

Visible Light Mediated-Cleavage of Polymer Chains under Physiological Conditions via Photoreduction and Quinone Trimethyl Lock

Vinh X. Truong,* Fanyi Li, Fran Ercole, John S. Forsythe.*

Supporting Information

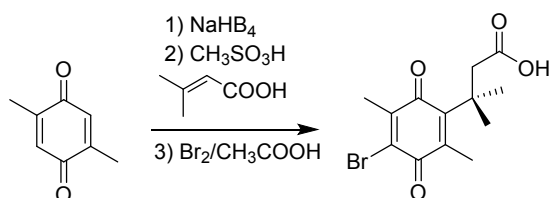
1. General Consideration

Solvents (CH_2Cl_2 , diethyl ether, petroleum ether, acetone, xylene, and methanol) were purchased from VWR in HPLC grade. PEG-SH (conversion of end-group was *ca.* 98%) and PEG-NH₂ (conversion of end-group was *ca.* 94%) precursors were prepared from 4-arm PEG_{10k}-OH (Jenkem Tech, USA) or MeO-PEG-OH (Sigma-Aldrich) using established procedures.^{1,2} Other chemicals were purchased from Sigma-Aldrich and used as received. All synthesised compounds were stored in a fridge at 2-4 °C.

Glassware for synthesis was first treated in a base bath (KOH in isopropanol 2 M) overnight, rinsed with water, submerged in an acid bath (HCl 1 M) for 2-4 h, rinsed with reverse osmosis water and dried in an oven at 120 °C for 6-12 h.

NMR spectra were recorded on a Bruker Advance III 400 or 600 with a 5 mm broadband auto-tunable probe with Z-gradients at 293 K. Chemical shifts are reported as δ in parts per million (ppm) and referenced to the chemical shift of the residual solvent resonances (CDCl_3 $\delta = 7.26$ ppm), couplings are shown as d: doublet, t: triplet, m: multiplet and bs: broad singlet. Polymer samples were prepared at a concentration of 20 mg mL⁻¹. In most spectra traces of water appears as a broad singlet at around 1.5-2.0 ppm. NMR spectra were processed using MestReNova software.

2. Materials Synthesis



Synthesis of 3-(4-bromo-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoic acid (S1).

This compound was synthesized following a previously reported method,³ in brief 2,5-dimethyl-1,4-benzoquinone (2 g, 15 mmol) was added to a solution of Et₂O (40 mL), MeOH (20 mL) and H₂O (80 mL). NaBH₄ (2.85 g, 75.4 mmol) was added in small portions with stirring. The solution colour quickly changed from deep yellow to light yellow. After 20 min, the solution was extracted with Et₂O (100 mL x3), the organic phase was washed with brine (100 mL), dried (MgSO₄) and concentrated in vacuo to give product as a pale yellow solid.

The above product and 3,3-dimethylacrylic acid (1.7 g, 7 mmol) were dissolved in methansulfonic acid (100 mL) and the resultant mixture was heated to 70 °C and stirred at this temperature overnight. The solution was then poured into ice and the mixture was extracted with EtOAc (100 mL

x 4). The organic phases were combined, dried (MgSO_4) and concentrated in vacuo to give product as a pale yellow solid.

The above product was suspended in acetic acid (100 mL) and a solution of Br_2 (1.7 mL) in acetic acid (18 mL) was added dropwise. The solution was then covered with aluminium foil and stirred overnight at ambient temperature. The resultant dark red solution was poured into ice water (100 mL) and extracted with CH_2Cl_2 (100 mL x 2). The combined organic phase was back-extracted with NaHCO_3 solution (*note: extreme gas was released from mixing, shake with caution*). The aqueous phase was neutralised with careful addition of concentrated HCl under vigorous stirring until pale yellow solid appeared. The solution was then extracted with EtOAc (100 mL x 3), dried (MgSO_4) and concentrated in vacuo to give product as a light brown solid (3.32 g, total yield: 63%). ^1H NMR (400 MHz, CDCl_3): 3.01 (s), 2.21 (s), 2.14 (s), 2.09 (s), 1.45 (s). Spectral data are in agreement with literature values.³

