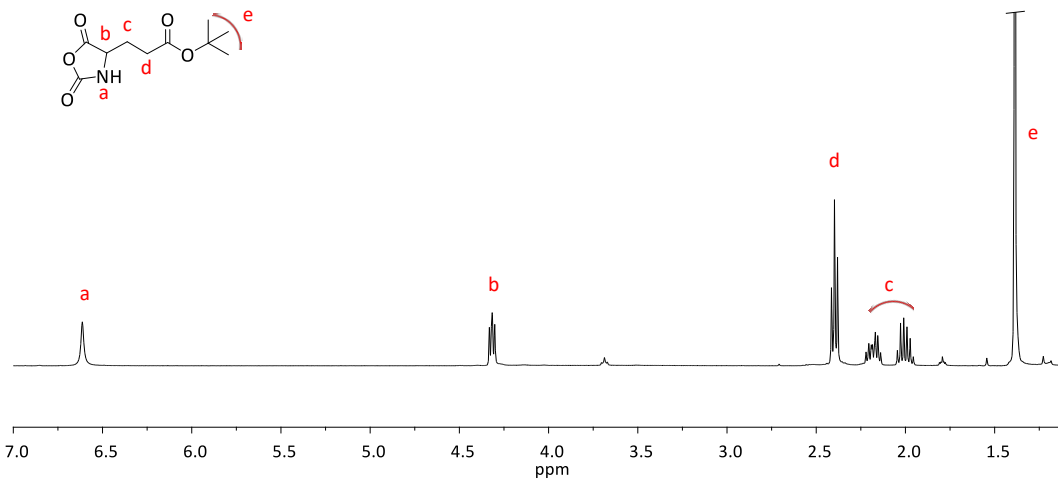

Fig. S2 ^{1}H-NMR spectra of γ-tert-butyl-L-glutamate (TBLG) NCA.

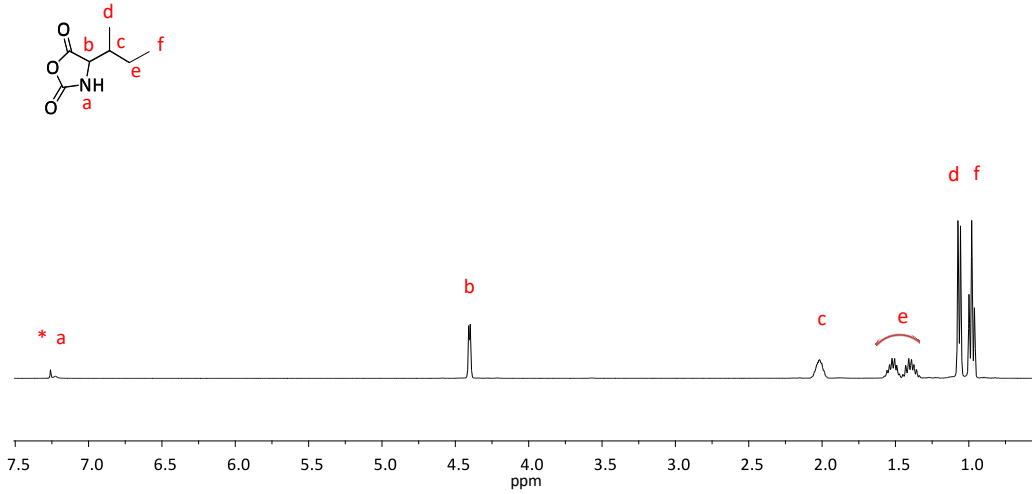

Fig. S3 ^{1}H-NMR spectra of L-isoleucine NCA.

