Electronic Supplementary Material (ESI) for Chemical Communications

Biocompatible macro-initiators controlling radical retention in microfluidic on-chip photo-polymerization of water-in-oil emulsions

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Experimental

All reagents and chemicals were used as received unless otherwise specified. Ultrapure MilliQ water was obtained from a Labconco Water Pro PS purification system with a resistivity of 18.2 MΩ. NMR spectra were recorded on a Varian Inova 400 spectrometer with $CDCl_3$ or D_2O as solvents and TMS as internal standard. Dialysis was performed using a Spectra/Por dialysis membrane (MWCO 1,000). Bright-field microscopy was performed on an IX71 microscope (Olympus) equipped with objectives ranging from 2x to 40x (air) and a Phanton MIRO high-speed camera (Vision Research Inc. U.K.) to detect single-droplet formation in the microfluidic devices. To determine the exact channel height of the channel network fabricated in SU-8 (Microchem) *via* photolithography, differential interference contrast (DIC) microscopy was performed using a Wyco NT1100 optical profiler (Bruker).

Microfluidic device fabrication

Microfluidic devices were fabricated using a combination of photo- and soft lithography, as shown in Figure S1.[1] The poly(dimethylsiloxane) (PDMS) replica of the microchannel network was sealed with a glass slide *via* oxygen plasma treatment. To form water-in-oil emulsions, we need the fluorinated oil to wet the microchannel walls of the PDMS-based microfluidic device. We thus coated the microfluidic devices with Aquapel® containing a fluorinated silane, which improves the hydrophobic surface properties of PDMS.[2]

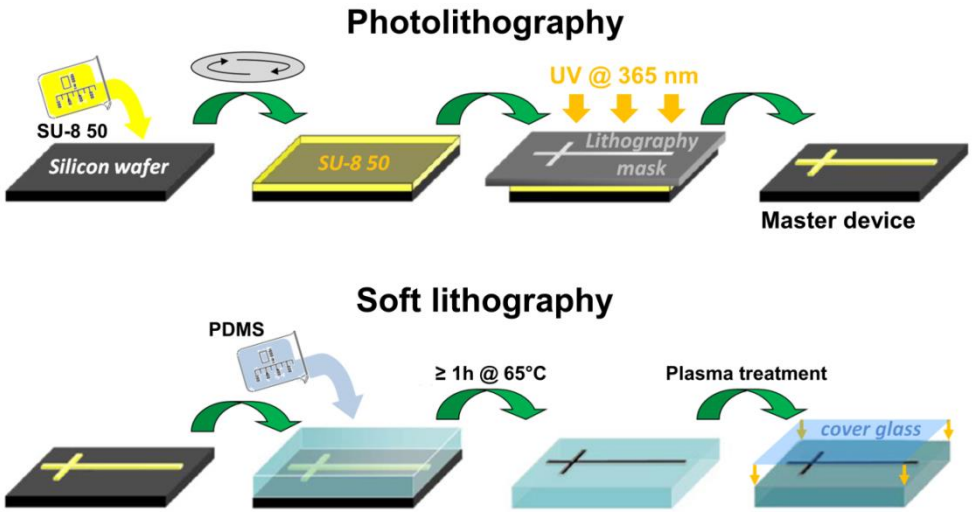

Fig. S1 Fabrication of microfluidic devices combining photolithography and soft lithography.

The microfluidic channel network designed in AutoCAD 2014 (Autodesk) is shown in Figure S2. The channel width is 100 µm at the cross-junction and 50 µm at the constriction which is included in the device design to improve droplet pinch-off. The height of the droplet formation area is 90 µm and 210 µm in the delay line, as determined by DIC microscopy. The device design is versatile and includes a second

inlet port for the inner aqueous phase to separately inject the HAMA solution and another aqueous phase, such as cell solutions or a second hydrogel building block.