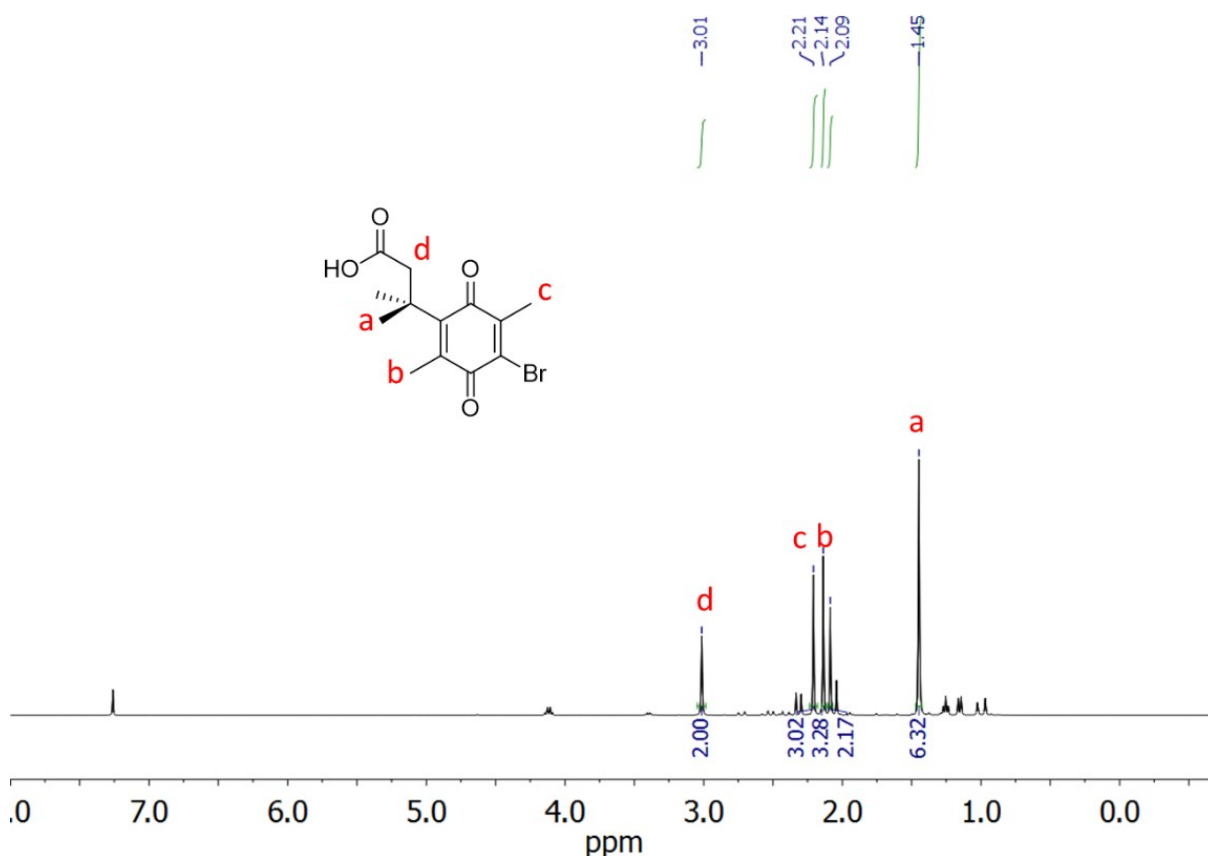
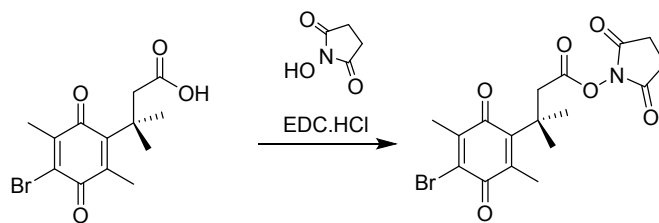


Figure S1. ^1H NMR (CDCl_3 , 400 MHz) of compound **S1**, traces of water and EtOAc are present.



Synthesis of 2,5-dioxopyrrolidin-1-yl 3-(4-bromo-2,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-methylbutanoate (**2**). This synthesis was undertaken under minimal lighting conditions; where possible the chemicals were covered with aluminium foil. Compound **S1** (2.34 g, 7.43 mmol) was dissolved in CH₂Cl₂ (50 mL), (3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.48 g, 7.5 mmol) and N-hydroxysuccinimide (0.85 g, 7.5 mmol) were added. The solution was stirred at ambient temperature and monitored by TLC until complete consumption of the acid starting materials. The solution was then washed with water (50 mL), brine (50 mL), dried (MgSO₄) and concentrated in vacuo. The residue was purified by column chromatography on SiO₂ and eluting with ethyl acetate. The yellow fractions were collected and dried in vacuo to give product as yellow solid (Yield 2.45 g, 79.3%) ¹H NMR: 3.14 (s), 2.73(s), 2.17 (s), 2.10 (s), 1.47 (s). ¹³C NMR: 187, 180, 169, 168, 151, 148, 141, 133, 44, 38, 29, 25, 17, 15. HRMS (ESI): m/z [M⁺] calcd 411.0317 found 411.0114

