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PHOTOMEDIATED OXIME LIGATION AS A BIOORTHOGONAL TOOL FOR SPATIOTEMPORALLY-CONTROLLED HYDROGEL FORMATION AND MODIFICATION

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SUPPORTING INFORMATION

Multi-arm PEG Crosslinker Syntheses

2-5-dioxopyrrolidin-1-yl 4-formylbenzoate (CHO-NHS; II). CHO-NHS was synthesized from 4-formylbenzoic acid as previously reported.[24]

2,5-dioxopyrrolidin-1-yl (2-(2-nitrophenyl)propyl) carbonate (V). 2,5-dioxopyrrolidin-1-yl (2-(2-nitrophenyl)propyl) carbonate was synthesized as previously reported.[24]

2-((((2-(2-nitrophenyl)propoxy)carbonyl)amino)oxy)acetic acid (VI). 2,5-dioxopyrrolidin-1-yl (2-(2-nitrophenyl)propyl) carbonate (750 mg, 2.33 mmol) and aminooxyacetic acid (593 mg, 4.65 mmol, Enamine) were added to a round bottom flask and dissolved in dichloromethane (23.4 mL, Sigma-Aldrich). Triethylamine (2.7 mL, Sigma-Aldrich) was added to the round bottom flask, which was then purged with argon gas. The reaction mixture was stirred overnight at room temperature protected from light. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in 50 mL dH_2O. The aqueous mixture was washed with ethyl acetate (50 mL, 2x, Macron), and the organic layers were discarded. The aqueous layer was acidified to a pH of 1 by titrating with 1 N HCl, and washed with ethyl acetate (50 mL, 3x). The organic layers were combined, dried with $MgSO_4$ (EMD Chemicals), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (20:1 ethyl acetate: acetic acid) on silica gel to yield a yellow solid (498 mg, 1.67 mmol, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.79 (d, J = 7.0 Hz, 1H), 7.63 (t, J = 7.1 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.44 (t, J = 7.1 Hz, 1H), 4.50 (s, 2H), 4.43 (dd, J = 10.6, 5.9 Hz, 1H), 4.31 (dd, J = 10.5, 8.4 Hz, 1H), 3.84 – 3.76 (m, 1H), 1.41 (d, J = 7.0 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 172.52, 158.24, 150.49, 136.50, 132.86, 128.01, 127.78, 124.25, 73.38, 70.47, 33.04, 17.35. HRMS (ESI+) Calculated for $C_{12}H_{14}N_2O_7$ $[M + {}^1H]^+$, 299.0874; observed 299.0878 (Δ = 1.4 ppm); Calculated for $C_{12}H_{18}N_3O_7$ $[M + {}^{14}N^1H_4]^+$, 316.1139; observed 316.1140 (Δ = 0.2 ppm).

2,5-dioxopyrrolidin-1-yl-2-((((2-(2-nitrophenyl)propoxy)carbonyl)amino)oxy)acetate (NPPOC-ONH-NHS; VII). 2-((((2-(2-nitrophenyl)propoxy)carbonyl)amino)oxy)acetic acid (320 mg, 1.07 mmol), N-hydroxysuccinimide (160 mg, 1.40 mmol, Sigma-Aldrich) and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (267 mg, 1.40 mmol, AK Scientific) were added to a round bottom flask, dissolved in anhydrous acetonitrile (3.40 mL, Acros Organics), and purged with argon gas. The reaction was stirred overnight at room temperature protected from light. The reaction was concentrated under reduced pressure and the residue was dissolved in dichloromethane (50 mL). The solution was washed with dH_2O (50 mL, 3x). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to yield a yellow oil (420 mg, 1.06 mmol, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 4.74 (s, 2H), 4.39 (dd, *J* = 10.7, 6.1 Hz, 1H), 4.33 – 4.24 (m, 1H), 3.75 (dt, *J* = 14.7, 6.2 Hz, 2H), 2.91 (s, 4H), 1.40 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.17, 168.58, 164.78, 156.63, 136.67, 132.79, 128.07, 127.69, 124.27, 70.85, 69.93, 33.12, 25.59, 17.50. HRMS (ESI+): Calculated for $C_{16}H_{18}N_3O_9$ $[M + {}^1H]^+$, 396.1038; observed 396.1041 (Δ = 0.9 ppm); Calculated for $C_{16}H_{21}N_4O_9$ $[M + {}^{14}N^1H_4]^+$, 413.1303; observed 413.1302 (Δ = 0.3 ppm).