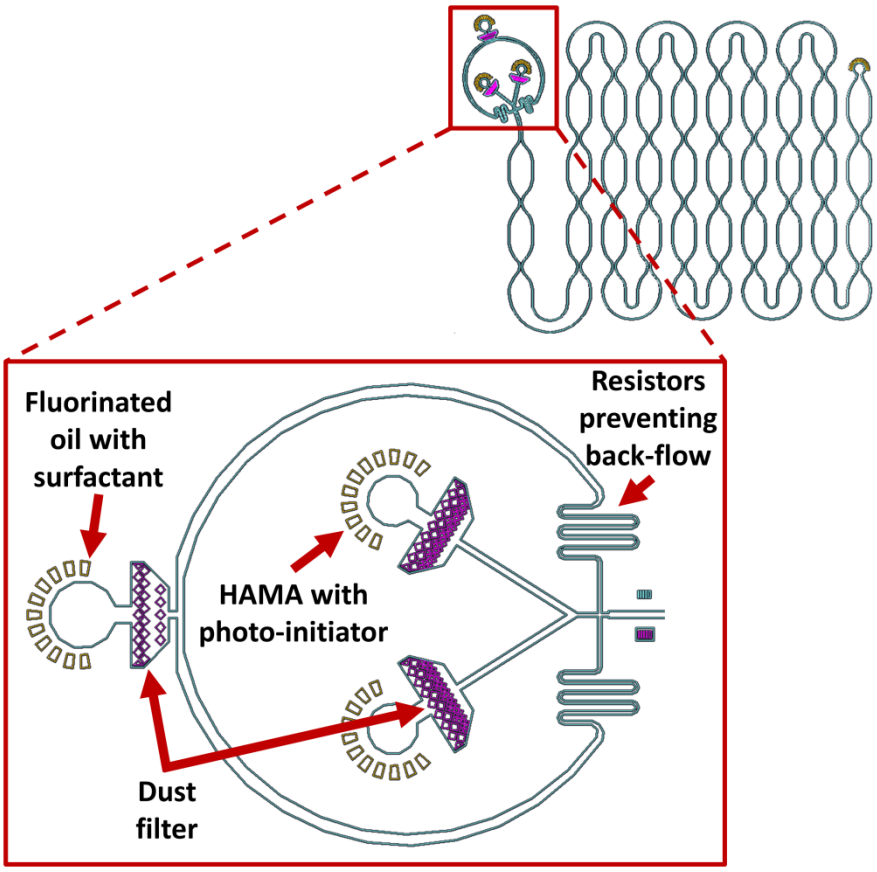

Fig. S2 Microfluidic device design developed in AutoCAD 2014. The delay line is 250.98 mm long corresponding to a delay / UV exposure time of approx. 5 min. The gap between the first and second meander prevents UV exposure of the droplet formation area that could cause polymerization of the HAMA solution in bulk.

On-chip photo-polymerization

Solutions were injected into the microfluidic device using gastight syringes (Hamilton 1700 series) mounted onto high-precision syringe pumps (Cetoni® neMESYS, 14.5 gear) connected to the device *via* PTFE tubing (Novodirect GmbH, ID = 0.53 mm, OD = 1.03 mm). We formed water-in-oil emulsions by injecting an aqueous solution of methacrylated hyaluronic acid (HAMA) with the respective photo-initiator together

with HFE 7500 (3M®) that contained a triblock copolymer surfactant (1 % w/w).[3] Droplet formation and on-chip photo-polymerization was performed on a modified IX71 microscope (Olympus) equipped with UV-transparent quartz glass optics. A UV-light source (Omnicure® S1000 series, 250-450 nm) with automated shutter was connected to the microscope and used to shine a focused UV beam onto the delay line of the microfluidic device. The droplets / HAMA hydrogel particles were collected in an Eppendorf tube (1.5 mL), and the beads were obtained from the emulsion by adding a demulsifier (1*H*, 1*H*, 2*H*, 2*H*-perfluoro-1-octanol, Sigma).

Photo-initiator syntheses

Lithium phenyl- 3-(chloromethyl)-2,4,6-trimethylbenzoylphosphinate (1)

Dimethyl phenylphosphonite (65 mg, 0.38 mmol, 1.0 equiv) was reacted with 3-(chloromethyl)-2,4,6-trimethylbenzoyl chloride (87 mg, 0.38 mmol, 1.0 equiv) *via* a Michaelis-Arbuzov reaction. At room temperature under argon atmosphere, a solution of 3-(chloromethyl)-2,4,6-trimethylbenzoyl chloride in dry THF was added dropwise over 10 hrs to an equimolar amount of continuously stirred dimethyl phenylphosphonite solution in THF. Subsequently, a four-fold excess of lithium bromide in 100 mL of 2-butanone was added. The reaction mixture was then heated to 50 ^{o}C. After 10 min, a solid precipitate formed. The filtrate was washed and filtered five times with 2-butanone to remove unreacted lithium bromide, and excess solvent was removed in vacuo. After column chromatography (H_2O-MeOH 1:10) / CH_2Cl_2, 1:20 → 1:1), methanol was removed and the desired product **1** (34 mg, 0.1 mmol,

27 % yield) was obtained after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ 7.65 – 7.20 (m, 5H), 6.69 (s, 1H), 3.24 – 3.07 (s, 2H), 1.94 (d, J = 79.9 Hz, 9H).