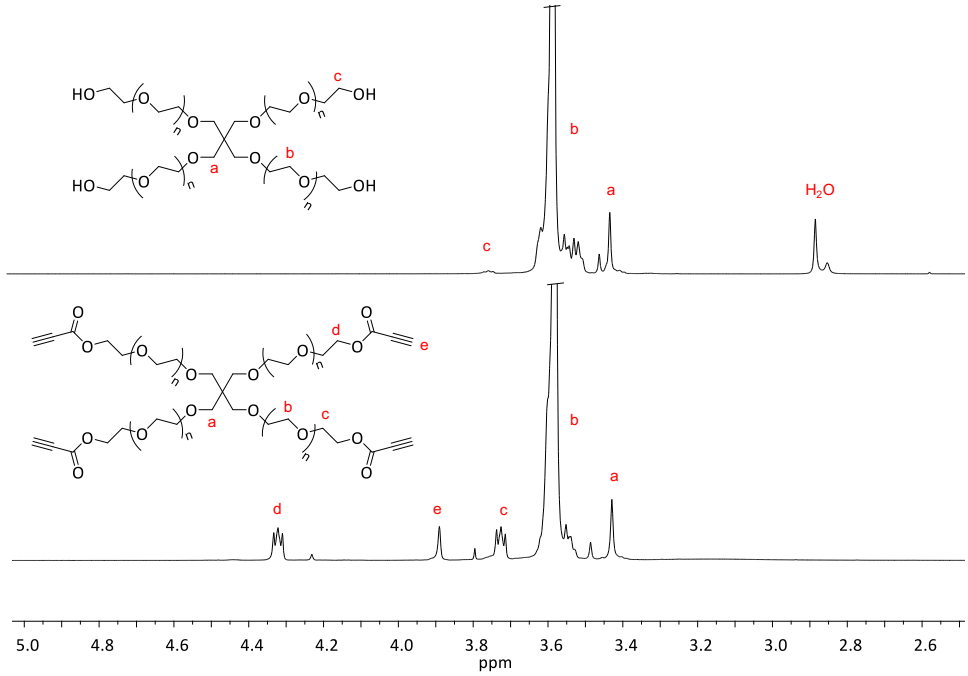

Fig. S4 ^{1}H-NMR spectrum of 4-arm PEG-OH and 4-arm PEG-propiolate in acetone-d_6.

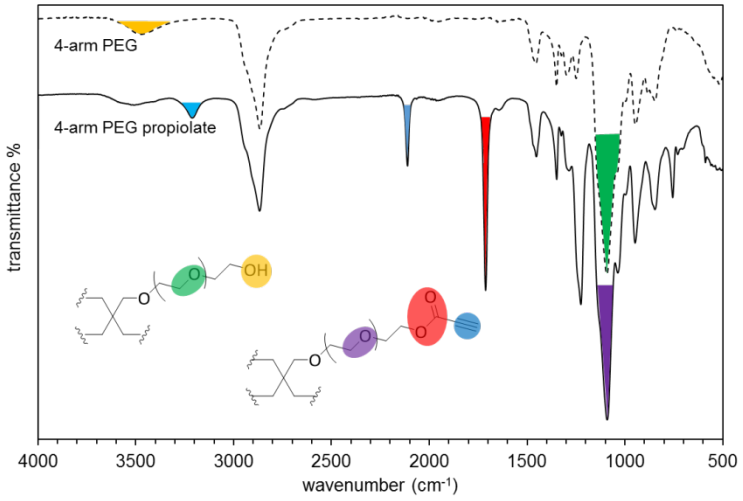

Fig. S5 FTIR spectrum of 4-arm PEG-OH and 4-arm PEG-propiolate with highlighted functional groups.