PEG-CHO. 8-arm PEG-amine (200 mg, 0.0200 mmol, M_n ~ 10 kDa, tripenterythritol core, JenKem) and CHO-NHS (79.1 mg, 0.320 mmol) were added to a round bottom flask purged with argon gas. Flask contents were dissolved in anhydrous DMF (1 mL, Acros Organics), and N,N-diisopropylethylamine (111 μL, 0.640 mmol, Sigma-Aldrich) was added. The reaction was stirred overnight at room temperature. dH_2O (9 mL) was added to the reaction and the solution was transferred to dialysis tubing (Spectra/Por 1 kDa MWCO) and dialyzed for 24 h in dH_2O. The dialysis tubing contents were filtered through 0.2 μm polyethersulfone syringe filters and lyophilized to yield a white powder (190 mg, 0.0169 mmol, 84% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 7.86 (m, 32H), 3.42 (s, 16H), 3.32 (s, 8H). Based on relative integrations of protons corresponding to the 8-arm PEG backbone (δ 3.42 and 3.32) and aromatic hydrogens introduced through 4-formylbenzoic acid (δ 8.06-7.86), end-group functionalization of PEG-CHO was estimated to be 86%.

PEG-ONH-NPPOC. 8-arm PEG-amine (158 mg, 0.0158 mmol, M_n ~ 10 kDa, tripenterythritol core) and NPPOC-ONH-NHS (100 mg, 0.253 mmol) were added to a round bottom flask purged with argon gas. Flask contents were dissolved in anhydrous DMF (1 mL), and N,N-Diisopropylethylamine (88 μL, 0.506 mmol) was added. The reaction was stirred overnight at room temperature. dH_2O (9 mL) was added to the reaction and the solution was transferred to dialysis tubing (Spectra/Por 1 kDa MWCO) and dialyzed for 24 h in dH_2O. The dialysis tubing contents were filtered through 0.2 μm polyethersulfone syringe filters and lyophilized to yield a white powder (139 mg, 0.0111 mmol, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 8H), 7.79 (d, *J* = 7.9 Hz, 8H), 7.62 (t, *J* = 7.2 Hz, 8H), 7.50 (d, *J* = 7.3 Hz, 8H), 7.42 (t, *J* = 7.7 Hz, 8H), 4.32 (s, 16H), 3.42 (s, 16H), 3.32 (s, 8H), 1.46 (d, *J* = 7.0 Hz, 2H), 1.39 (d, *J* = 6.9 Hz, 22H). Based on relative integrations of protons corresponding to the 8-arm PEG backbone (δ 3.42 and 3.32) and ONH-NPPOC (δ 1.46 and 1.39), end-group functionalization of PEG-ONH-NPPOC was estimated to be 100%.

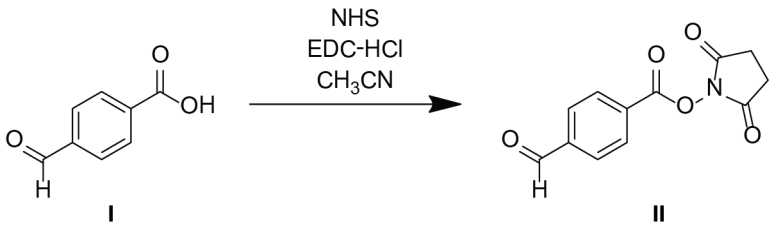

Fig. S1 Synthesis of CHO-NHS (II).

