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Supporting Information

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Photodegradable Hydrogels for Capture, Detection, and Release of Live Cells**

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Materials and Methods

Glass slides (75 ×25 mm^2) were purchased from Fisher Scientific (Pittsburg, PA). (3-Aminopropyl)trimethoxysilane, (3-acryloxypropyl)trimethoxysilane and (3-acryloxypropyl)-trichlorosilane were purchased from Gelest (Morrisville, PA). Methacryloxyethyl thiocarbamoyl rhodamine B (Acryl-Rho) was purchased from Polysciences (Warrington, PA). 4-{4-[1-(9-fluorenylmethyloxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy} butyric acid (Fmoc-photolabile linker: Fmoc-PLL) was purchased from Advanced ChemTech (Louisville, KY). *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*´,*N*´-tetramethyluronium hexafluorophosphate (HATU) was purchased from GL Biochem (Shanghai, China). *O*,*O*'-Bis-(2-aminopropyl) polypropylene glycol-*block*-polyethylene glycol-*block*-polypropylene glycol 1900 (Jeffamine ED-2001), *N*,*N*-diisopropylethylamine (DIPEA), *N*,*N*-dimethylforamide (DMF) and piperidine were purchased from Aldrich Chemicals (Milwaukee, WI). Photolabile crosslinker (PCL) (Figure S1) was synthesized and characterized by the method as described previously.[1] Acrylate-PEG-*N*-hydroxysuccinimide ester (Acryl-PEG-NHS, MW=3400) was purchased from Laysan Bio (Arab, AL). Mouse anti-human CD4 antibody (anti-CD4 Ab) and mouse anti-human CD8 antibody (anti-CD8 Ab) were purchased from Beckman Coulter (Miami, FL). Biotinylated anti-human CD4 antibody (b-anti-CD4 Ab), biotinylated mouse anti-human CD8 antibody (b-anti-CD8 Ab) and CD4/CD8/CD3 antibody, FITC, PE, Cy5-PE conjugate were purchased from Thermo Fisher Scientific (Rockford, IL). Mouse anti-human TNF-α antibody (anti-TNF-α Ab) and biotinylated goat anti-human TNF-α antibody (b-anti-TNF-α Ab) were purchased from R&D Systems. LIVE/DEAD® Viability/Cytotoxicity Kit, CellTracker Green, Alexa Fluor 546 donkey anti-mouse IgG, streptavidin R-Phycoerythrin conjugate, streptavidin-Alexa Fluor 546 conjugate and 1 ×Phosphate buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA). All other chemicals were obtained from Sigma (St. Louis, MO) or Aldrich Chemicals (Milwaukee, WI). Molt-3 T-lymphocytes and NIH 3T3 mouse fibroblasts were purchased from American Type Culture Collection (ATCC). Molt-3 T-lymphocytes were culutured in 10% (v/v) fetal bovine serum (FBS, Invitrogen, Carlsbad, CA), 100 U/mL penicillin and 100 μg/mL streptomycin in RPMI-1640 media (VWR, West Chester, PA) at 37 ^oC in a humidified 5% CO_2 atmosphere. NIH 3T3 mouse fibroblasts were culutured in 10% (v/v) FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in Dulbecco's Modified Eagle Medium (DMEM, VWR, West Chester, PA) at 37 ^oC in a humidified 5% CO_2 atmosphere. Blood was collected from healthy adult donors through

venipuncture under sterile conditions with informed consent and approval of the Institutional Review Board of the University of California at Davis (IRB protocol number 222894, revised 02/11/2011) and peripheral blood mononuclear cells (PBMCs) were isolated from whole blood with Lymphoprep (Stemcell Technologies, Vancouver, Canada).[2] These cells were concentrated by centrifugation and resuspended in whole RPMI-1640 media.
Scanning electron microscopy (SEM) images were obtained using a Philips XL 30 scanning electron microscope. Fluorescence images were obtained with a confocal microscope (Zeiss LSM 5 Pascal, Carl Zeiss, Göttingen, Germany). UV illumination (365 nm) was carried out by a fiber optic light source (OmniCure® Series 1000, Lumen Dynamics Group, Mississauga, Ontario, Canada) and an epi-fluorescence microscope (Zeiss 200 M, Carl Zeiss MicroImaging, Inc. Thornwood, NY). Ab spots were printed on the photogel surface using a microarrayer (Spotbot 3, Arrayit, Sunnyvale, CA).

Surface Modification with Silane Coupling

Glass slides were placed in an oxygen plasma chamber for 3 min at 300 W and immersed in solution of 0.1% (v/v) (3-acryloxypropyl)trichlorosilane, in toluene for 1 h under nitrogen gas. To introduce amino groups on the glass surface, the plasma treated glass slides were immersed in solution of 0.1% (v/v) (3-aminopropyl)trimethoxysilane, 0.1% (v/v) (3-acryloxypropyl) trimethoxysilane in toluene for 5 h under nitrogen gas. The slides were rinsed in toluene and dried under nitrogen.

