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Supporting Information

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Micropatterned Photodegradable Hydrogels for the Sorting of Microbeads and Cells**

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Supplemental Material

Materials

NIH 3T3 mouse fibroblasts were purchased from American Type Culture Collection (ATCC) and culutured in 10% (v/v) fetal bovine serum (FBS, Invitrogen, Carlsbad, CA), 100 U/mL penicillin and 100 μg/mL streptomycin in Dulbecco's Modified Eagle Medium (DMEM, VWR, West Chester, PA) at 37 ^{o}C in a humidified 5% CO_2 atmosphere. Glass slides (75 × 25 mm^2) were purchased from Fisher Scientific (Pittsburg, PA). 3-Acryloxypropyltrichlorosilane was purchased from Gelest (Morrisville, PA). LIVE/DEAD® Viability/Cytotoxicity Kit was purchased from Invitrogen (Carlsbad, CA). PEG grafted aminofunctionalized polystyrene microbeads (TentaGel beads, d=75 μm, 0.4 - 0.6 mmol $NH_2 \cdot g^{-1}$) was purchased from Rapp Polymere (Tuebingen, Germany). *O*,*O*'-Bis-(2-aminopropyl) polypropylene glycol-*block*-polyethylene glycol-*block*-polypropylene glycol 1900 (Jeffamine ED-2001) was purchased from Aldrich (Milwaukee, WI). Maleimide-*N*-hydroxysuccinimide ester bifunctional linker (MAL-dPEG2-NHS ester: MAL-NHS linker) was purchased from Quanta Biodesign (Powell, OH). Peptide (Gly-Arg-Gly-Asp-Ser-Cys; GRGDSC) and *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) were purchased from GL Biochem (Shanghai, China). 4-{4-[1-(9-fluorenylmethyloxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy} butyric acid (Fmoc-photolabile linker: Fmoc-PLL) was purchased from Advanced ChemTech (Louisville, KY). All other chemicals were purchased from Sigma (St. Louis, MO) or Aldrich Chemicals (Milwaukee, WI).

PCL synthesis

1) Fmoc-PLL coupling

Fmoc-PLL (1.91 g, 3.67 mmol) was preactivated in a solution of HATU (1.40 g, 3.67 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 1.28 mL, 7.34 mmol) in *N*,*N*-dimethyl foramide (DMF, 20 mL) for 5 min. The reaction mixture was poured into a solution of Jeffamine ED-2001 (3.34g, 1.67 mol) in DMF (20 mL) and stirred magnetically for 16 h at room temperature. The reaction mixture was slowly added to 4×50 mL cold diethylether containing tubes (-20 °C) and centrifuged for 3 min at room temparature at 2500 rpm. The yellow bottom layer was collected to a glass vial.

2) Fmoc removal

The product from step 1) was used without further purification. To the solution of yellow bottom layer from step 1), 20% (v/v) piperidine in DMF (30 mL) was added and stirred for 16 h at room temparature. The product was isolated by the same method described in step 1).

3) methacryl group coupling

The product from step 2) was used without further purification. To the solution from step 2), methacrylic anhydride (0.994 mL, 6.67 mmol) and DIPEA (2.32 mL, 13.34 mmol) in DMF was added and stirred for 16 h at room temparature. After ether precipitation, the crude product was diluted with water (90 mL) and dialyzed using a dialysis tubing (MWCO=2k) for 3 d. The dialyzed product was collected and lyophilized for 2 d. Overal isolated yield of the final lyophilized product was 50 % (mol/mol) comparing to Jeffamine ED-2001. The structure of the final product was confirmed by ^{1}H NMR.

^{1}H NMR (600 MHz, DMSO-d6, δ): 8.45 (d, 1H, C(=O)N<u>H</u>), 7.5 (s, 1H, Ar <u>H</u>), 7.3 (s, 1H, Ar <u>H</u>), 5.7 (s, 1H, CH(CH_3)=C<u>H_2</u>), 5.5 (m, 1H, Ar C<u>H</u>), 5.4 (s, 1H, CH(CH_3)=C<u>H_2</u>), 4.05 (t, 2H, Ar

$OC\underline{H_2}CH_2CH_2$), 3.9 (s, 3H, Ar $OC\underline{H_3}$), 3.2-3.6 (m, ~180H, $C\underline{H_2}C\underline{H_2}O$ in Jeffamine), 2.2 (t, 2H, $C(=O)C\underline{H_2}CH_2CH_2$), 1.9 (m, 2H, $CH_2C\underline{H_2}CH_2$), 1.8 (s, 3H, $CH(C\underline{H_3})=CH_2$), 1.5 (d, 3H, Ar $CHC\underline{H_3}$), 1.0 (m, 16H, $CH_2CH(C\underline{H_3})O$ in Jeffamine).

