Electronic Supplementary Information (ESI)

Photolabile micropatterned surfaces for cell capture and release

Dong-Sik Shin,[a] Jeong Hyun Seo,[a] Julie L. Sutcliffe[a,b,c] & Alexander Revzin[a*]

[a]*Department of Biomedical Engineering, University of California, Davis, CA 95616, USA*

[b]*Division of Hematology/Oncology, Department of Internal Medicine, University of California, Davis, CA 95616, USA*

[c]*Center for Molecular and Genomic Imaging, University of California, Davis, CA 95616, USA*

arevzin@ucdavis.edu

1. Experimental Details

Materials and Methods

Molt-3 T-lymphocytes 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 RPMI-1640 media (VWR, West Chester, PA) at 37 ^{o}C in a humidified 5% CO_2 atmosphere. 20× Phosphate buffered saline (PBS) was purchased from TEKnova (Hollister, CA). Biotin conjugated mouse anti-human CD4 antibody (biotin-anti-CD4 Ab) was purchased from Beckman Coulter (Miami, FL). LIVE/DEAD® Viability/Cytotoxicity Kit and all streptavidin and neutravidin conjugates were purchased from Invitrogen (Carlsbad, CA).

Glass slides (75 × 25 mm^2) were purchased from Fisher Scientific (Pittsburg, PA). NHS-dPEG®12-biotin was purchased from Quanta Biodesign (Powell, OH). 3-Aminopropyl triethoxysilane and 3-acryloxypropyl triethoxysilane were purchased from Gelest (Morrisville, PA). Fmoc-photolabile linker (Fmoc-PLL: 4-{4-[1-(9-fluorenylmethyloxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy} butyric acid) 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). All other chemicals were obtained from Sigma (St. Louis, MO) or Aldrich Chemicals (Milwaukee, WI).

Ellipsometry was carried out using a Elli2000 imaging system (Nanofilm Technologie, Göttingen, Germany) at the incidence angle of 60º, with a frequency-doubled Nd:YAG laser at 532.8 nm [1]. Fluorescence images were observed by using a confocal microscope (Zeiss LSM 5 Pascal, Carl Zeiss, Göttingen, Germany) and fluorescence profilings were analyzed by a software program (Zeiss LSM Image Browser). The exposure of 365-nm UV was carried out by a fiber optic light source (OmniCure® Series 1000, Lumen Dynamics Group, Mississauga, Ontario, Canada) and a UV illuminator (FOTO/Phoresis® UV Transilluminator, Fotodyne, Hartland, WI).

Modification of Glass Substrates with Amino-/Acryl- Mixed Silane

Glass slides were placed in an oxygen plasma chamber for 3 min at 300W and immersed in solution of 0.1 % (v/v) 3-aminopropyl trimethoxysilane, 0.1% (v/v) 3-acryloxypropyl

trimethoxysilane and 0.1 % (v/v) *N*, *N*-diisopropylethylamine (DIPEA) in toluene for 5 hr under nitrogen gas. The slides were rinsed in toluene and dried using nitrogen gas.

PEG Microwell Patterning on Silane-Modified Glass Substrates

A solution of 2% (v/v) photoinitiator (2-hydroxy-2-methylpropiophenone) in PEG-diacrylate (DA) (MW 575) was spin-coated on the amino- and acryl- mixed glass slides at 800 rpm for 5 sec. The slides were irradiated through a photomask for 5 sec by a 365 nm-UV light source (80 mW/cm^2), washed with DI water and dried using nitrogen gas.

Coupling of Photolabile Linker and Biotin on the PEG Patterned Surface

The PEG patterned slides were placed in a mixture of 10 mM Fmoc-photolabile linker (PLL), 10 mM HATU and 20 mM DIPEA in *N*, *N*-dimethylformamide (DMF) for 2 hr, washed with DMF and methanol and dried using nitrogen gas. The slides were placed in 20 % (v/v) piperidine in DMF for 50 min, washed with DMF and methanol and dried using nitrogen gas. The slides were placed in 10 mM NHS-dPEG®12-biotin and 20 mM DIPEA in DMF for 2 hr, washed with DMF and methanol and dried using nitrogen gas.

Cell Capture and Release

Neutravidin (20 μg/mL in 1×PBS) was incubated with the PLL- and biotin-functionalized glass slides for 1 hr and washed with 1×PBS. Biotin-anti-CD4 Ab (20 μg/mL in 1×PBS) was incubated with the slides for 1 hr and washed with 1×PBS. Molt-3 cells suspended in the culturing media were incubated with the slides for 10 min in a petridish or in a microchannel followed by washing with the media [2]. After the cells were attached on the surface, the cells were released by the exposure of 365-nm UV from the illuminator (500 mW/cm^2).