Photogel Coating on Acryl Surface

200 mM tretramethylethylenediamine (TEMED) in DI water (1.5 μL) was added to a mixture of 20% (v/v) PCL in DI water (5 μL), 20% (v/v) poly(ethylene glycol) methyl ether acrylate (PEG-A, M_n=480) in DI water (2 μL) and 200 mM ammonium persulfate in DI water (AP) (1.5 μL) and mixed carefully to prevent the generation of bubbles inside the solution. For rhodamine incorporated gel, 20 mM Acryl-Rho in DI water (0.2 μL) was added to the mixture. The solution (5 μL) was dropped onto the acrylated glass slides (cut to 1.25 cm ×1.25 cm sized pieces) and cover slips were placed over the solution. Cover slips were removed after 1 h and the photogel coated surfaces were washed with DI water and dried with nitrogen.

Optimization for Photodegradation of Photogel

A rhodamine incorporated photogel surface was exposed to UV from a fluorescence microscope for varying periods of time (600 mW/cm^2, exposure diameter=200 μm). Fluorescence images were obtained from the fluorescence microscope after 1 h for diffusion of the photogel. The degraded areas were measured by ZEN 2011 software provided by Carl Zeiss and normalized to complete degradation.

Attachment of Collagen and NIH 3T3 Mouse Fibroblasts on Photogel Removed Surfaces

Photogel coated surfaces on amino-/acryl-functionalized glass slides were exposed to 365 nm UV through a photomask from an illuminator (500 mW/cm^2, 30 s). The surfaces were treated with 50 μg/mL FITC-collagen in PBS for 1 h at RT for FITC-collagen coating. The fluorescence images were obtained by a confocal microscope. Prior to seeding cells on the surfaces, NIH 3T3 mouse fibroblasts were stained by CellTracker Green following the protocol provided by Invitrogen Co. Briefly, 10 mM CellTracker Green in DMSO was diluted to 10 μM by adding serum-free DMEM medium. The cell suspension was incubated in the working solution for 30 min, washed with serum-free DMEM medium and incubated in whole medium 30 min. The cells were then trypsinized and seeded onto the photogel patterned surfaces (100,000 cells/mL). Fluorescence images were obtained by a confocal microscope 1 d after initial seeding.

Cell Sorting on Ab-Printed Photogel Surface

NHS-functionalized photogel was prepared with a mixture of 20% (v/v) PCL in DI water (5 μL), 20% (v/v) PEG-A in DI water (1 μL), 20% (w/v) Acryl-PEG-NHS in DI water (1 μL), 200 mM AP in DI water (1.5 μL) and 200 mM TEMED in DI water (1.5 μL). The solution was incubated between an acrylated slide and a cover slip for 1 h and the photogel was washed with DI water and dried with nitrogen. Anti-CD4 and CD8 Abs (200 μg/mL, respectively) supplemented with 0.5% (v/v) BSA and 0.005% (v/v) Tween20 were printed alternately on the photogel with a microarrayer (spot diameter=150 μm, center-to-center=300 μm). After incubated with 1% BSA for 30 min, the Ab-printed photogel surface was incubated with PBMCs inside a PDMS microfluidic chamber (dimension: (3 mm (width) × 10 mm (length) × 0.1 mm (height)) for 30 min and flushed with fresh media.[3] The cells on a CD4 or CD8 Ab spotted region were exposed

to 365 nm UV from an epi-fluorescence microscope (600 mW/cm^2) for 30 s with a constant flow of media (1 μL/min). The release of cells was monitored over time under the microscope.
To check the purity of cells on anti-CD4 and CD8 Ab spots, the patterned cells on photogel were stained with CD4/CD8/CD3 antibody, FITC, PE, Cy5-PE conjugate (Thermo Fisher Scientific, Rockford, IL) in 1% BSA/PBS (1:10). The surface was visualized in FITC (green), PE (yellow) and Cy5-PE (red) channels by a confocal microscope (Figure S4). Cells on both CD4 and CD8 Ab spots were stained red since CD3 antigen is present on all T-cells. We could observe that the cells on anti-CD4 Ab spots were stained green whereas the cells on anti-CD8 Ab spots showed yellow signals. As seen from the merged image, the purity of $CD3^+CD4^+$ and $CD3^+CD8^+$ expressing cells on the anti-CD4 Ab and the anti-CD8 Ab spots is determined to be more than 95%, which is comparable to our previous results on PEG surfaces.[2]

Cell Viability Test

Molt-3 T-lymphocytes captured on anti-CD4 Ab printed photogel were released by UV illumination (500 mW/cm^2, 30 s). The suspension was collected into a tube, centrifuged and decanted. The cells were placed into a mixture of 4 mM calcein AM and 2 mM ethidium homodimer-1 in PBS for 30 min. The stained cells were washed with PBS and fluorescence images were obtained by a confocal microscope. We did not see loss of ruptured cells during centrifugation. The numbers of dead and live cells were counted and the viability was calculated.