Photogel base layer fabrication

Glass surfaces were first functionalized with an acrylated silane to promote adhesion of photogel. Briefly, glass slides were exposed to O_2 plasma for 5 min at 300 W, placed into a nitrogen filled glovebag, and immersed in 0.1% (v/v) 3-acryloxypropyltrichlorosilane in anhydrous toluene for 1 h at room temparature. Aqueous photogel precursor solution (10% (v/v) PCL, 8% (v/v) PEG-A) and initiator (ammonium persulfate (30 mM), tretramethyl ethylene diamine (30 mM)) were then placed between acrylated glass surfaces and a glass cover slip for polymerization. After 20 min at room temperature, the cover slip was removed and photogel was washed with deionized water.

Orthogonal photopolymerization/photodegradation

Phenylbis(2,4,6- trimethylbenzoyl)phosphine oxide photoinitiator (Irgacure 819) was used for photogel polymerization under 420-nm light that minimizes degradation of photosensitive *o*-nitrobenzyl groups.[1] Using 1% (w/v) Irgacure 819 and 40% (w/v) PCL in DMF, photogel was polymerized on acryl-silane treated surfaces *via* covalent grafting by photolithography through a photomask with 500-µm sized circular patterns. The exposure for polymerization was carried out by using an illuminator (70 mW/cm^2, 2 min) through a 420-nm band pass filter (Thorlabs, Newton, NJ). The patterned photogel was degraded with 365-nm light projected from an epi-fluorescence microscope. The size of the exposed region could be controlled by changing the

aperture equipped in the microscope (50-200 μm). To check the efficiency of photodegration, photogel microarrays were patterned on the acrylated surface (**Figure S2**). After the microphotogel array was exposed to UV for varying periods of time, degradation efficiency was quantified by normalizing the remaining area. Interestingly, the photogel did not disappear right after exposure but gradually diffused into the solution. The photocleavage reaction was completed after 10 sec exposure to UV (600W) and the diffusion of the gel was observed after 1 h.

Peptide immobilization on microbeads

TentaGel beads (50 mg) were incubated in a 1 mL solution of 13.8 μM MAL-NHS linker and 27.6 μM DIPEA in DMF for 1 h at 37 °C. After 3× alternate washing with fresh DMF and methanol, beads were re-incubated in a solution of 6.9 μM GRGDSC peptide and 2.4 μM DIPEA in DMF for 1 h at 37 °C. Finally, beads were again washed with DMF and methanol, then stored in a vacuum desiccator.

Microbead array fabrication by μTM

PDMS prepolymer was mixed at an elastomer to crosslinker ratio of 15:1, poured over an SU-8 negative photoresist master mold, then baked at 70 °C for 2 h to form "soft" PDMS microwell arrays (diam = 125 μm, height = 100μm, center-center spacing = 300 μm). PDMS mold surfaces were exposed to O_2 plasma for 5 min at 300 W prior to bead seeding. Beads were mixed in a solution of photogel precursor (20% (v/v) PCL, 8% (v/v) PEG-A in water) and initiator (30 mM ammonium persulfate (AP), 30 mM tretramethyl ethylene diamine (TEMED) in water), then dropped onto PDMS microwell arrays. Excess prepolymer was removed by scraping the mold

surface with the edge of a second piece of PDMS. Seeded arrays were compressed against the preformed photogel layer on glass (400 Pa) and immediately placed in a nitrogen filled glove bag for 4 h. After polymerization, molds were gently removed and bead arrays were washed with phosphate buffered saline (PBS), then sterilized in 70% ethanol for 1 min.

3T3 cell capture

Trypsin harvested NIH 3T3 cells were seeded onto bead arrays such that the entire array was covered by a layer of cells, then gently washed with PBS after 1 h incubation. Samples were re-incubated in culture media for 24-48 h for peptide mediated cell spreading over bead surfaces.

Imaging and Bead Release

Array screening and bead release studies were performed under a Zeiss A.1 Axioscope upright fluorescent microscope. To collect isolated beads, arrays were inverted in a two layer PDMS chip (see **Figure S4**) and regions of interest were exposed at 600 $mW \cdot cm^{-2}$ for 2 min. Selected beads were then allowed to settle by gravity to the bottom of the PDMS chip following diffusion of degraded photogel monomers (1-2 h).