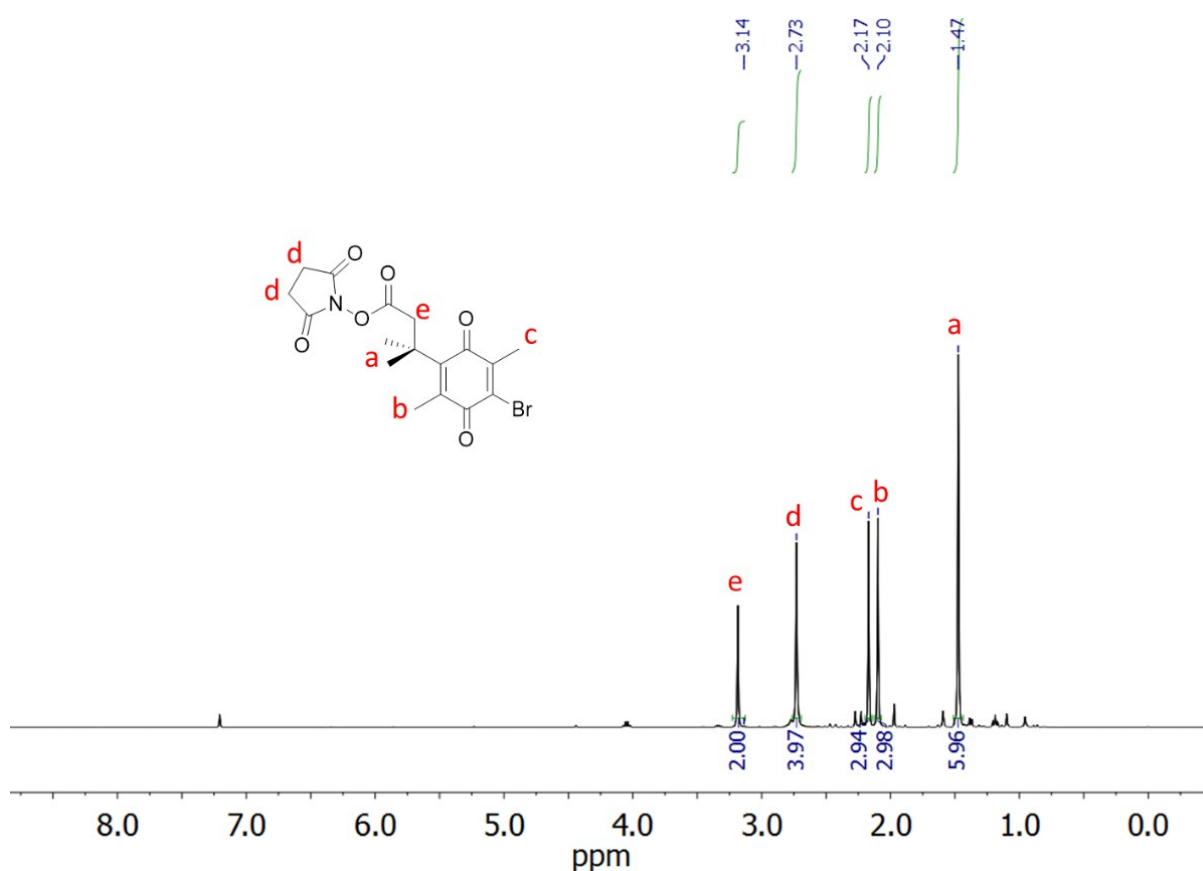


Figure S2. ¹H NMR (CDCl₃, 400 MHz) of compound **2**, traces of water and EtOAc are present.

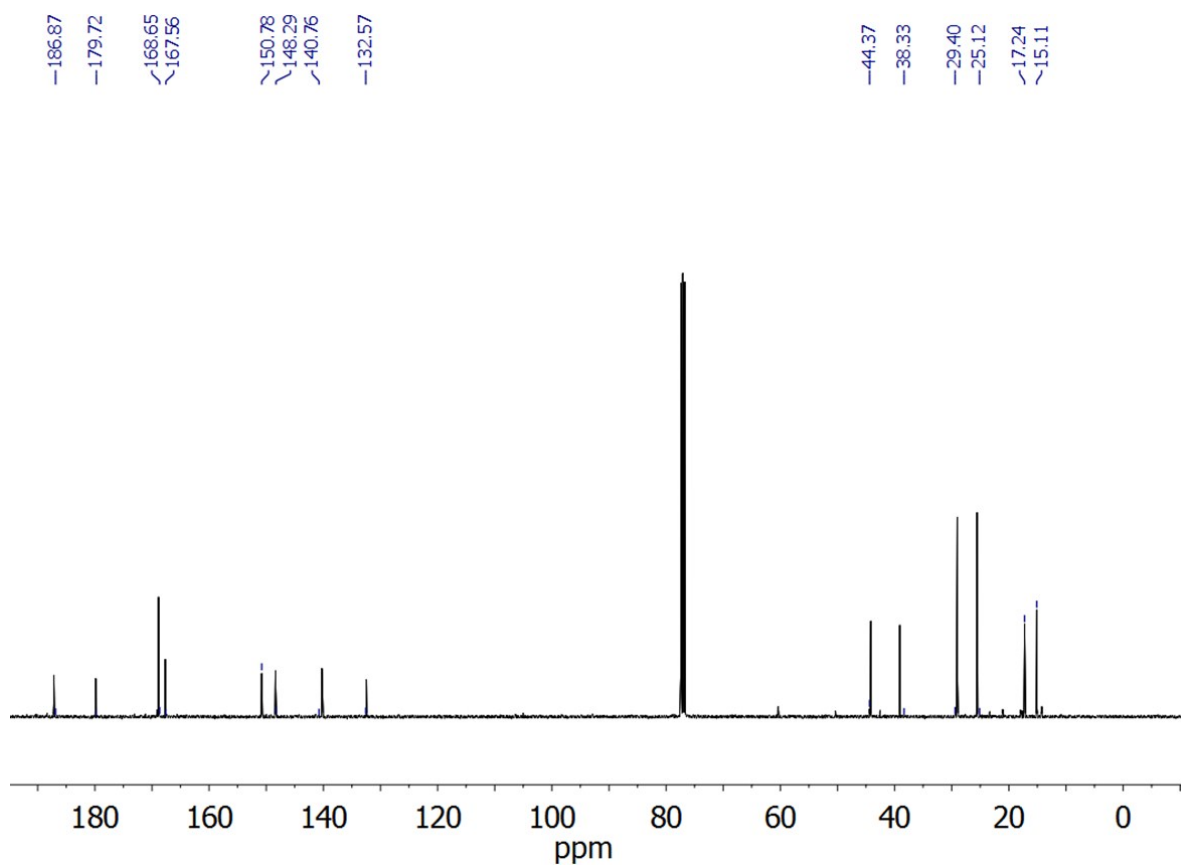


Figure S3. ^{13}C NMR (CDCl_3 , 100 MHz) of compound **2**.

Synthesis of MeO-PEG-bromobenzoquinone (compound 3). This reaction was carried out under minimal light exposure. MeO-PEG-NH₂ (1g, 0.5 mmol) and **2** (0.25 g, 0.6 mmol) were dissolved in CH₂Cl₂ (5 mL) followed by addition of N-Ethyldiisopropylamine (50 μL) and the solution was stirred overnight. This solution was then added to Et₂O (50 mL) and the mixture was filtered to give polymer product as yellow powder (yield: 0.97 g, 84.3%). ^1H NMR: 6.21 (bs), 3.65 (bs), 3.32 (s), 2.78 (s), 2.17 (d), 1.34 (s). $M_n = 4451 \text{ g mol}^{-1}$, $\mathcal{D} = 1.07$.