Avidin Patterning by Photolithography (Scheme S1)

PLL and biotin were immobilized on unpatterned silane-modified glass slides as described above. To demonstrate cleavage of biomolecular constructs, the biotin and PLL coupled surface was exposed to 365-nm UV at 1.2 W/cm^2 for 0.5 sec with a photomask followed by treating 20 μg/mL of Alexa Fluor® 546-streptavidin in PBS for 30 min (Scheme S1A). To characterize avidin detachment from photolabile surfaces, 20 μg/mL of neutravidin in PBS was incubated for

30 min on biotin- and PLL-modified surface. This surface was exposed to 365-nm UV at 1.2 W/cm^2 for 0.5 sec through a photomask followed by incubation in 5 μg/mL of Atto 565-Biotin in PBS for 30 min (Scheme S1B). The slides prepared by each method were washed with 1x PBS, dried using nitrogen gas, and scanned by a confocal microscope.

Testing Cell Viability After UV Exposure

To test cell viability we prepared surfaces containing biotin, neutravidin and Ab layers without photolabile spacer. After capture on the surface, Molt-3 cells were exposed to 365-nm UV light for 10 min. Besides increased exposure time, conditions were identical to those used in a cell release experiment described above. The cells were not released because of the lack of photolabile linker and could be easily assayed for viability on the slide using a LIVE/DEAD® Viability/Cytotoxicity Kit. For this experiment, glass slides were placed into the mixture of 4 mM of calcein AM and 2 mM of ethidium homodimer-1 in PBS for 30 min. The slide was washed gently with PBS and dried. Fluorescence imaging was performed using a confocal microscrope (excitation with Ar laser 488nm and HeNe laser 543 nm).

2. Supplementary Results and Discussion

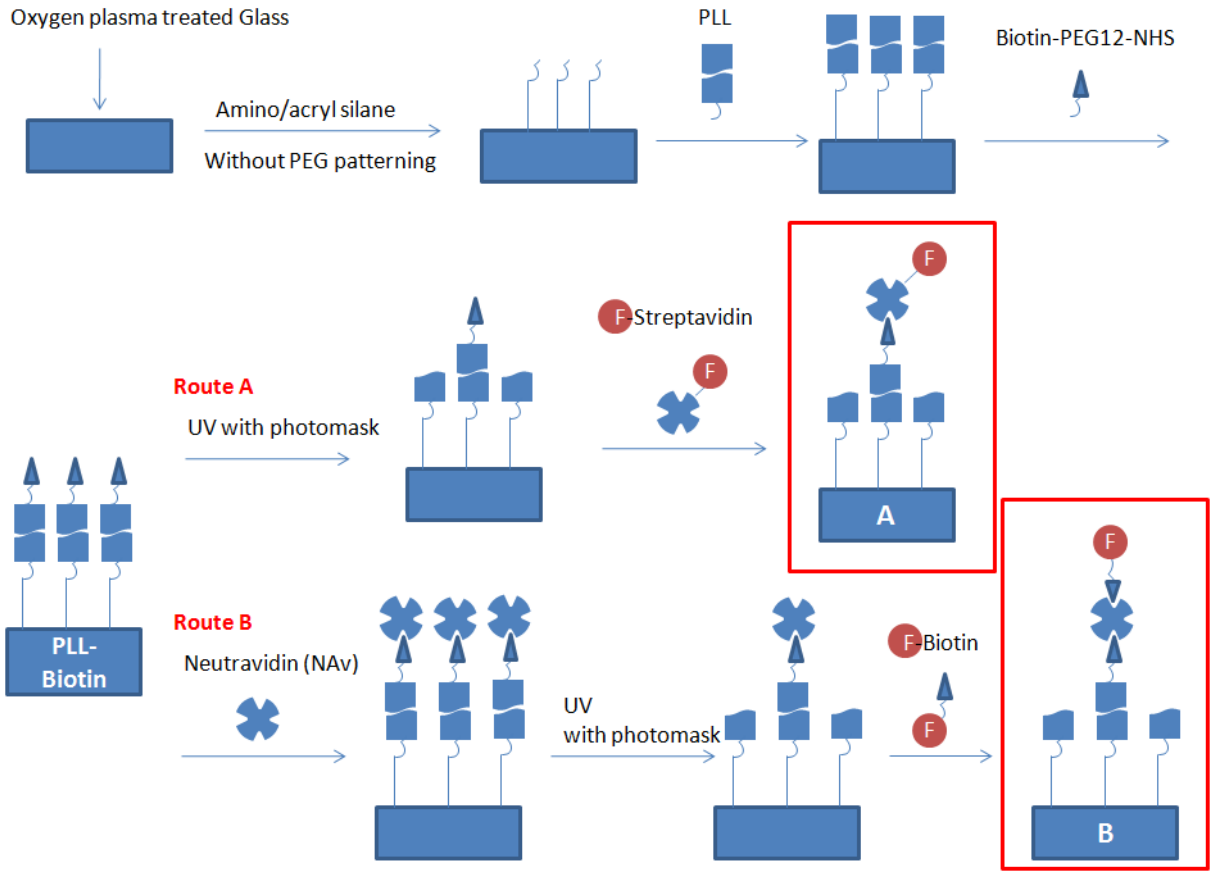
PEG Patterning on Silanized Glass Surfaces

Upon characterizing biomolecule cleavage, we proceeded to fabricate microwells incorporating photolabile surface chemistry. Fig. S1A shows an array of 100-µm diameter PEG hydrogel wells with biotin-containing attachment sites. After incubation of the microwell array with fluorescently-labeled avidin, fluorescence was localized inside the microwells, pointing to the non-fouling properties of PEG hydrogel walls (Fig. S1B). Based on these results, that biotin-Ab conjugates would selectively bind in the glass attachment sites and will enable capture of T-cells within the microwells.