Supplemental Figures

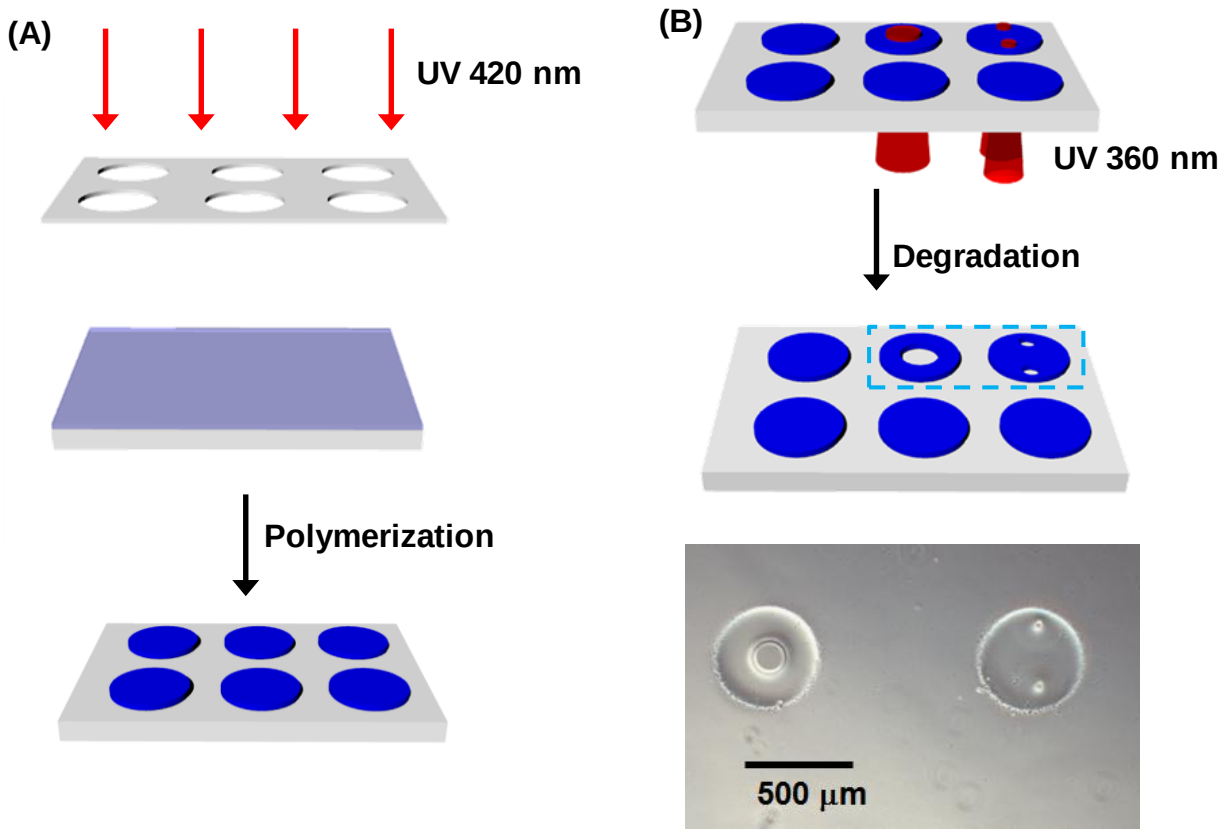

Figure S1: Polymerization and photodegradation of photogel. a) Photogel microarrays were photolithographically patterned with a photoinitiator activated with 420 nm light, without cleaving photolabile *o*-nitrobenzyl groups. a) Photogel was selectively degraded by exposing 365 nm light with a fluorescence microscope. The area of degradation was controlled by narrowing the microscope aperature.