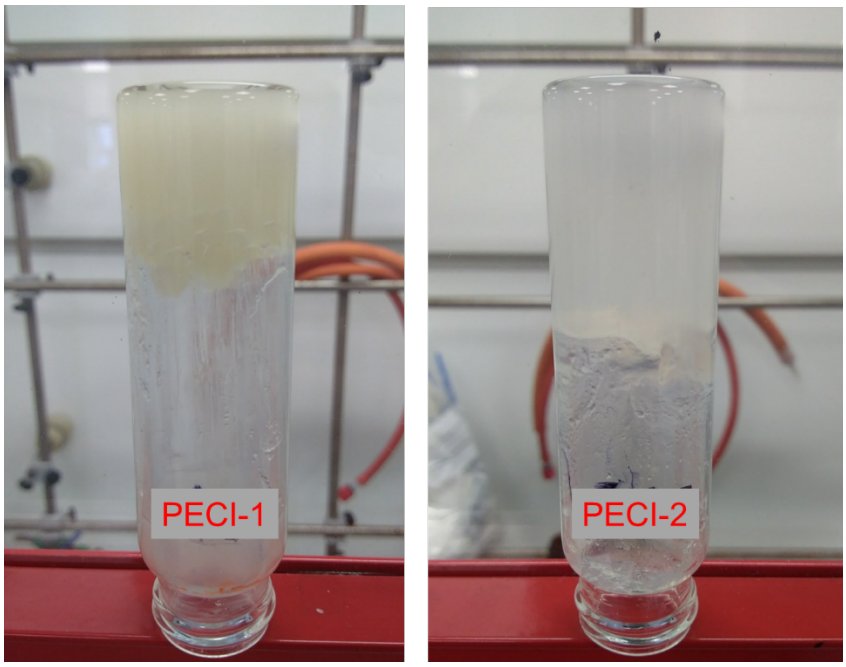

Fig. S6 Vials containing the polymerisation mixture after complete consumption of monomers (18 hours).

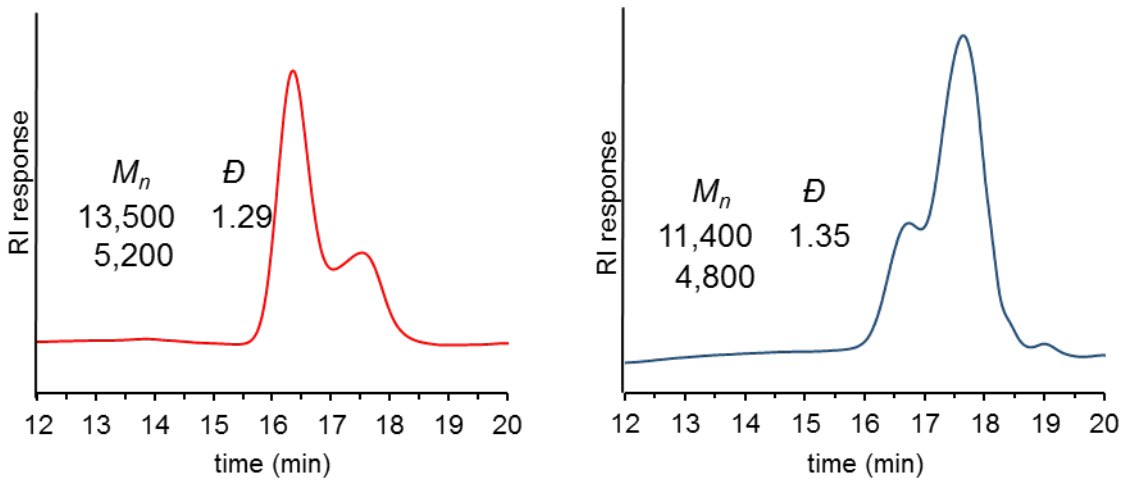

Fig. S7 Size exclusion chromatography (SEC) traces of PECI-1 (red) and PECI-2 (blue).