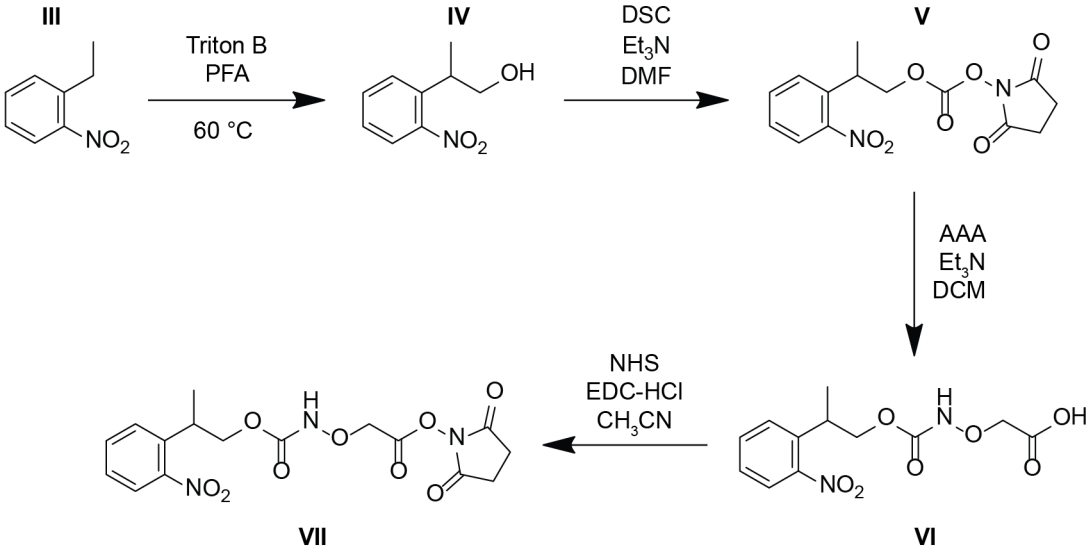

Fig. S2 Synthesis of NPPOC-ONH-NHS (VII).