Polyethylene glycol-lithium phenyl-2,4,6-trimethylbenzoylphosphinate (PEG-LAP) (2 and 3)

A solution of potassium hydroxide in THF (1 mL) was added dropwise into a flask containing PEG (Mw 550 and 2k) (0.2 mmol, 2.0 equiv). The reaction mixture was stirred at room temperature for 12 hrs, after which compound **1** (34 mg, 0.1 mmol, 1.0 equiv) dissolved in a mixture of H_2O and THF (1:10 (v/v)) was added dropwise into the reaction mixture. THF was evaporated and H_2O was removed by freeze-drying to obtain a white solid. Column chromatography (H_2O-MeOH-Acetone 1:4:5) / CH_2Cl_2, 1:20→1:1). Subsequently, the organic solvents were evaporated under vacuum and residual H_2O was removed by freeze-drying to obtain white solid **2** (PEG M_W 550, 6 mg, 0.0076 mmol, 2 % yield) and **3** (PEG M_W 2,000, 18 mg, 0.011 mmol, 3 % yield). ^{1}H NMR (400 MHz, D_2O) δ 7.74 – 7.11 (m, 5H), 6.71 (s, 1H), 4.23 (s, 2H), 3.64 – 3.31 (br. m, 136H), 3.21 (s, 3H), 1.98 (d, J = 76.4 Hz, 9H). ^{13}C NMR (101 MHz, D_2O) δ 227.61, 139.58, 133.60, 132.19, 131.90, 128.33, 128.21, 128.04, 71.62 (s), 69.69 – 69.28 (m), 57.96 (s), 20.17 (s), 18.56 (s).

Poly (ethylene glycol) methyl ether tosylate (4 and 5)

A solution of PEG methyl ether (M_W 550 and 2,000) (0.2 mmol, 1.0 equiv) in dry THF (10 mL) was loaded in a 3-neck round bottom flask. Pyridine (19 mg, 0.24 mmol, 1.2 equiv) was added dropwise and was allowed to stir for 12 hrs. 4-toulenesulfonyl chloride (46 mg, 0.24 mmol, 1.2 equiv) was added to the reaction mixture and stirred for 10 hrs at room temperature under argon atmosphere. Afterwards, the reaction mixture was transferred to a dialysis bag (M_W cut-off 1,000) and dialyzed against a mixture of MilliQ and THF (1:1 v/v) for 24 hrs, followed by dialysis against MilliQ for 72 hrs to remove THF and any excess reagents. Finally, water was removed by freeze-drying to obtain a white solid **4** (PEG M_W 550, 108 mg, 0.146 mmol, 73 % yield) and **5** (PEG M_W 2,000, 350 mg, 0.16 mmol, 80 % yield). ^{1}H NMR (400 MHz, D_2O) δ 7.69 (d, J = 8.4 Hz, 2H), 7.35 (dd, J = 8.6, 0.6 Hz, 2H), 4.15 – 4.06 (m, 2H), 3.58 – 3.43 (m, 219H), 3.20 (s, 3H), 2.29 (s, 3H).

Poly (ethylene glycol) methyl ether 2-hydroxy-1[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (PEG-I2959) (6 and 7)

2-hydroxy-1[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (I2959) (27 mg, 0.12 mmol, 1.2 equiv) was dissolved in THF (2 mL) in a three neck round bottom flask, followed by dropwise addition of pyridine (10 mg, 0.12 mg, 1.2 equiv) and was allowed to stir for 12 hrs. Compound **4** or **5** (0.1 mmol, 1.0 equiv) was dissolved in

THF (10 mL) and added dropwise into the reaction mixture. The reaction mixture was allowed to stir for 10 hrs at room temperature under argon atmosphere. The reaction mixture was then transferred to a dialysis bag (M_W cut-off 1,000) and dialyzed against MilliQ and THF (1:1 v/v) for 24 hrs, followed by dialysis against MilliQ for 72 hrs to remove THF and any excess reagents. Finally, water was removed by freeze-drying method to obtain **6** (PEG M_W 550 14 mg, 0.02 mmol, 13 % yield) and **7** (PEG M_W 2,000, 160 mg, 0.07 mmol, 58 % yield) as a white solid ^{1}H NMR (400 MHz, D_2O) δ 7.93 (d, J = 9.1 Hz, 2H), 6.90 (d, J = 9.1 Hz, 2H), 4.11 – 3.97 (m, 2H), 3.80 – 3.73 (m, 2H), 3.53 (d, J = 5.8 Hz, 174H), 3.20 (s, 3H), 1.40 (s, 6H). ^{13}C NMR (101 MHz, D_2O) δ 206.3, 162.2, 132.2, 127.1, 114.1, 109.9, 77.4, 71.2, 69.3, 60.0, 57.9, 27.1.