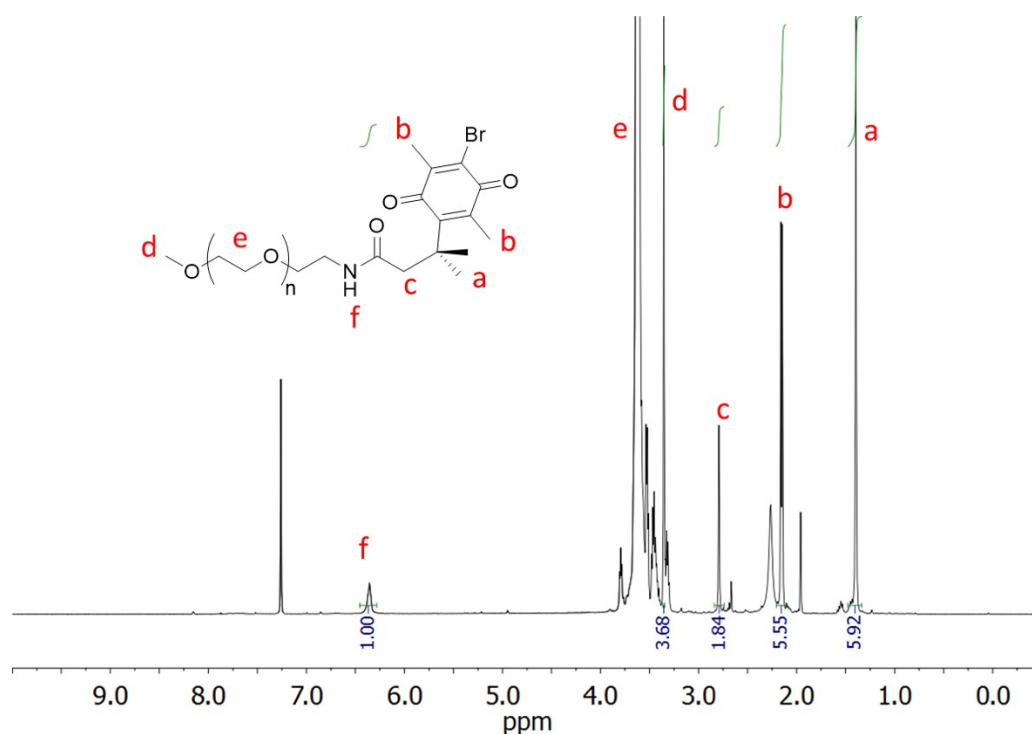


Figure S4. ^1H NMR (CDCl_3 , 400 MHz) of polymer **3**.

Preparation of polymer 5. Compound **3** and MeO-PEG-SH (compound **4**) were each dissolved in PBS pH 8.0 to form solutions with polymer concentration of 100 mg mL^{-1} (mM). The two solutions were mixed and stirred for 30 min after which it was lyophilized. The collected yellow solid was redissolved in CDCl_3 for ^1H NMR and GPC analysis without further purification.

4-arm PEG-bromobenzoquinone (compound **9**) was synthesized from 4-arm PEG- NH_2 following a similar procedure to the synthesis of polymer **3** to produce polymer **9** as a slight yellow solid with a yield of 92.1%.

3. Polymer and hydrogel characterizations

For the photolysis study, polymer **5** which was collected from lyophilisation was redissolved in water (concentration of 50 mg mL^{-1}) and subjected to irradiation of blue light (420 nm , 20 mW cm^{-2}) for 30 min after which it was lyophilized for further ^1H NMR and GPC analysis.

Size Exclusion Chromatography (SEC) analyses of polymer samples were performed using a Shimadzu modular system comprising a DGU-20A3R degasser unit, a SIL-20A HT autosampler, a $10.0 \mu\text{m}$ bead-size guard column ($50 \times 7.8 \text{ mm}$) followed by three KF-805L columns ($300 \times 8 \text{ mm}$, bead size: $10 \mu\text{m}$, pore size maximum: 5000 \AA) and an RID-10A differential refractive-index detector. The temperature of columns was maintained at $40 \text{ }^\circ\text{C}$ using a CTO-20A oven. The eluent was dimethylacetamide

(CHROMASOLV Plus for HPLC) and the flow rate was kept at 1.0 mL min^{-1} using a LC-20AD pump. A molecular weight calibration curve was produced using commercial narrow molecular weight distribution polystyrene standards with molecular weights ranging from 500 to $2 \times 10^6 \text{ g mol}^{-1}$. Polymer solutions at approx. 2 mg mL^{-1} were prepared and filtered through $0.45 \mu\text{m}$ PTFE filters before injection.

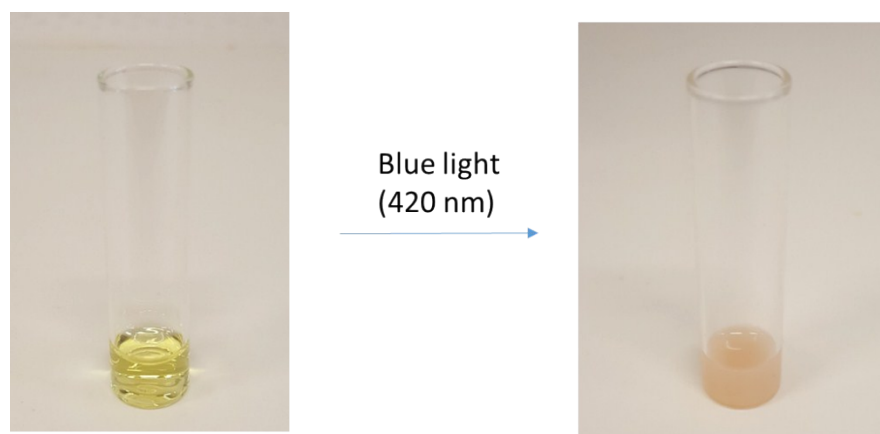


Figure S5. The polymer solution before and after treatment with blue light.