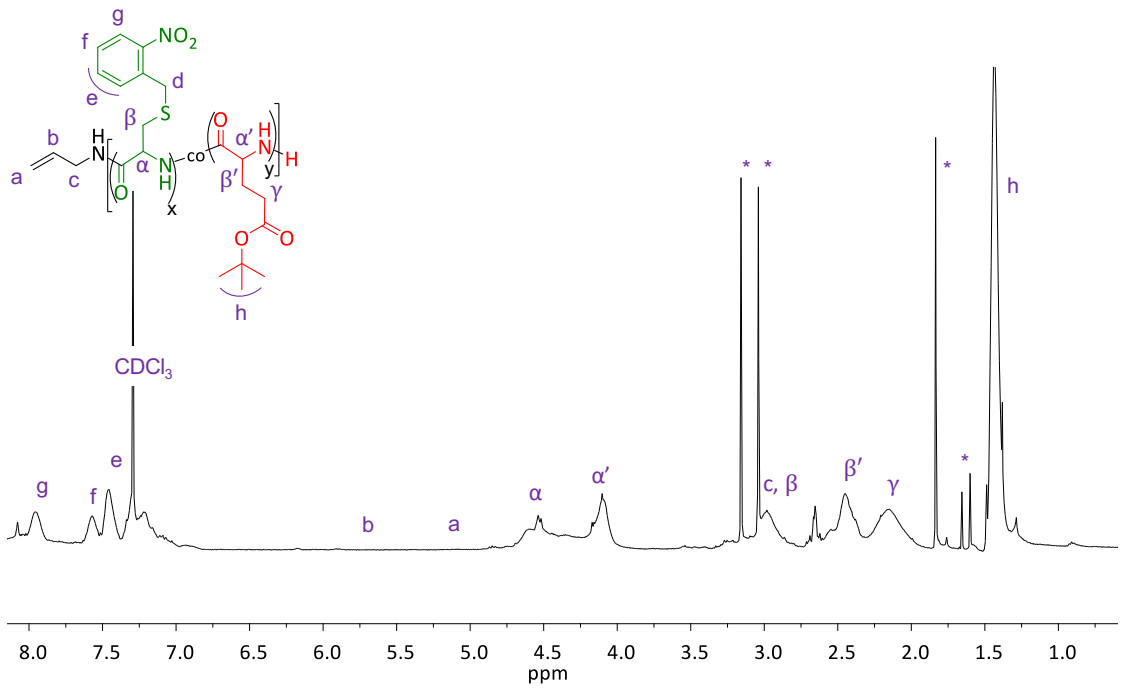

Fig. S8 ^{1}H-NMR spectrum of first block of PECl-2 polymer ($CDCl_3$ with 5%TFA-d).