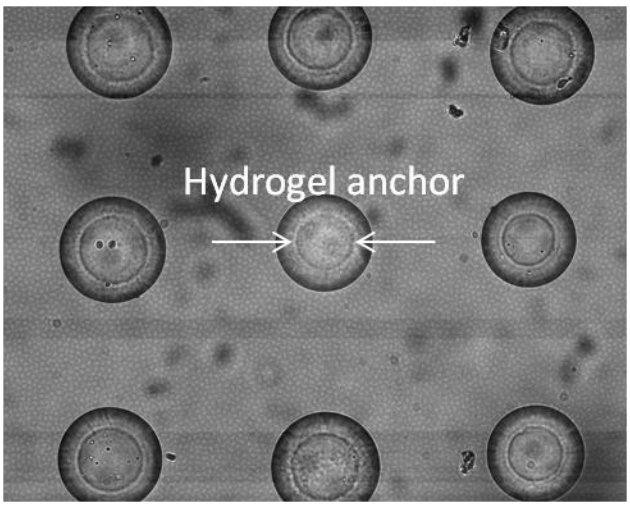

Figure S2: The photogel anchor diameter depends on the polymerization "inhibition layer" thickness during µTM, and determines both bead transfer yield and degree of bead surface exposure. Under conditions described, the average anchor diameter was 75 µm, corresponding to > 90% bead surface exposure (for an average swollen bead diameter of ~130 µm). These conditions yielded 68 ± 11% total bead transfer efficiency (n = 3).