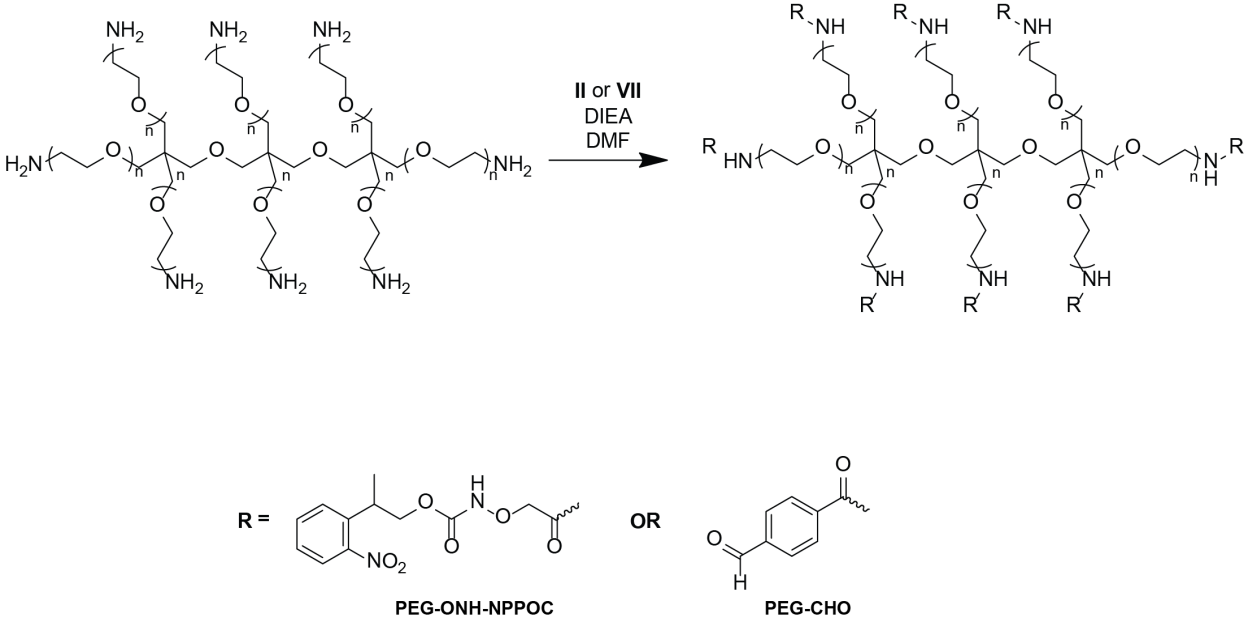

Fig. S3 Functionalization of 8arm-PEGs *via* NHS chemistry.

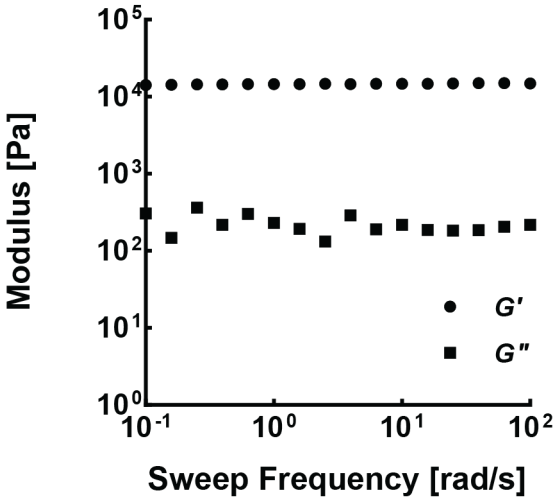

Fig. S4 A frequency sweep shows that data collected at 1% strain, and a frequency of 1 rad/s lie within the linear viscoelastic region.

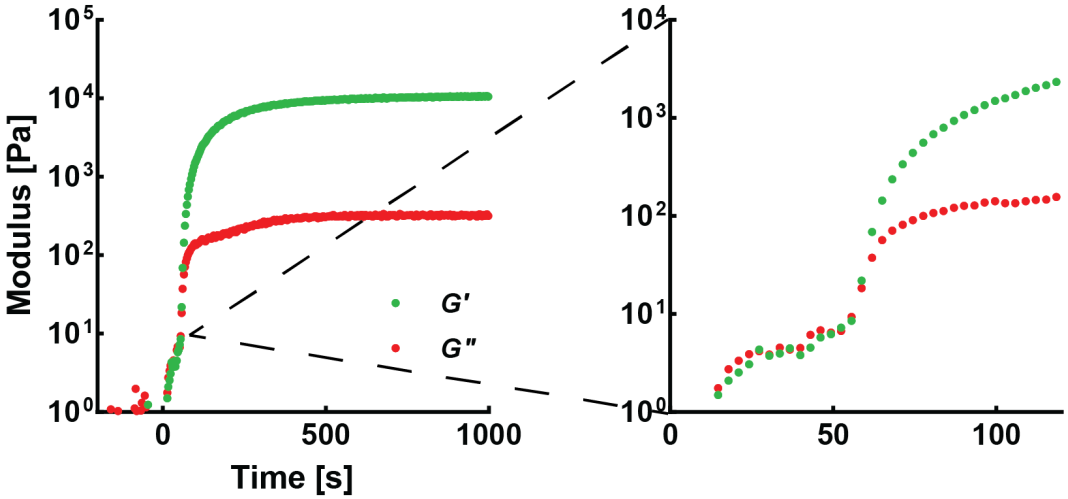

Fig. S5 Representative G' and G'' gel evolution profiles. Data collection begins at t = -180 s and continuous UV irradiation begins at t = 0 s. Before irradiation, G' and G'' are low, noisy, and indistinguishable. The curves rapidly diverge about 1 min after initiating light irradiation.