Flow Cytometry of Isolated CD4 or CD8 Cells

Primary cells retrieved from anti-CD4 Ab or anti-CD8 Ab printed photogel were suspended in a mixture of b-anti-CD4 Ab or b-anti-CD8 Ab (200 μg/mL) and 1% BSA in PBS (1: 10) for 1 h. The cells were washed with PBS and re-suspended in streptavidin R-Phycoerythrin conjugate (1 mg/mL), FITC-anti-CD3 Ab and 1% BSA/PBS (0.1:1:10) for 1 h. The cells were washed with PBS and re-suspended in 1% BSA/PBS. Flow cytometry was performed by a flow cytrometry instrument FACScan (Becton Dickinson, Franklin Lakes, NJ). Non-isolated PBMCs were used as control samples stained in the same way. The data were analyzed by a flow cytometry program FlowJo (TreeStar Inc. Ashland, OR).

Single Cell Capture, Detection and Release

A mixture of anti-CD4 Ab and anti-TNF-α Ab (200 μg/mL, respectively) supplemented with 0.5% (v/v) BSA and 0.005% (v/v) Tween20 was printed on the photogel (1 mm diameter). After incubated with 1% BSA for 30 min, the Ab-printed photogel surface was incubated with PBMCs (0.5 million cells/mL) inside a PDMS microfluidic chamber (dimension: (3 mm (width) × 10 mm (length) × 0.1 mm (height)) for 30 min and flushed with fresh media. The cells were incubated in PMA (50 ng/ml) and ionomycin (1 μg/mL) in RPMI 1640 with 10% (v/v) FBS and 100 U/mL penicillin and 100 μg/mL streptomycin at physiological conditions (37 ^{o}C in a humidified 5% CO_2 atmosphere) for 5 h. Biotinylated goat anti-human TNF-α antibody (b-anti-TNF-α Ab, 10 μg/mL in 1% BSA with PBS) was incubated in the channel for 1 h followed by streptavidin-Alexa Fluor 546 conjugate (10 μg/mL in 1% BSA with PBS) for 30 min. The chamber was rinsed with PBS between each step. Fluorescence images were observed from an epi-fluorescence microscope and single cells expressing strong fluorescence signals were released from the photogel by UV light from the epifluorescnece microsocpe (500 mW/cm^2, 30 s).

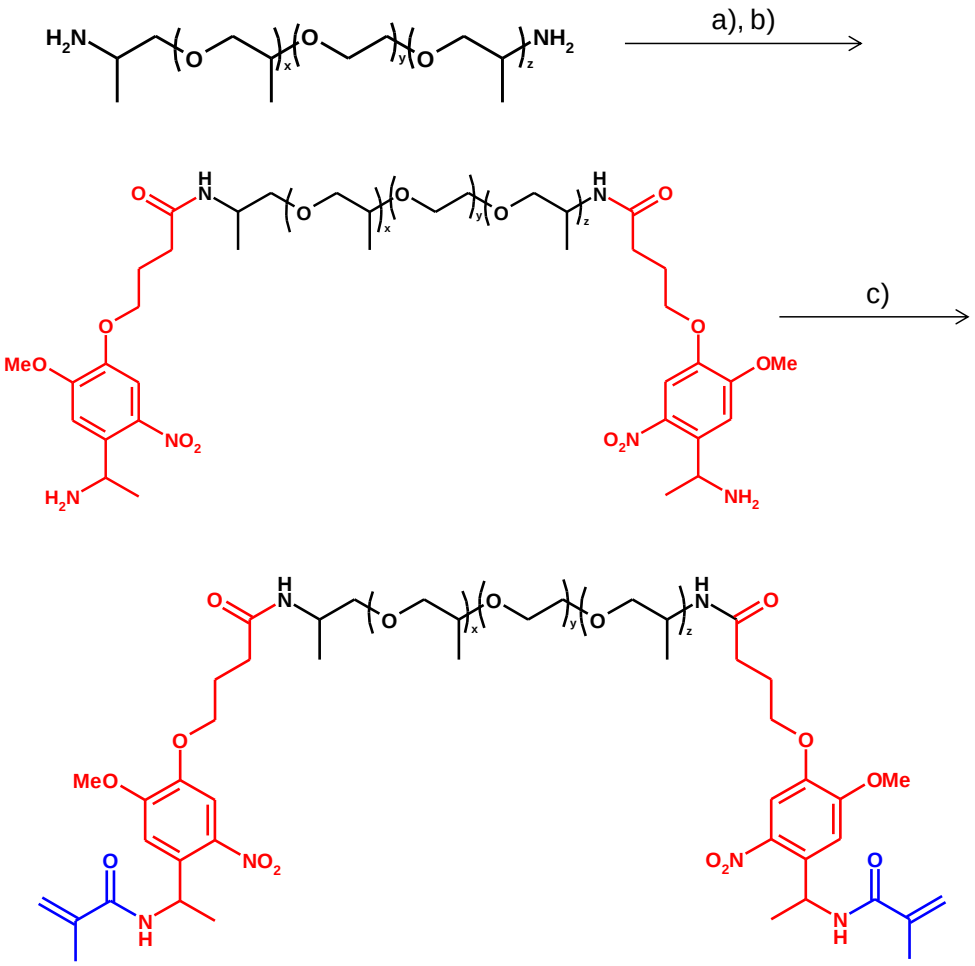

Figure S1. Synthesis of photolabile crosslinker (PCL). Reagents: a) Fmoc-PLL, HATU, DIPEA, DMF; b) piperidine, DMF; c) methacrylic anhydride, DIPEA, DMF.[1]