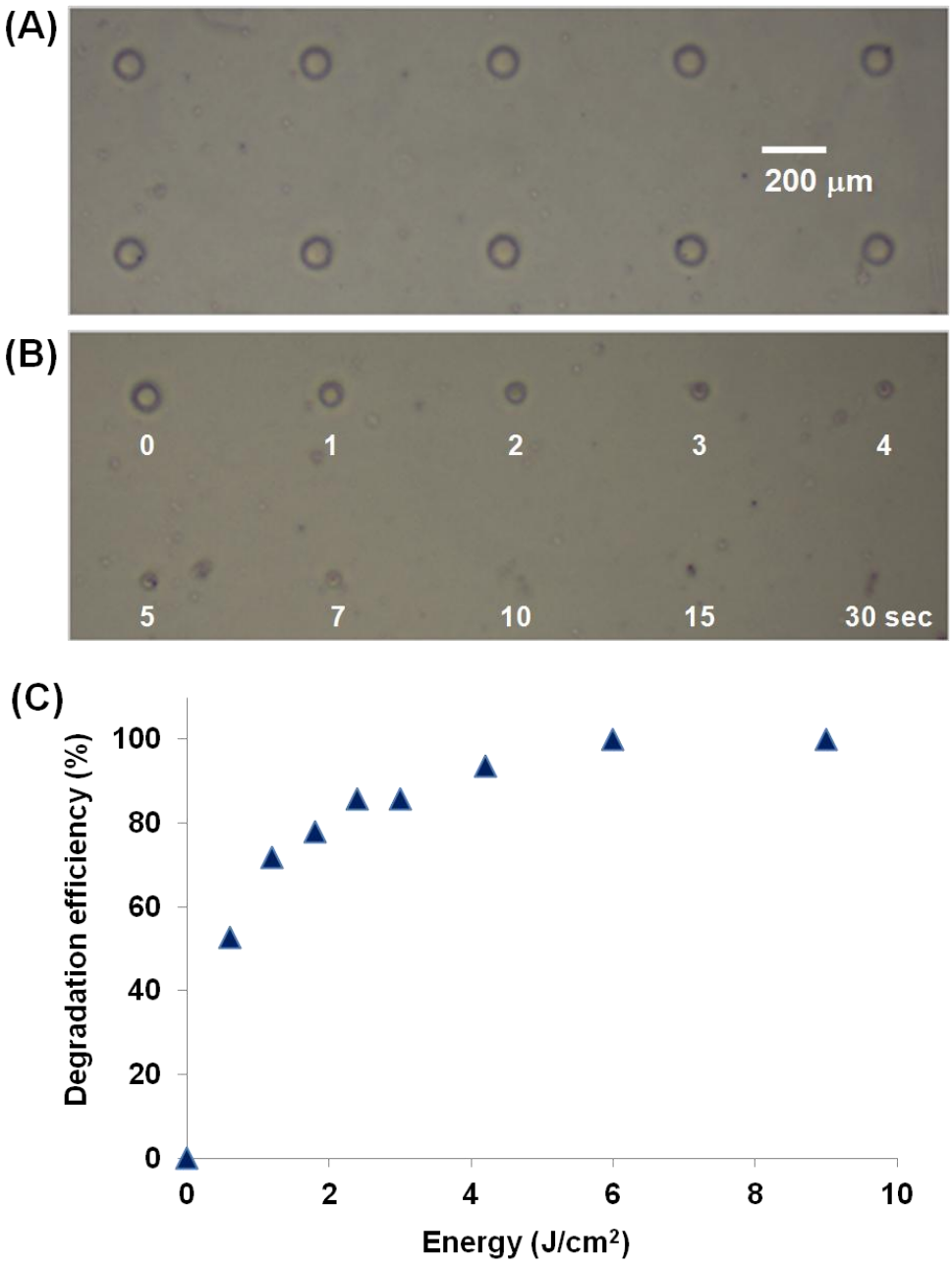

Figure S3: Photodegradation efficiency. a) Photogel microarrays patterned with photolithograpy (420 nm light). b) Photogel features were then exposed to 365 nm light at 600 $mW \cdot cm^{-2}$ for varying periods of time to observe degradation. c) After 1 h degraded monomers diffused into solution. Remaining gel was normalized to orignal area to determine degradation efficiency.

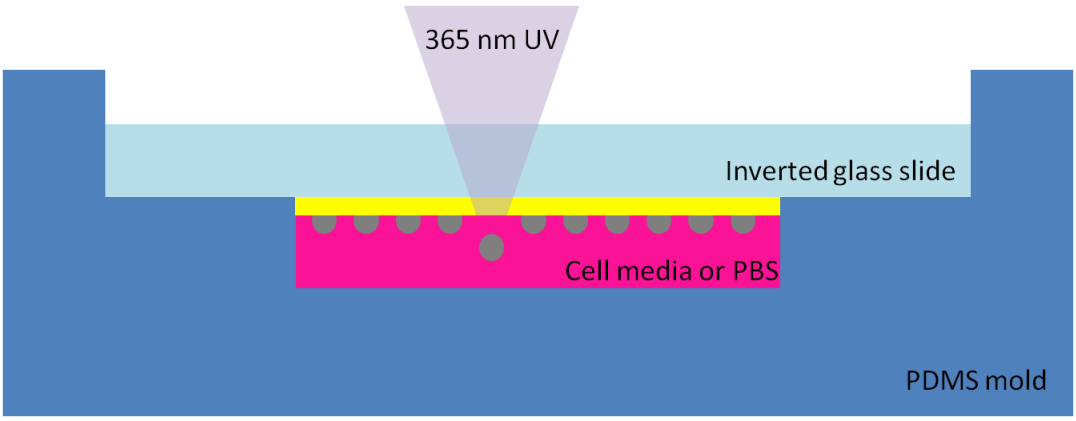

Figure S4: Photogel degradation and bead release scheme. Bead arrays were inverted and placed in a PDMS mold containing a liquid medium (culture media or PBS). An upright fluorescent microscope exposes UV light to degrade photogel anchor, and selected beads sediment to the bottom PDMS surface.

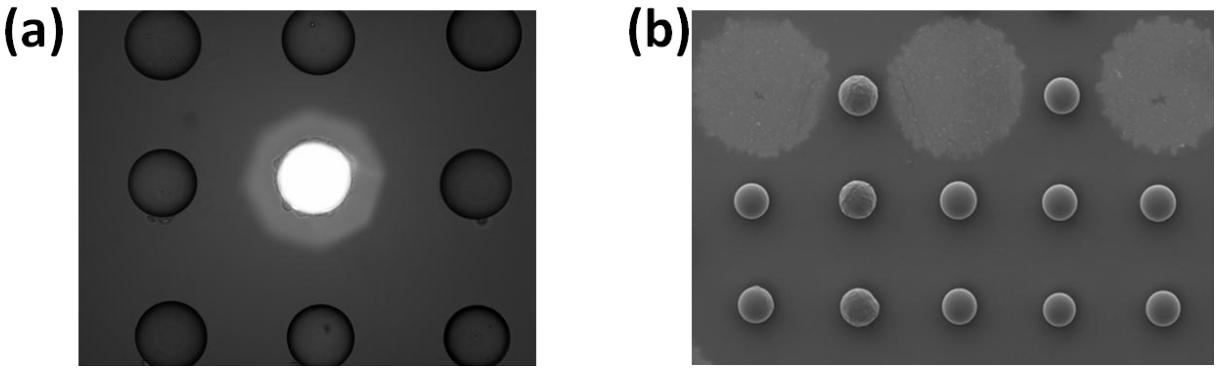

Figure S5: Bead release by local UV exposure for photogel anchor degradation (a) UV spot area is controlled via microscope aperture. (b) SEM image shows degraded photogel anchor and base layer adjacent to unaffected neighboring beads.

References:

[1] A. Kloxin, M. Tibbitt, K. Anseth, Nature protocols 2010, 5, 1867.