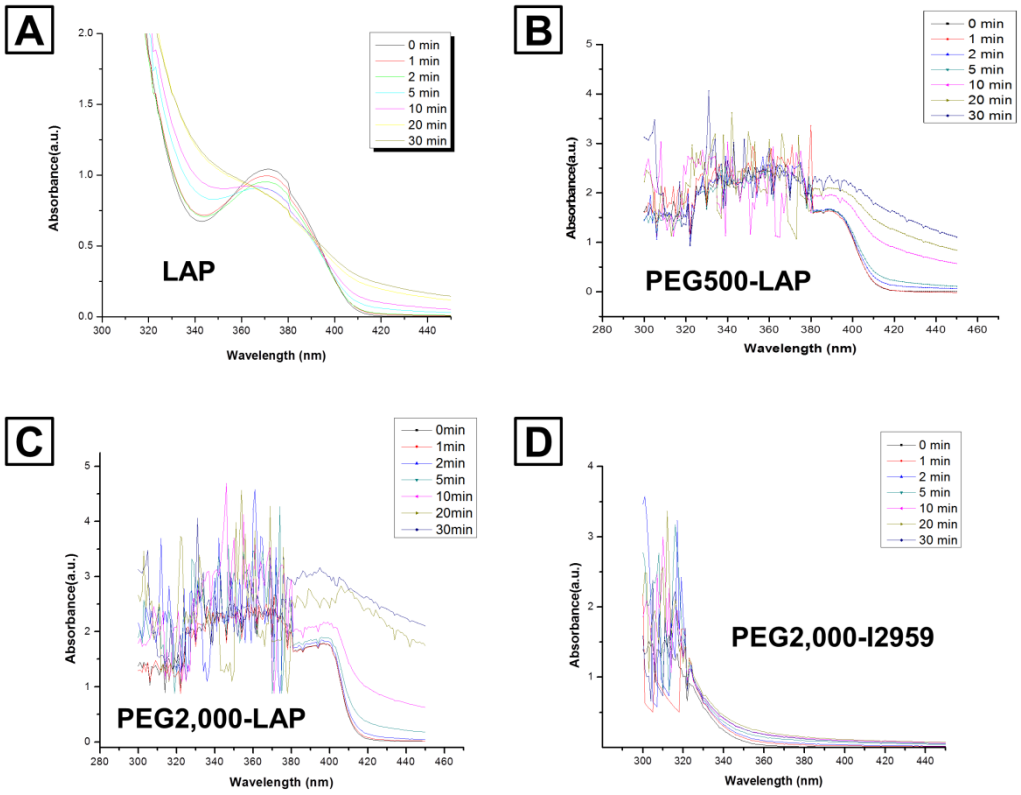

Fig. S3 Degradation of photo-initiators after 5 min. of UV irradiation reassembling the exposure time in the respective microfluidic online-polymerization experiments.

Morphology and size distribution of HAMA beads when PEG-LAP and PEG-I2959 were used

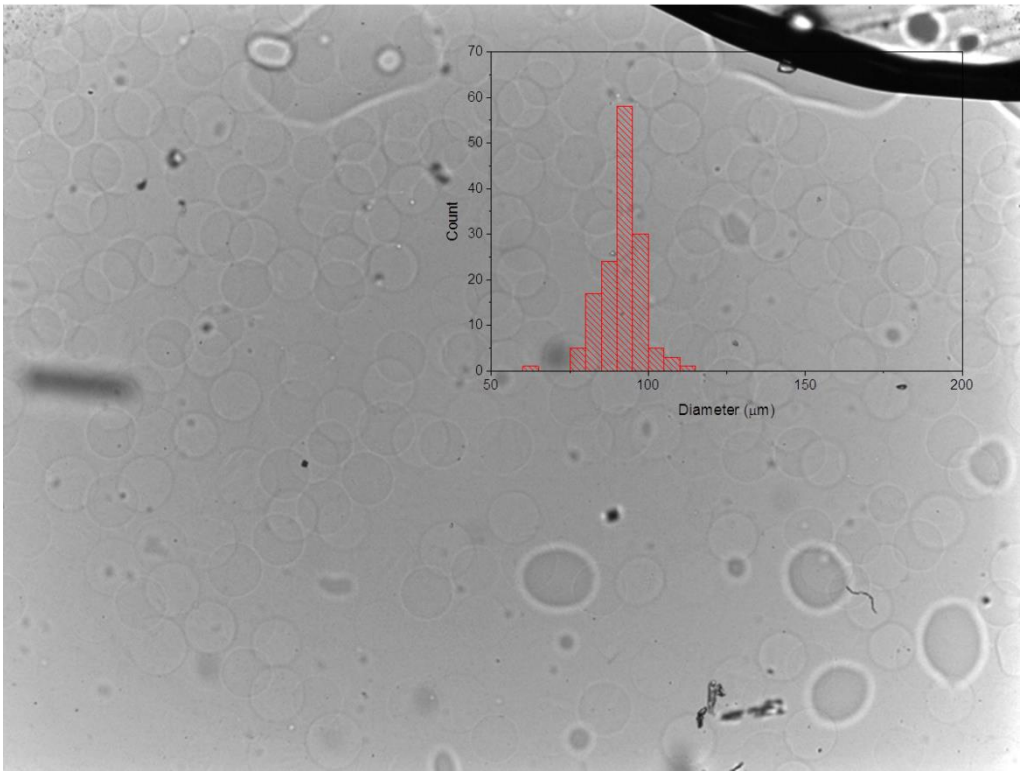

Figure S4 Optical micrograph of HAMA beads obtained when using PEG2000-LAP as the photoinitiator and corresponding particle size distribution.