Table S1. SEC data of investigated polymers

Polymer	M_n (g mol^{-1})	\bar{D}
1	3135	1.08
3	4451	1.07
4	4839	1.13
5	6795	1.12
6-8	5269	1.22

UV-vis spectra were measured using a Carry60 spectrometer. A quartz cuvette with a transparent window above 220 nm was used and the recorded absorbance values were corrected for background and solvent absorbance.

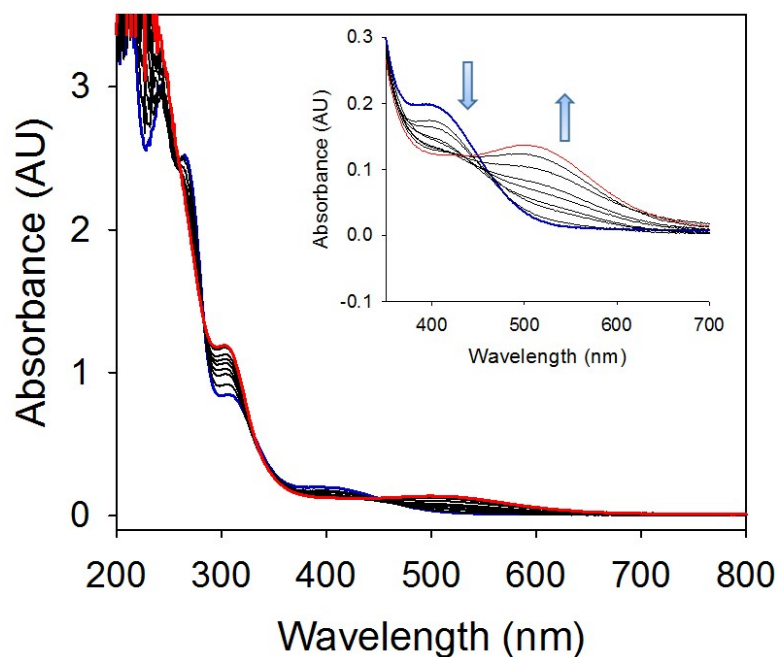


Figure S6. Time course UV-Vis spectra of the photolysis by irradiation of **5** with blue light (420 nm) in water.

Table S2. Spectroscopic data of **5** in water for irradiated wavelength

	365 nm	420 nm	445 nm	470 nm
$\epsilon(\text{M}^{-1} \text{cm}^{-1})$	896	680	512	316

Rheological analysis. Tests were carried out on an Anton Paar Physica rheometer with a plate-plate configuration - the lower plate is made of quartz and the upper plate is made of stainless steel with a diameter of 15 mm. A liquid light guide, which was connected to the light source (Wavelength-Switchable LED Sources), was equipped below the quartz plate. In a typical experiment, PBS solution (with pH range of 7.4-8.6) of polymer **8** (10 wt%, 25 μL) and polymer **9** (10 wt%, 25 μL) was mixed and the solution was quickly vortexed for 5 seconds. 25 μL of the resultant mixture was then placed on the lower plate and the upper plate was lowered to a measurement gap of 0.2 mm. A layer of paraffin oil was applied on the edge of the stainless steel plate to prevent dehydration of hydrogel and the test was quickly started by applying a 1% strain with the frequency of 1 Hz on the sample.

For the photo-degradation study, the storage modulus of the gel formed within the rheometer was allowed to reach equilibrium when no further increase in the G' value was observed, then the light at the investigated wavelength was turned on. The intensity of light irradiance on the gel sample was

tuned to the desired intensity using either a Cole Parmer Series 9811 365 nm Radiometer (for 365 nm wavelength) or a RM-12 radiometer (Opsytec) with a sensor VISBG 400-570 nm (for 420 nm, 455 nm, and 470 nm wavelength).