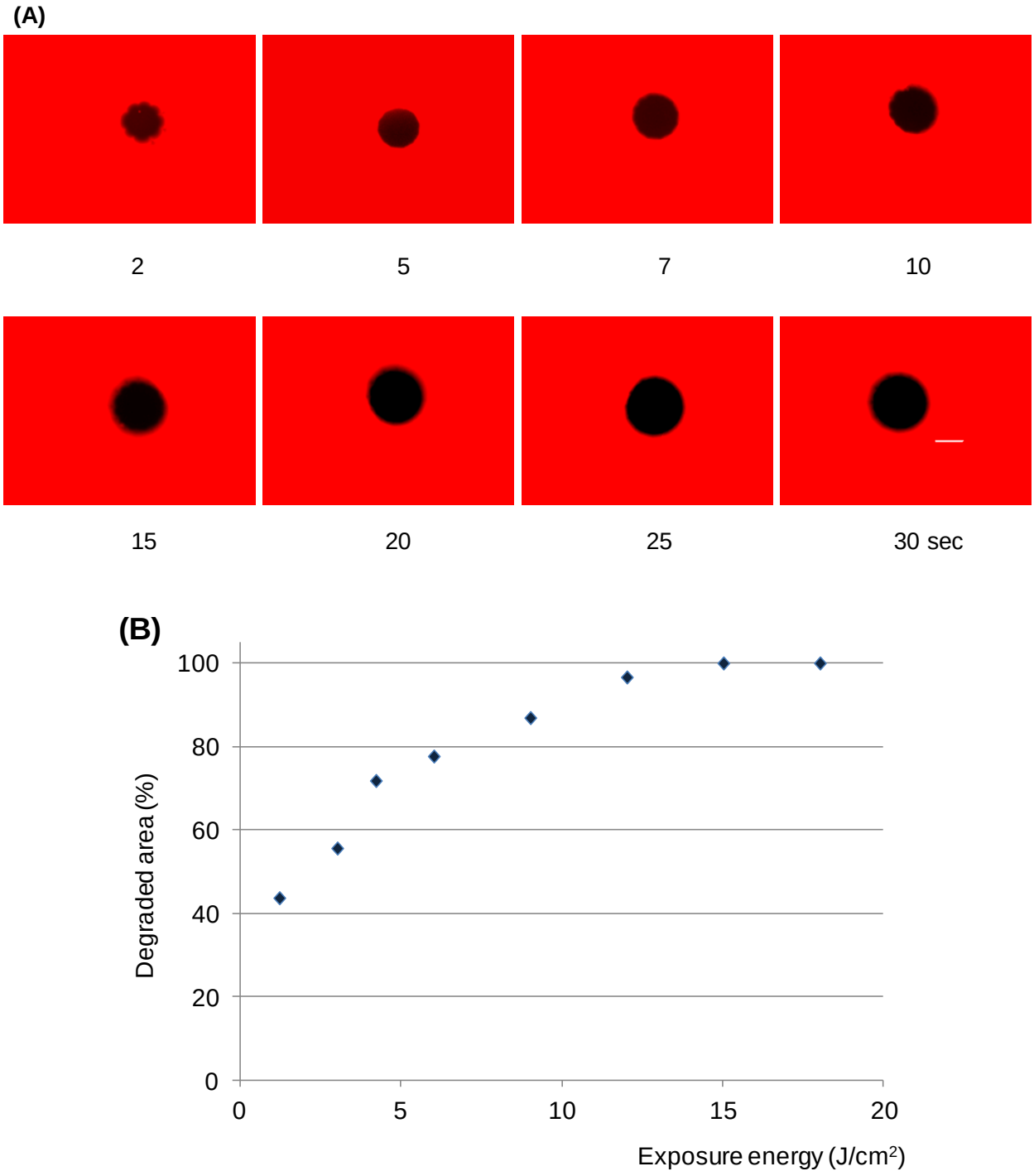

Figure S2. Optimization for photodegradation: (A) Fluorescence images of rhodamine incorporated photogel with temporal UV exposure (scale bar=100 μm), (B) The areas of degraded gel were obtained from the images and normalized to complete degradation.