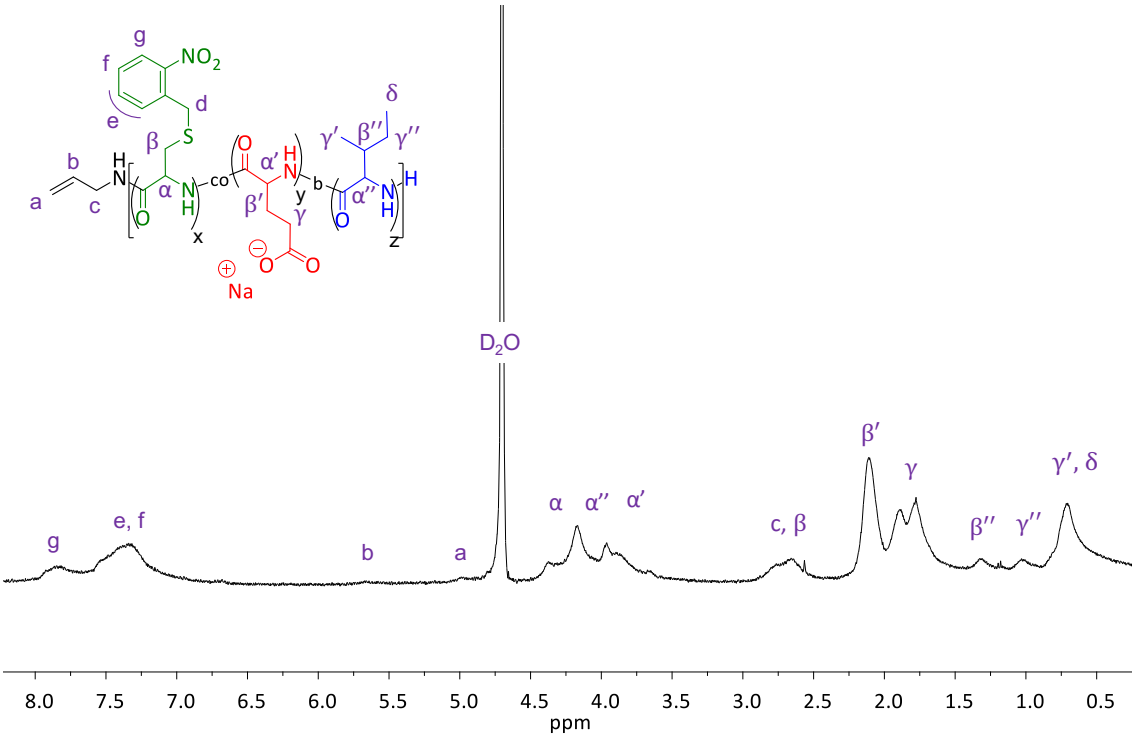

Fig. S9 ^{1}H-NMR spectrum of deprotected PECl-2 polymer (D_2O).