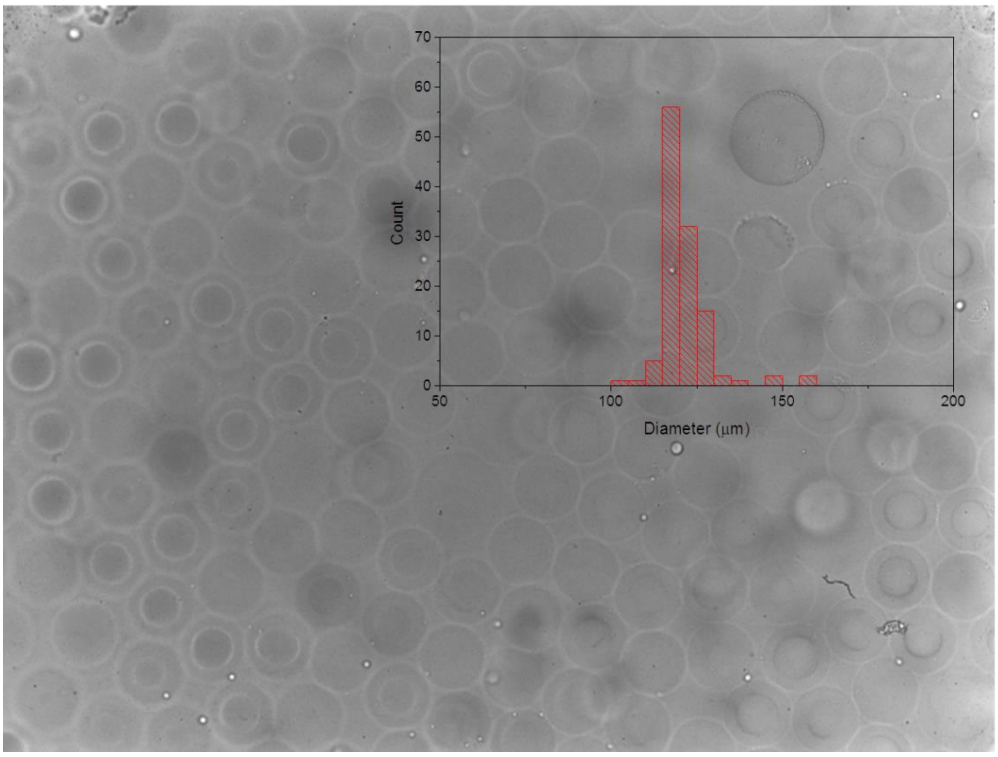

Figure S5 Optical micrograph of HAMA beads obtained when using PEG2000-I2959 as the photoinitiator and corresponding particle size distribution.

MTT assay

NIH/3T3 fibroblast cells were seeded at a density of ~ 2,500 cells cm^{-2} in 96 well plates and incubated in DMEM at 37 ^{o}C, 5 % CO_2 for 48 hrs. The medium was then removed, all 5 different photo-initiators dissolved in DMEM were added to the cells to reach desired concentrations. The cells were incubated in DMEM at 37 ^{o}C, 5 % CO_2 for another 48 hrs before the MTT assay was performed. An overview over the results is given in Figure S4.[4]