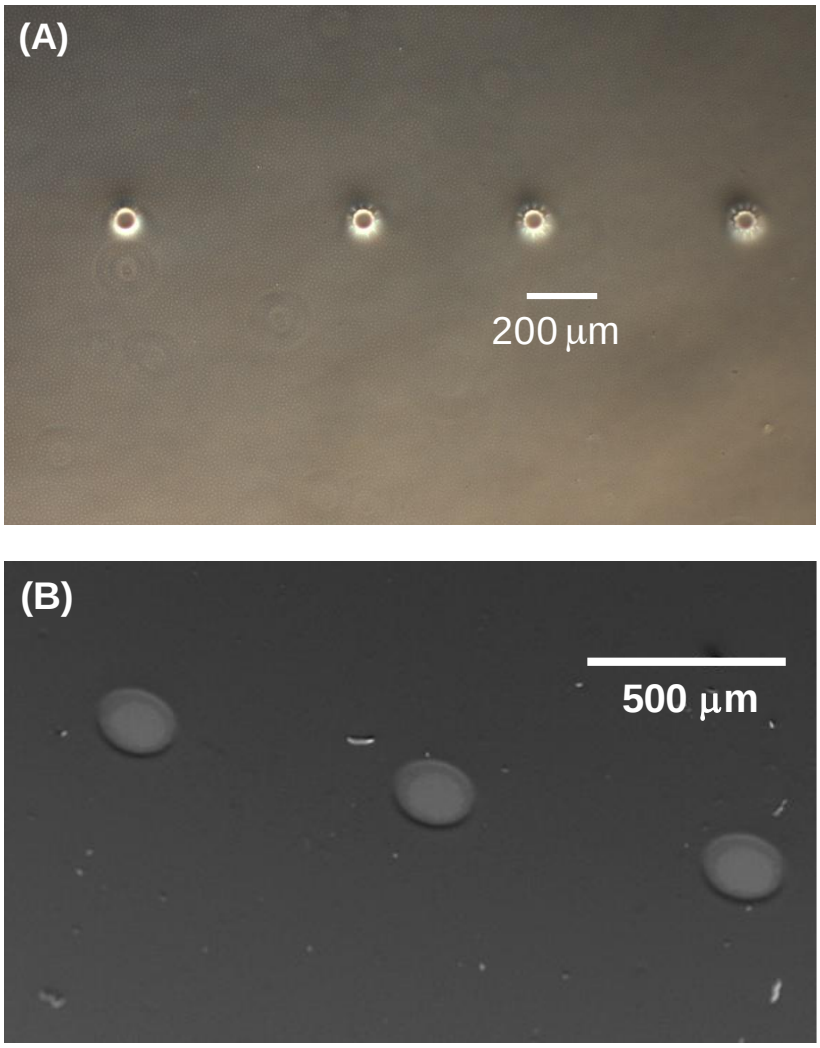

Figure S3. (A) Bright field image (each diameter=50 μm) and (B) scanning electron microscope (SEM) image (each diameter=200 μm) of degraded photogel upon UV exposure from a fluorescence microscope.