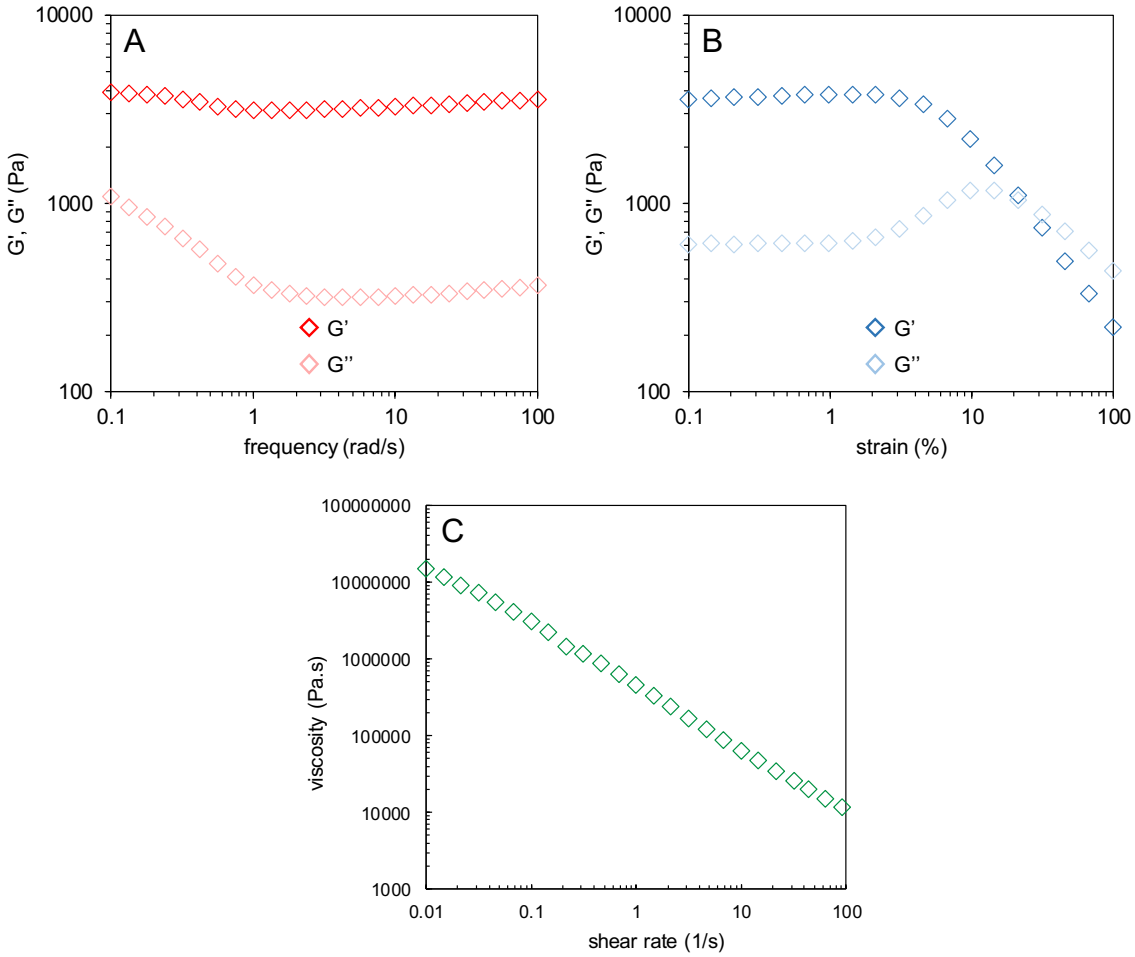

Fig. S10 Rheological properties of PECI-1 6.5 wt% hydrogel ink. A) Frequency sweep showing effect of alternating oscillation frequencies on hydrogel strength. B) Strain amplitude time sweep characterising the shear-thinning and recovery behaviour over time. C) Shear rate sweep showing effect of increasing shear on material viscosity.

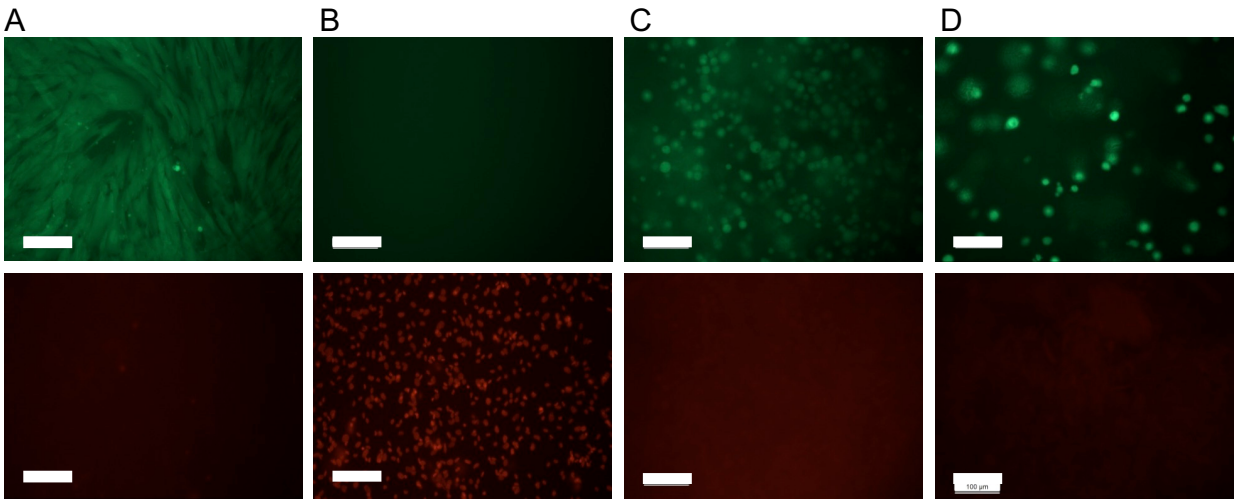

Fig. S11 A live/dead stain of human dermal fibroblasts after a 24h incubation time showing 2D cultured: (A) untreated cells, (B) untreated cells + 1% lysis buffer, (C) cells on top of PECI-2 hydrogel and (D) cells encapsulated within PECI-2 hydrogel (top panel live cells in green; bottom panel dead cells in red).