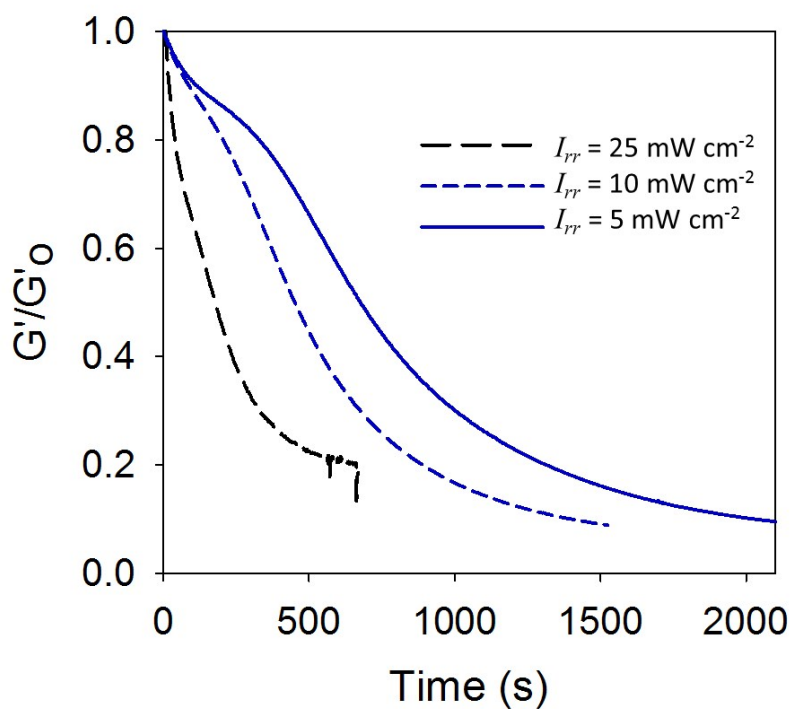
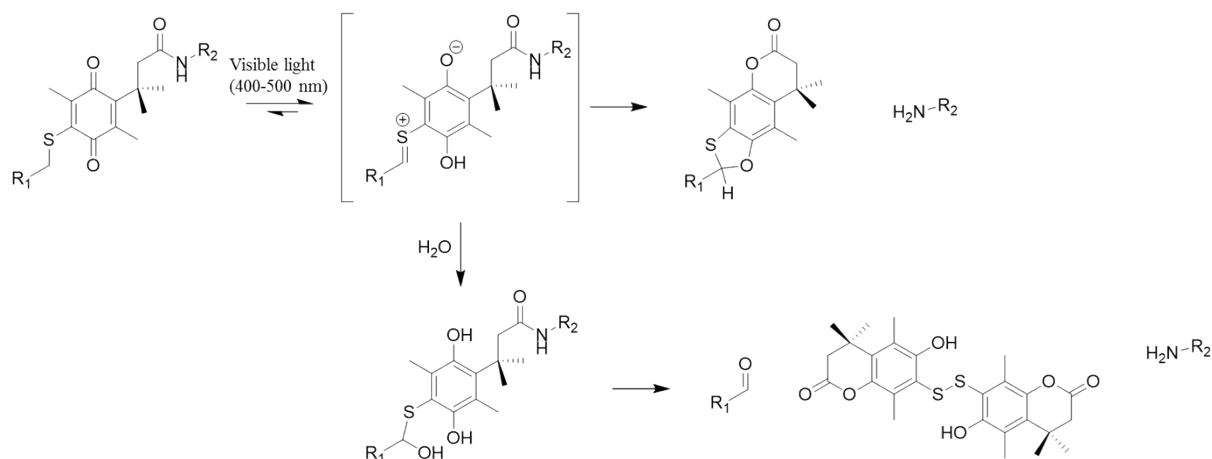


Figure S7. Rheological profiles of hydrogel degradation under irradiation of light at 420 nm at different intensities.

Scheme S1. Photolysis of hydroquinone is via photoreduction to form a zwitterionic intermediate which can then undergo direct trimethyl lock lactonization to give cyclic product or solvent trapping and hydrolysis to produce a carbonyl and disulfide adduct, both pathways release an amine group.



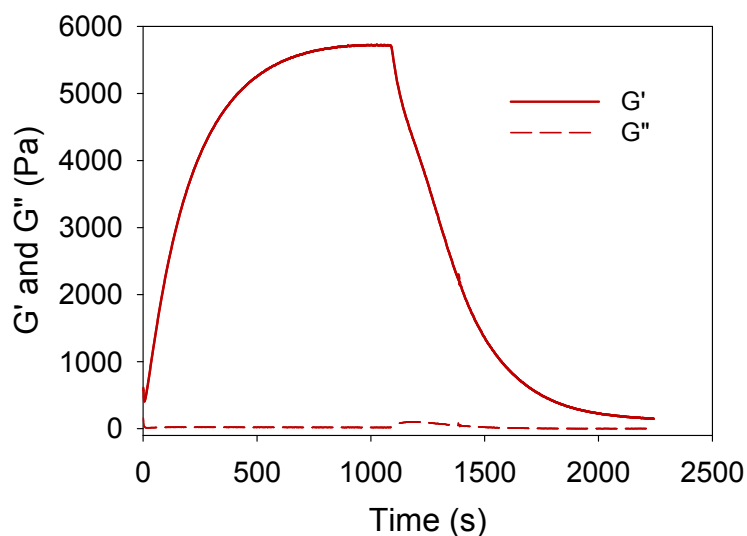


Figure S8. Rheology profiles for the formation and subsequent photodegradation of hydrogel used in cell encapsulation and release. Preparation conditions: polymer concentration of 10 wt% in DMEM PBS pH 8.4 solution. Light source for degradation is from an OmniCure Series 2000 lamp with a filter bandpass of 400-500 nm and the intensity was 20 mW cm⁻².

4. Cell maintenance and encapsulation

Mouse fibroblast L929 cells (L929) were maintained in cell culture media comprising of Dulbecco's Minimum Eagle medium (DMEM) (1 g L⁻¹ D-Glucose and 110 mg L⁻¹ Sodium pyruvate) (Life Technologies, USA) with 10% FBS (Scientifix Life, USA), 1% (v/v) penicillin-streptomycin (Life Technologies, UK) and 1% non-essential amino acid (NEAA) (Life Technologies, USA). Human foreskin fibroblast (HFF) cells were cultured in DMEM (1X) GlutaMAX™-1 (4.5 g L⁻¹ D-Glucose, L-Glutamine and 110 mg L⁻¹ Sodium pyruvate) with 10% FBS and 1% (v/v) penicillin-streptomycin. Human mesenchymal stem cells (hMSC) were cultured in DMEM (1 g L⁻¹ D-Glucose and 110 mg L⁻¹ Sodium pyruvate) with 10% FBS and 1% (v/v) penicillin-streptomycin. All cells were incubated at 37 °C with 5% CO₂ and passaged at 80% confluency. In the following studies, L929 at passage 27, HFF at passage 16 and hMSC at passage 6 were used.