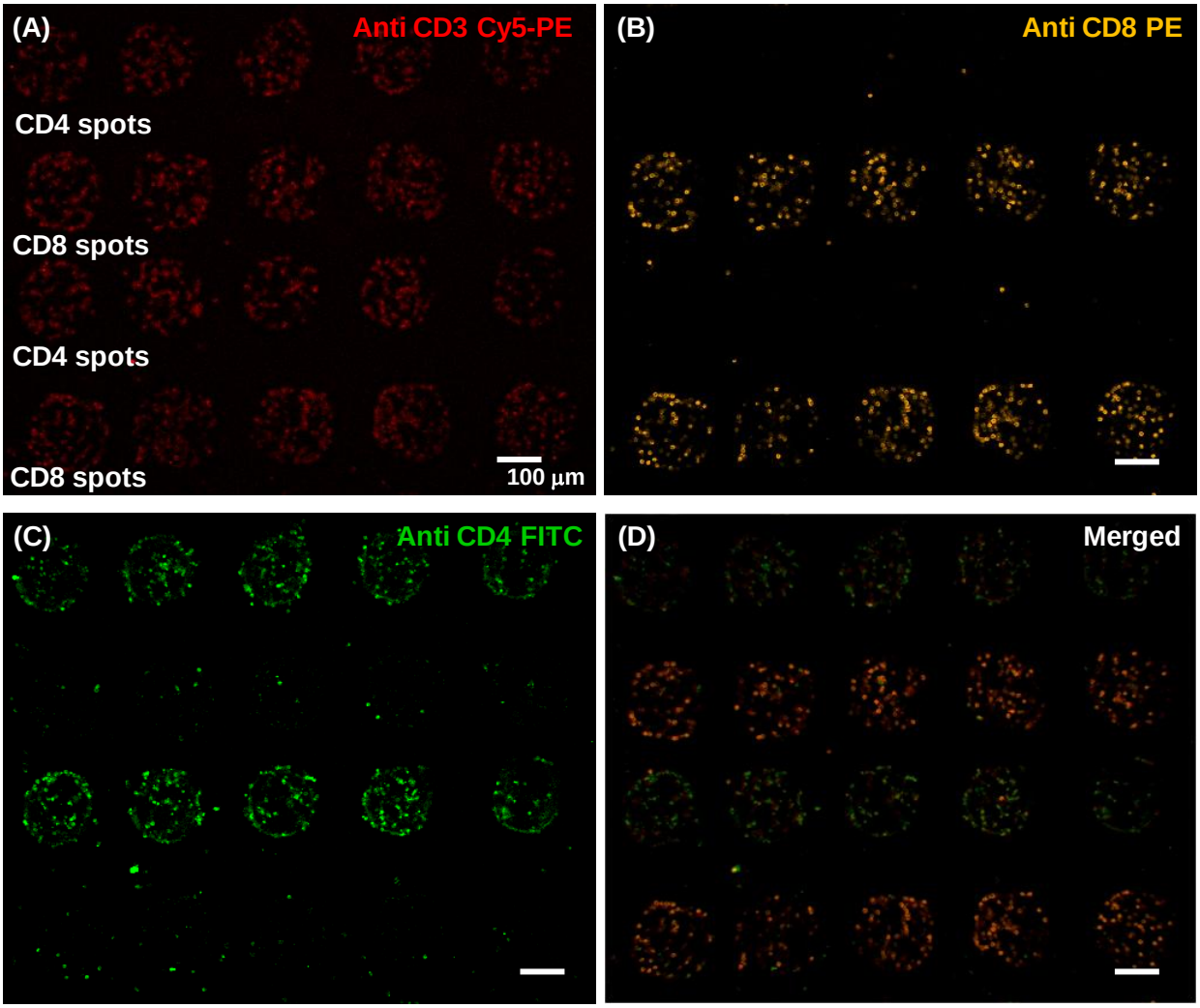

Figure S4. Cell capture on CD4 and CD8 Ab spots: Cells were stained by PE-Cy5-anti-CD3 Ab, PE-anti-CD8 Ab and FITC-anti-CD4 Ab. (A) Cy5-PE, (B) PE, (C) FITC channels, (D) 3-color merged image.

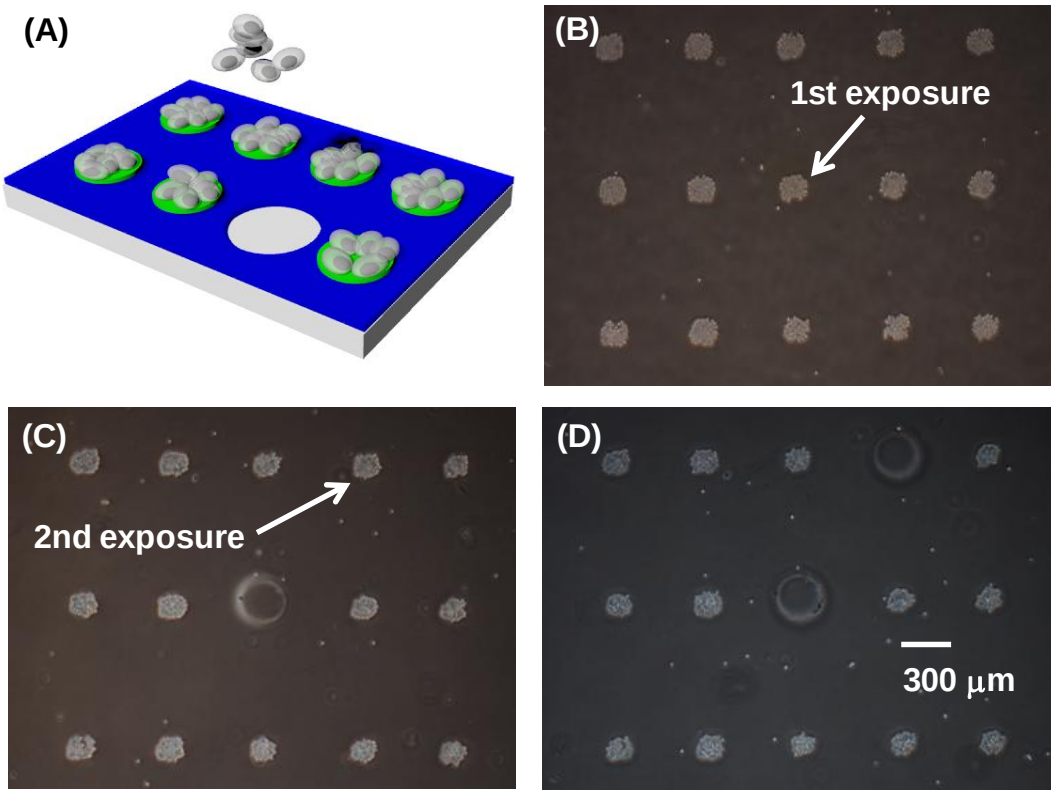

Figure S5. Capture of Molt-3 cells and sequential release. (A) Schematic representation of cell capture and release on anti-CD4 Ab-attached photogel. (B) Bright field image after Molt-3 cell capture. (C) (D) Sequential 365 nm light exposures from a microscope through an aperture (200 μm diameter).