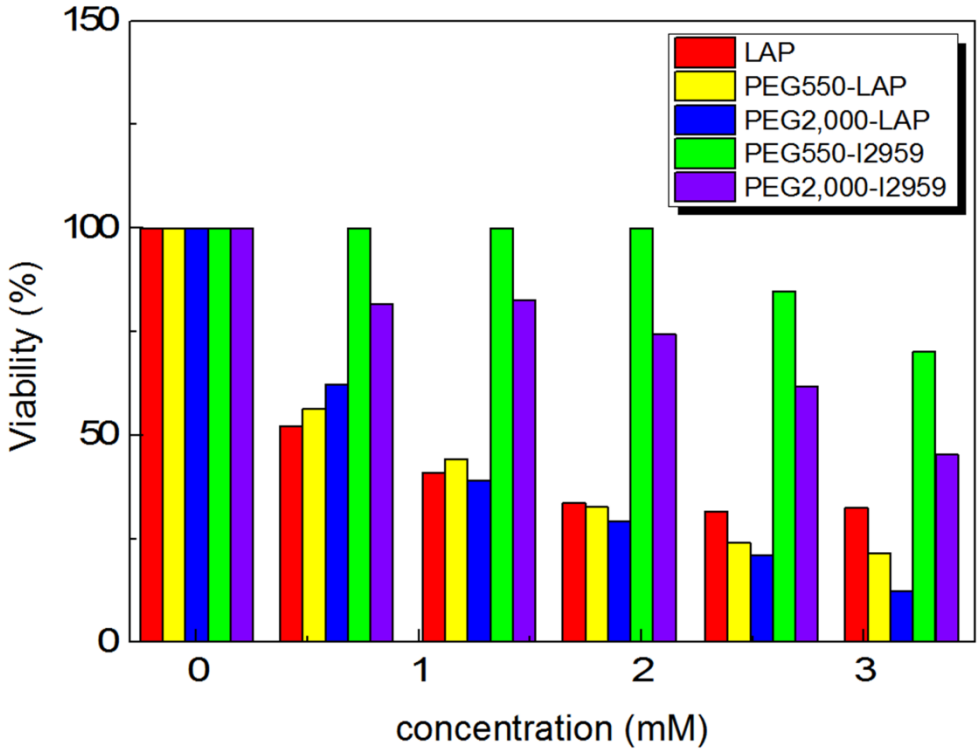

Fig. S6 Biocompatibility of macro-initiators based on a MTT cytotoxicity assay that determines the relative survival / viability of NIH/3T3 cells in the presence of the photo-initiators at different concentrations.

3D cell viability

For 3D cell viability studies, NIH/3T3 fibroblasts were mixed in PBS solution containing 0.5 % (w/w) HAMA and different initiators (~ 2 mM) at a seeding density of 10^6 cells mL^{-1}. The mixture was irradiated by UV (300~400 nm, 4 mW cm^{-2}) for 5 min. The cell-laden HAMA gel block was incubated in DMEM at 37 ^oC, 5 % CO_2 for 24 hrs. Afterwards cell viability was determined using a standard Live/Dead cytotoxicity assay kit (Invitrogen). Gels were incubated for 30 min in PBS containing 2 μM calcein AM and 4 μM ethidium homodimer 1 (EthD-1). Calcein AM stains live cells through its enzymatic transformation to green fluorescence emitting calcein and EthD-1 stains the nuclei of dead cells red, as shown in Figure S5. The cells in gels

were imaged under a Leica SP2 confocal microscope and the number of green and red fluorescence emitting cells were counted to obtain statistics on the cell viability in the 3D gel.

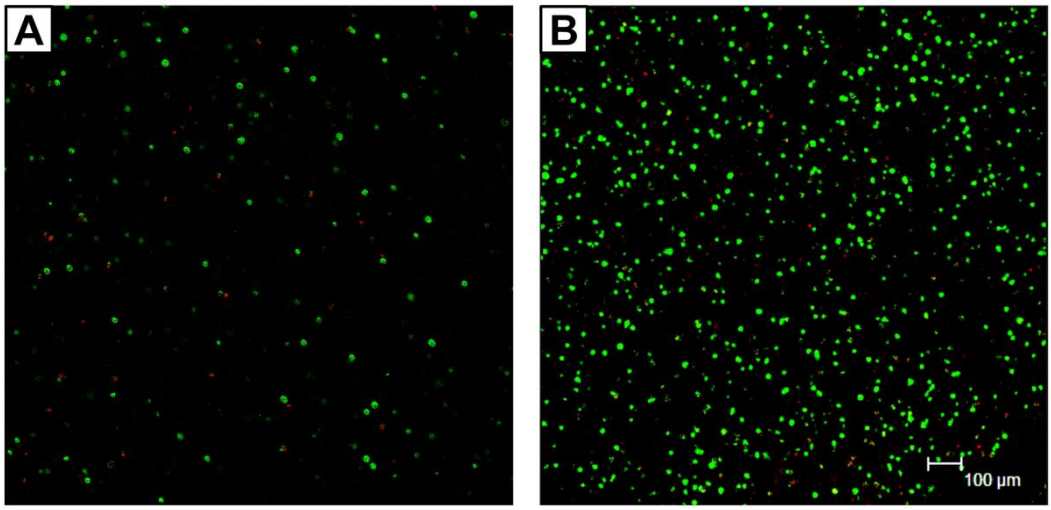

Fig. S7 A: Live/dead staining on the viability of NIH 3T3 cells embedded in HAMA (0.5 % w/w) hydrogels 24 hrs after UV-initiated gelation using PEG550-LAP as the initiator. B: Live/dead staining on the viability of NIH 3T3 cells embedded in HAMA (0.5 % w/w) hydrogels 24 hrs after UV-initiated gelation using PEG2000-I2959 as the initiator.