For cell encapsulation experiments, 4-arm PEG-bromoquinone (molar mass of 10 000 g mol⁻¹) was dissolved in DMEM and 4-arm PEG-SH (molar mass of 10 000 g mol⁻¹) was dissolved in Dulbecco's phosphate-buffered saline (DPBS) (pH = 9.0) to give two stock solutions of 6 wt% (6 mM) –bromo and 6 wt% (6 mM) –SH. L929, HFF and hMSC were trypsinized and resuspended in culture media. The final gel/cell mixture was prepared by mixing the corresponding amounts of stock solutions and

cell suspension to achieve the final solution (pH = 8.4) with 5 wt% (5 mM) –bromo and 5 wt% (5 mM) –SH at a density of 2 million cells mL⁻¹. The mixture was subsequently pipetted on the bottom of the well dishes and kept in the incubator at 37 °C for 30 minutes for gelation. The fully cured cell-laden hydrogels were then rinsed 5 times with culture media and maintained in the corresponding culture media depending on the cell types as previously discussed.

Cell viabilities were assessed by Live/Dead staining post 1 day of cell encapsulation and samples were imaged via a Nikon inverted confocal microscope taking z-stacks through 500 µm depth as shown in **Figure S9**. The z-stack images were formed using ImageJ software and 3D images were reconstructed via Icy software. The cell viability measurements are shown as mean ± standard deviation with one-way ANOVA and Tukey pairwise comparisons followed for the statistical analysis via Prism 7 software.

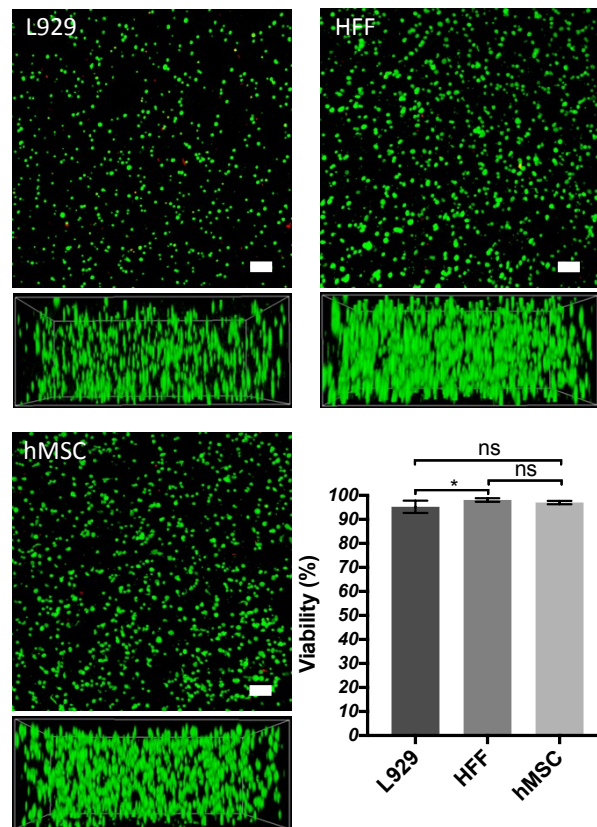


Figure S9. Live/dead staining fluorescent image (with 3D z-stack) and quantified viability of the accessed cell types 24 h post-encapsulation (scale bar = 100 µm; live = green; dead = red)

5. Cell retrieval via photoreduction/trimethyl lock

Post 24 hours of encapsulation, the cell-laden hydrogels were exposed to visible light irradiation (400 – 500 nm) at 20 mW cm⁻² for 10 minutes as shown in **Figure S10**.

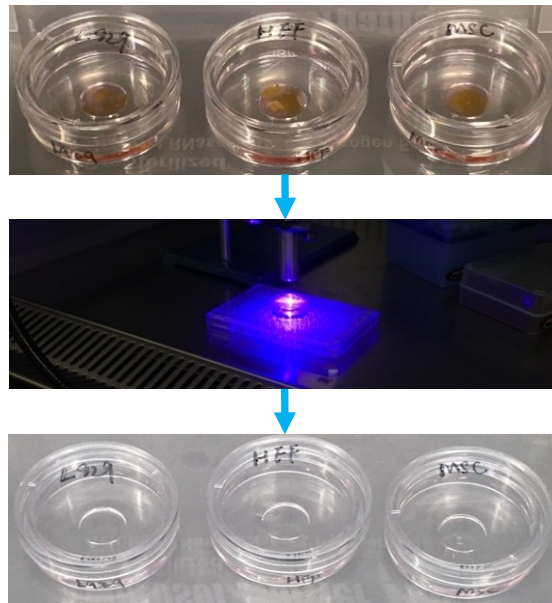


Figure S10. L929, HFF and hMSC were encapsulated and cultured in the well dishes; visible light source was used to degrade hydrogels to release cells; Hydrogels were degraded and retrieved cells were collected.

The gels were disintegrated and cells were released into the culture media. The released cell suspensions were subsequently generated and centrifuged into pellet with culture media and degradative material discarded. The cell pellet was then resuspended into the culture media with certain amount of density (L929 – 4 000 cells well⁻¹; HFF – 8 000 cells well⁻¹; hMSC – 8 000 cells well⁻¹) and seeded onto the 96 well-plate. In the meantime, a group of L929, HFF and hMSC directly harvested from the culture flasks were seeded on 96 well-plate at the same densities as retrieved groups as control groups. Cell viabilities of retrieved and controlled groups were assessed by Live/Dead staining at time points of 1, 3 and 7 days post-seeding (**Figure S11**).