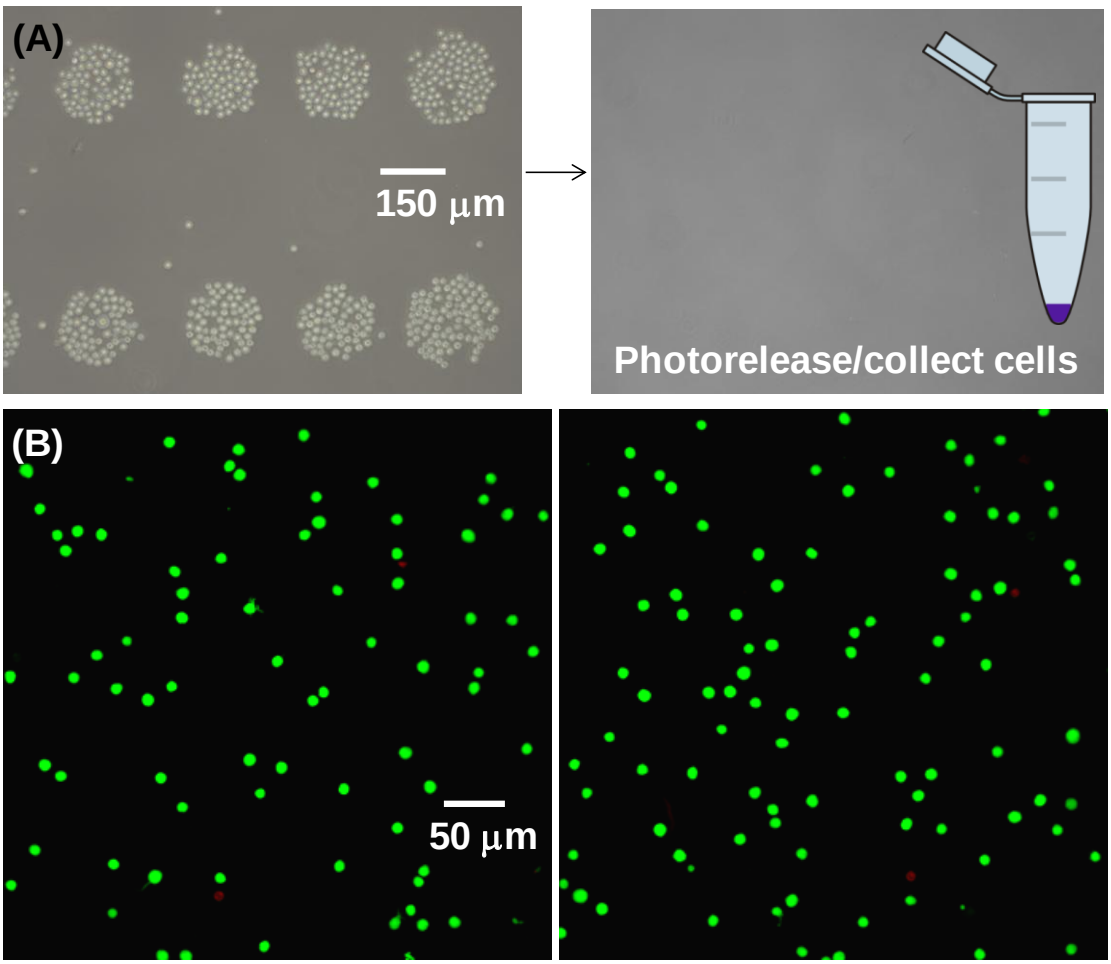

Figure S6. Viability test of Molt-3 cells after release. (A) Molt-3 cells were captured on anti-CD4 Ab attached photogel and released by exposing the whole photogel to UV from an illuminator. The cells were collected into a tube. (B) The collected Molt-3 cells were stained by using live/dead staining dyes (live cells: green, dead cells: red).