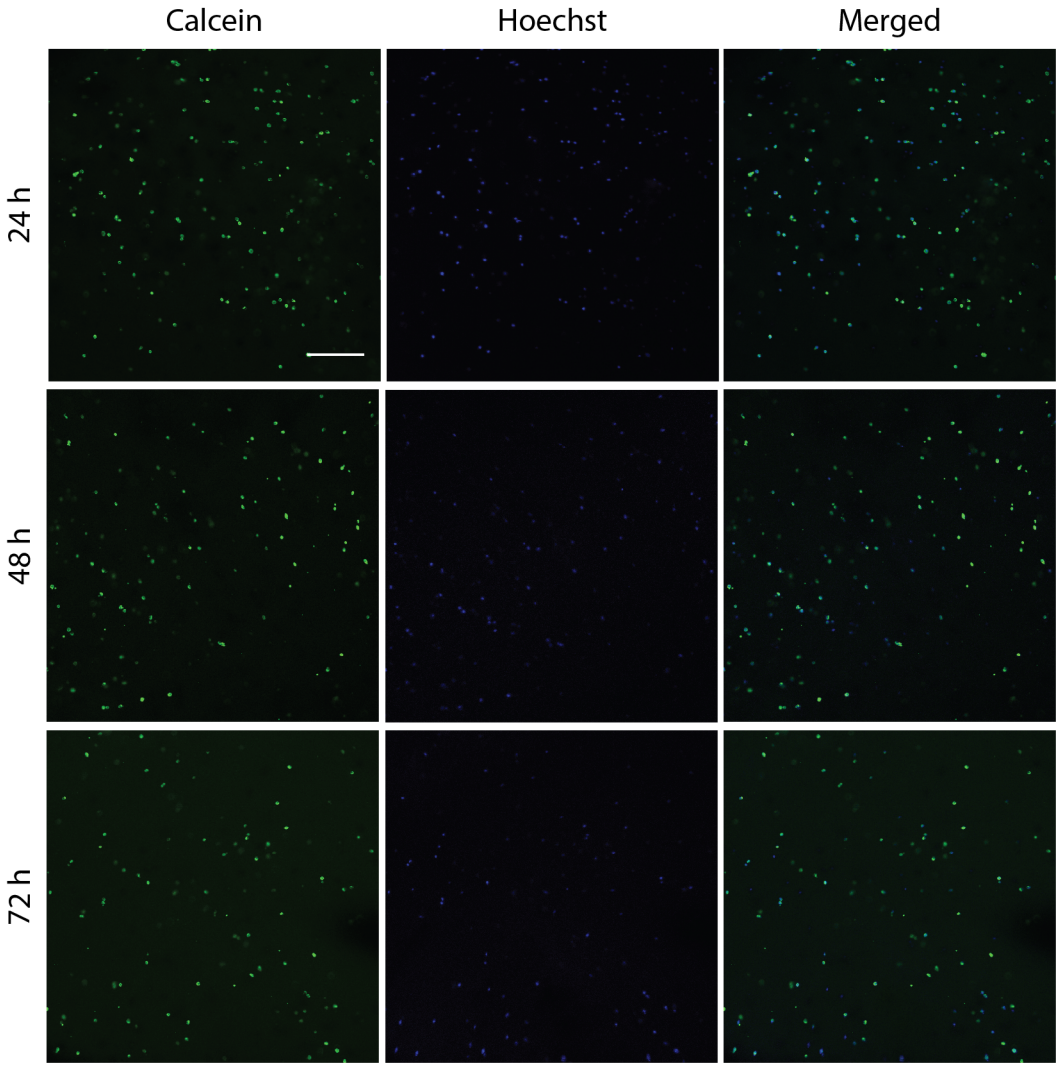

Fig. S6 Cells were suspended in gel precursor solution, irradiated, and encapsulated in photopolymerized oxime-based hydrogels. Cells were subsequently monitored for up to 72 h after encapsulation. Live cells were visualized with a calcein stain (green) and all cells were stained with Hoechst 33342 (blue) (Scale bar = 200 μm). After imaging, the number of live cells was compared to the total number of cells to obtain the fraction of cells alive in the material.