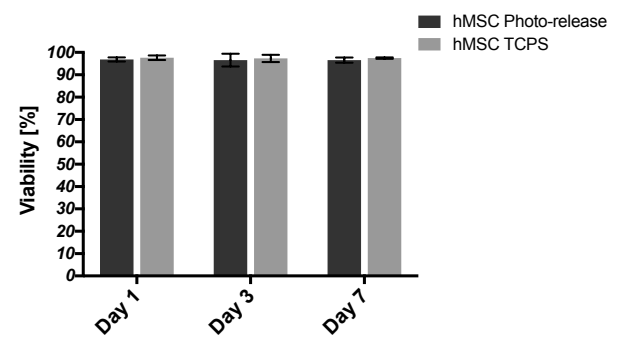
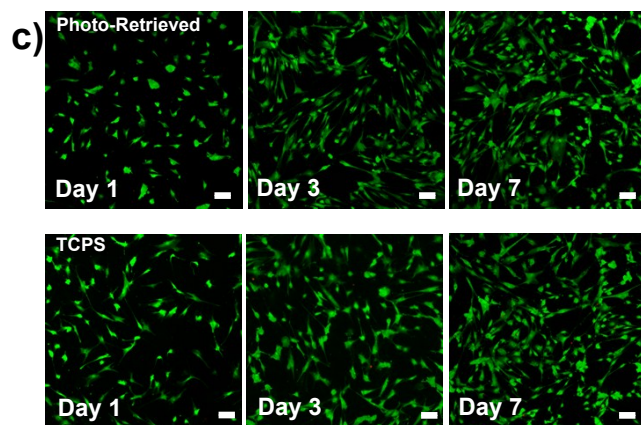
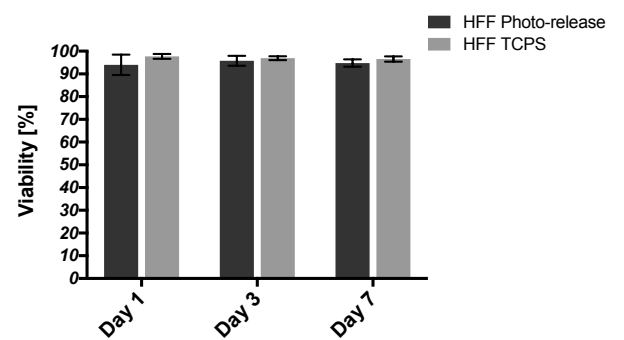
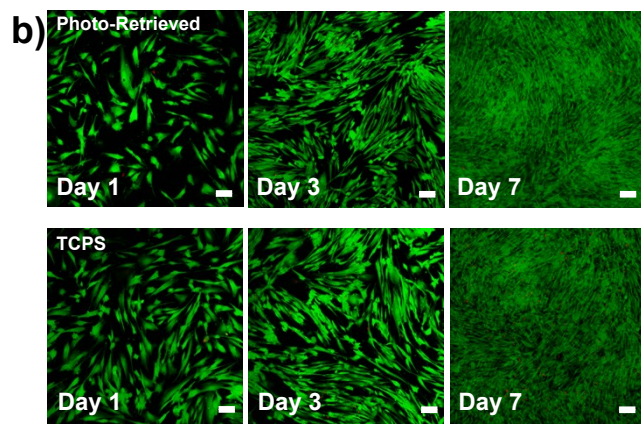
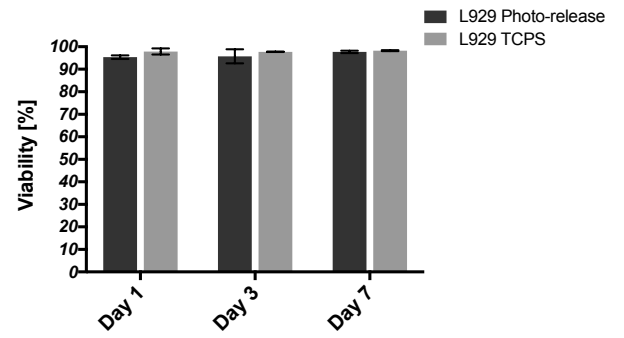
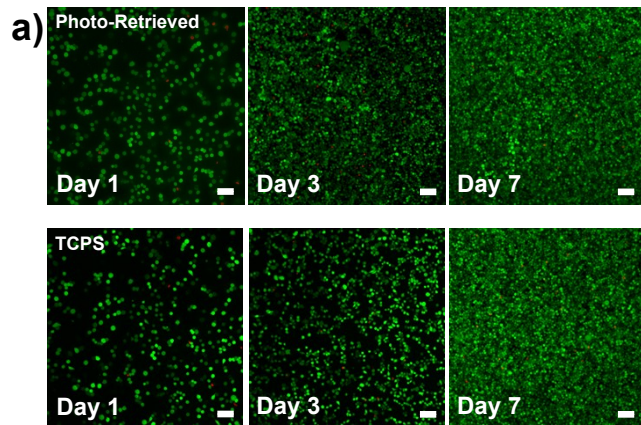


Figure S11. Live/Dead staining fluorescent images of L929, HFF, and hMSC cells retrieved from hydrogels after blue light irradiation, cultured on tissue culture poly(styrene) plates and without any treatment cultured on 96 well-plates for 1, 3, and 7 days (scale bar = 100 μ m; live = green; dead = red).

References:

1. V. X. Truong, K. M. Tsang, F. Ercole and J. S. Forsythe, *Chemistry of Materials*, 2017, **29**, 3678-3685.
2. V. X. Truong, K. M. Tsang and J. S. Forsythe, *Biomacromolecules*, 2017, **18**, 757-766.
3. D. P. Walton and D. A. Dougherty, *Journal of the American Chemical Society*, 2017, **139**, 4655-4658.