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2. L. Mazutis, J. Gilbert, W. L. Ung, D. A. Weitz, A. D. Griffiths, J. A. Heyman, *Nature Protocols* 2013, **8**, 870-891.
3. C. Holtze, A. C. Rowat, J. J. Agresti, J. B. Hutchison, F. E. Angilé, C. H. J. Schmitz, S. Köster, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. Pisignano, D. A. Weitz, *Lab on a Chip* 2008, **8**, 1632-1637.
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NMR spectra

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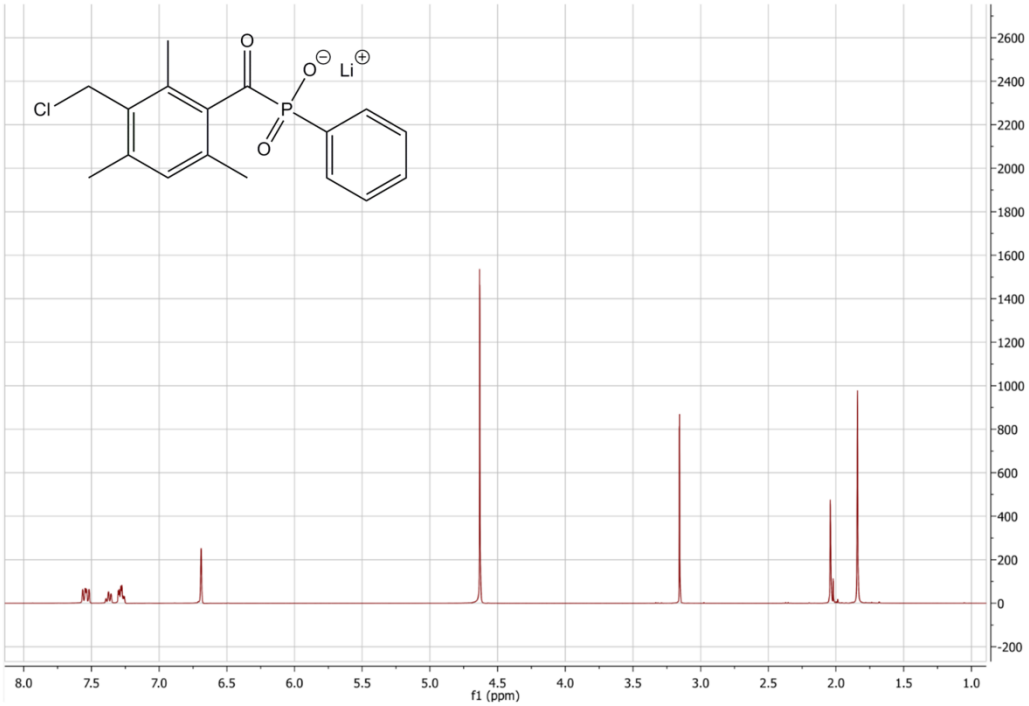
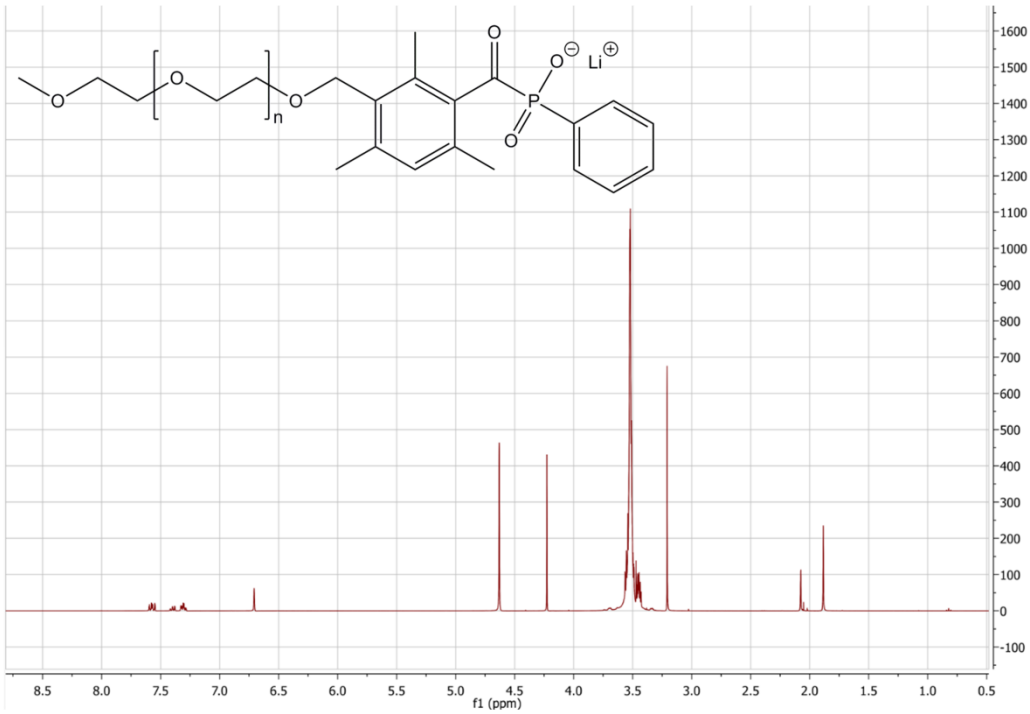
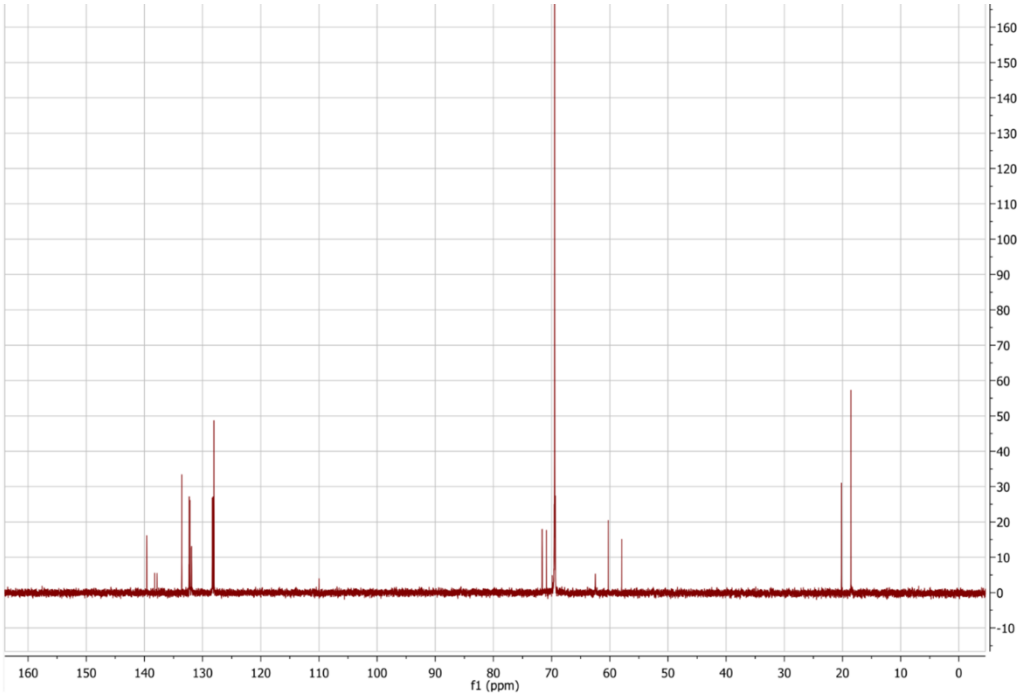