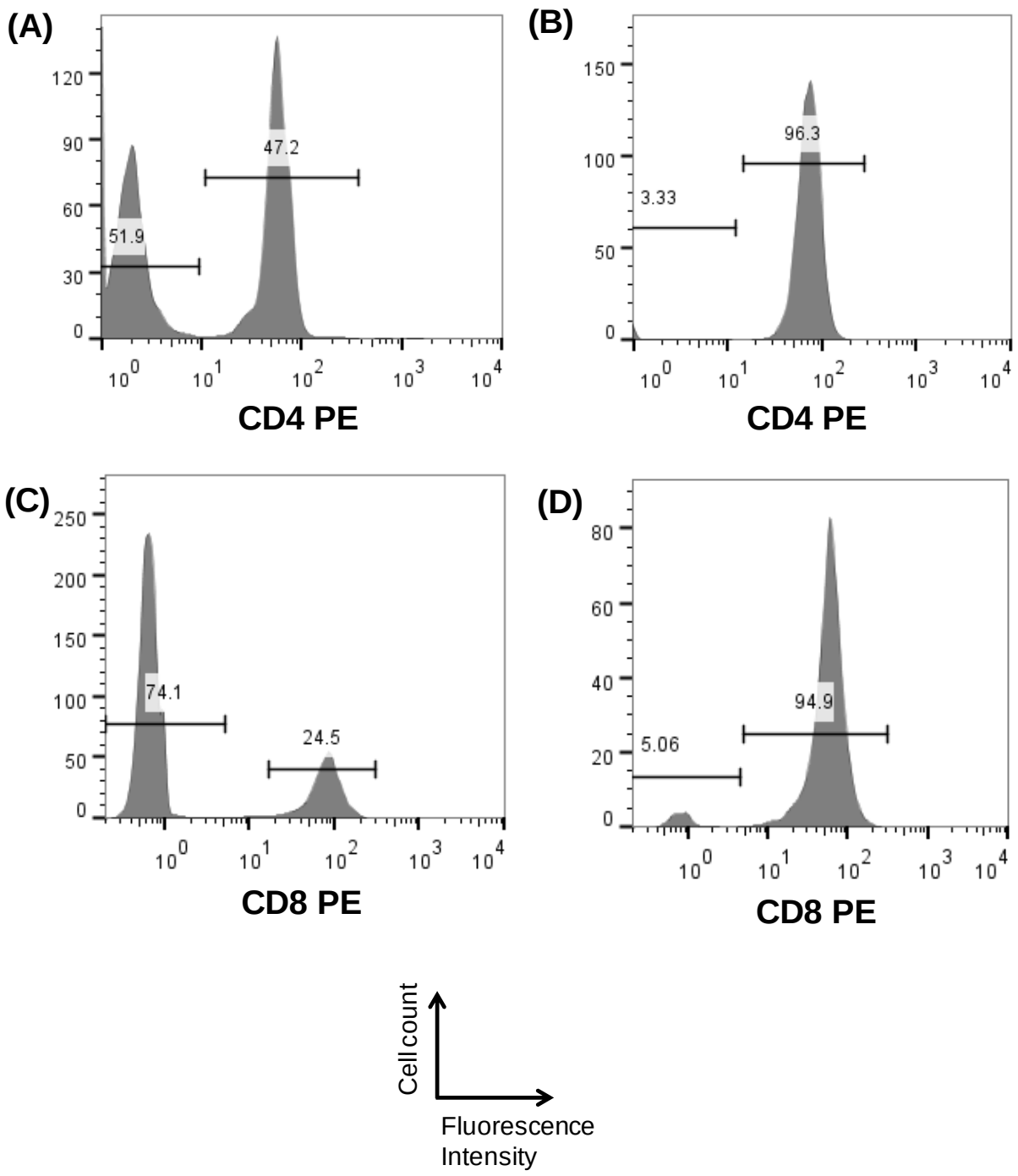

Figure S7. Histograms of CD4 and CD8 expressions in PBMCs. Flow cytometry was performed (A) before and (B) after isolation from anti-CD4 Ab printed photogel and (C) before and (D) after isolation from anti-CD8 Ab printed photogel.

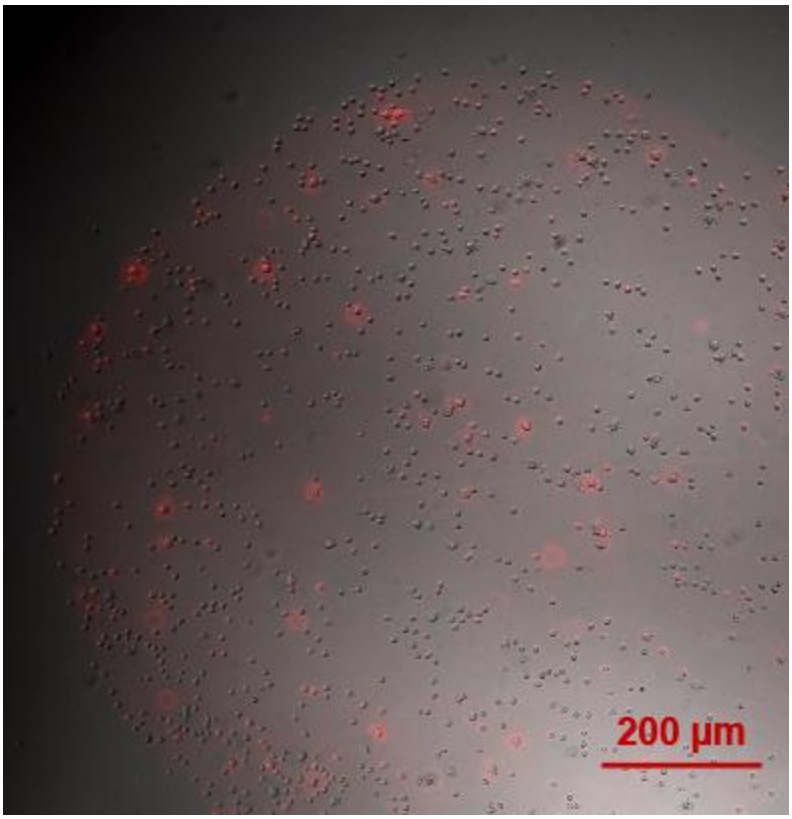

Figure S8. Local detection of TNF-α release from CD4 cells on photodegradable gel. Immuno-staining with anti-TNF-α-biotin and streptavidin-Alexa546 shows detection of TNF-α around single CD4 cells (red fluorescence).

References

[1] C. Siltanen, D. S. Shin, J. Sutcliffe, A. Revzin, *Angew. Chem. Int. Ed.* **2013**, *52*, 9224–9228.

[2] A. Chen, T. Vu, G. Stybayeva, T. Pan, A. Revzin, *Biomicrofluidics* **2013**, *7*, 024105.

[3] H. Zhu, G. Stybayeva, M. Macal, E. Ramanculov, M. D. George, S. Danekar, A. Revzin, *Lab Chip* **2008**, *8*, 2197-2205.