Cell Viability After UV Exposure

One application of photolabile micropatterned surfaces may be in retrieval of cells for re-cultivation. It is therefore important that UV-triggered release does not compromise cell viability. To check for viability after UV exposure, T-cells were immobilized in PEG microwells using the same procedure as shown in Scheme 1 but without photolabile linker. Cells were then exposed to UV for 10 min and stained using live/dead assay. Despite the fact that this exposure was 10 times greater than what is required for cell release, viability was determined to be >95% (Fig. S2). These results demonstrate that UV light used to trigger cell release did not adversely affect viability of cells.

3. Supplementary Scheme and Figures



Scheme S1 Assessment of photocleavage reaction by (A) immobilization of fluorescence conjugated streptavidin immobilization after photocleavage reaction and (B) attachment of fluorescence conjugated biotin after release of pre-immobilized avidin by photocleavage reaction.

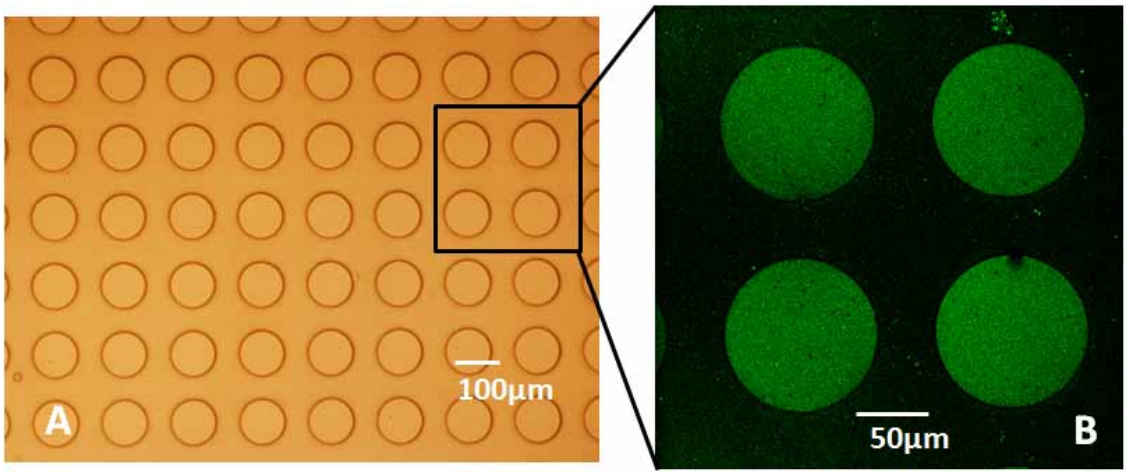


Fig. S1 Fabrication of microwells: A) Image of PEG patterning on amino- and acryl-mixed silane surface; B) immobilization of Alexa Fluor 488 conjugated streptavidin on a biotin-coupled surface with a PEG background.

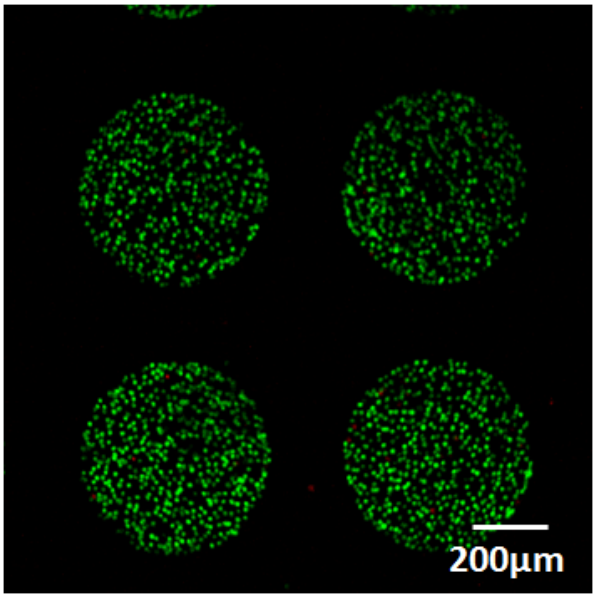


Fig. S2 Cell viability test: Immobilized cells without PLL were stained by using live/dead assay after UV exposure.

4. References

[1] M. C. Howland, A. W. Szmodis, B. Sanii, A. N. Parikh, *Biophys. J.* **2007**, *92*, 1306-1317.

[2] H. Zhu, J. Yan, A. Revzin, *Colloids Surf. B* **2008**, *64*, 260-268.