Polyethylene glycol-lithium phenyl-2,4,6-trimethylbenzoylphosphinate (PEG-LAP) (2 and 3)





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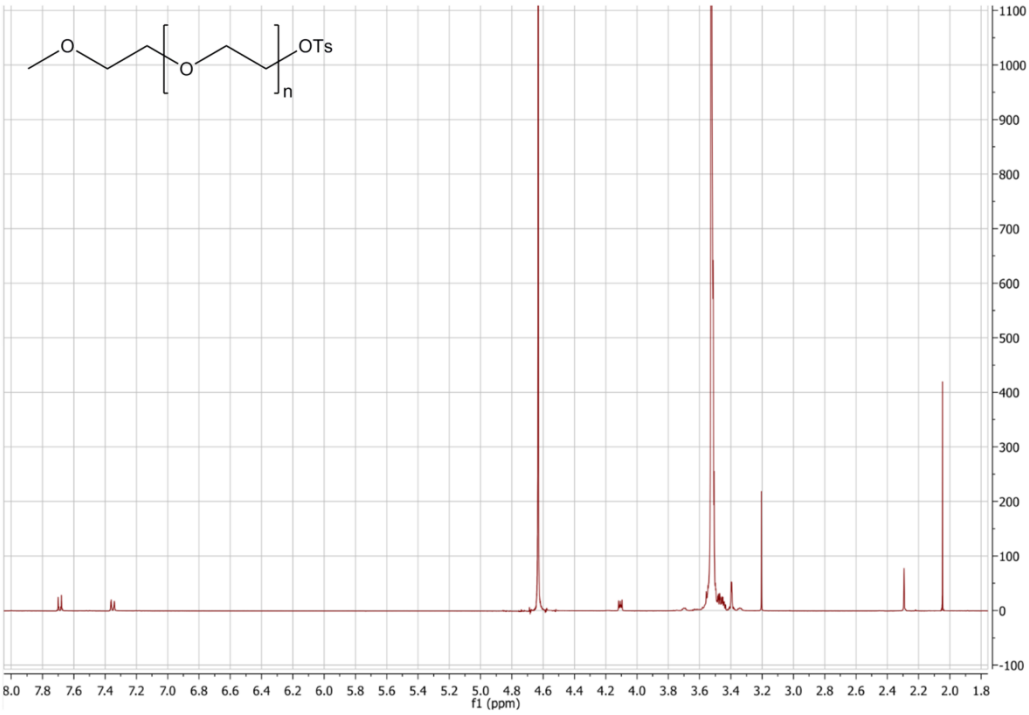

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