Supporting Information

Functional Changes during Electron-Beam Lithography of Biotinylated Poly(ethylene glycol) Thin Films

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Supporting Information.

This supporting information provides a more detailed description of the experimental procedures used in our experiments.

It also includes Figure S1 and S2. Figure S1 schematically shows the dose used to pattern the 10 μm-diameter PEG pads in the 5x5 array used here. Figure S2 provides dose-dependent SA-Cy5 and SAMSA-FITC intensity data for the three experiments contributing to the averaged data summarized by figure 4.

Detailed Experimental Methods

Hydroxy-terminated poly (ethylene glycol) [PEG-OH; M_w=6 kDa; Sigma Aldrich], biotin-terminated PEG (PEG-B; M_w=5 kDa; Creative PEGWorks], and alkyne-terminated PEG [PEG-alkyne; Creative PEGWorks; Mw=5 kDa] were used as received. 5-((2-(and-3)-S-(acetylmercapto) succinoyl) amino) fluorescein [SAMSA-FITC], Streptavidin-Cy5 [SA-Cy5], and Azide-Cy5 were purchased from Invitrogen, Thermo Fisher Scientific, and Lumiprobe, respectively. Copper (II) sulfate and sodium ascorbate were purchased from Sigma-Aldrich. THPTA (tris-hydroxypropyltriazolylmethylamine) was purchased from Click Chemistry Tools. Type I deionized (DI) water was provided using a Millipore Direct Q system.

Silicon wafers (5 mm × 5 mm, Pella) were immersed for 12 h in piranha solution (3:1 H_2SO_4/H_2O_2, use with caution), then rinsed multiple times with DI water and dried with gently flowing nitrogen gas. Immediately prior to spin coating, these wafers were exposed to an oxygen plasma for 10 min. Thin polymer films with were prepared by dropping 50 μL PEG solution (1 wt% PEG in tetrahydrofuran [THF]) onto plasma-treated silicon substrates spinning at 3250 rpm.

A Zeiss Auriga field-emission gun (FEG) scanning electron microscope (SEM) with an electrostatic beam-blanking system and a Nanometer Pattern Generation System (NPGS, Nabity) was used for patterning. We patterned circular arrays (10 μm diameter) with an interpixel spacing of 250 nm using a focused electron beam with an incident energy of 2 keV and current of ~200 pA. Exposure doses ranged from 1-500 μC/cm^2. After exposure, insufficiently cross-linked polymer was removed by immersing the substrates in 2−3 mL of DI water for 10 min with gentle rotary shaking (60 rpm). The patterned wafers were then immersed twice into DI water for 5 min with shaking and dried using flowing nitrogen gas.

The SAMSA-FITC solution was activated by dissolving 1 mg SAMSA-FITC in 0.1 mL of 0.1 M NaOH solution and then incubating it at room temperature for 15 min to remove the acetyl protecting group. The solution was neutralized with a mixture of 1.4 μL of 6 M HCl and 20 μL of PBS, which was then diluted using PBS to form a 20 μM SAMSA solution. A drop of 15 μL of SAMSA-FITC solution was placed on each patterned substrate. A second drop of 15 μL of SA-Cy5 (5 μM in PBS) was then added. After 2 h exposure at room temperature the samples were immersed for 5 min in 5 mL PBS with gentle shaking followed by similar washing, twice, in DI water.

The alkyne-azide reaction was operated following published protocols.[1] 10 μL of Azide-Cy5 solution (5 mM in PBS) was blended with a premixed solution of 6.3 μL of $CuSO_4$ (20 mM in DI water) and 12.5 μL of THPTA ligand (50 mM in DI water). 25 μL of sodium ascorbate (100 mM in DI water) was added to this mixture. 30 μL Azide-Cy5 (20 μM in DI water) mixture was placed on a patterned substrate. After 2 h exposure at room temperature the samples were immersed for 5 min in 5 mL of PBS with gentle shaking followed by similar washing, twice, in DI water.

The fluorescence emitted by the various samples was quantified using a Nikon E1000 upright optical microscope with an X-cite 120 LED light source and a sCMOS Camera (pco.panda). Imaging of hydrated specimens was carried out using a Nikon Plan Apo 40X lens and 0.170 mm glass coverslip. A typical exposure time was 300 ms. ImageJ[2] was used to determine the total fluorescent intensity within a given area of a particular microgel pad. From this total, the background intensity was subtracted using the total intensity from an identical area of adjacent unpatterned surface. The intensity per pixel was then determined by dividing the background-subtracted intensity by the number of pixels within the sampling area.

AFM images and thickness data from dry samples were collected using a Bruker Bioscope Resolve microscope in tapping mode (Silicon Nitride Cantilever, spring constant $\approx$ 40 N/m, tip radius < 12 nm, scan rate = 0.5 Hz) and analyzed using NanoScope Analysis V1.90 software.

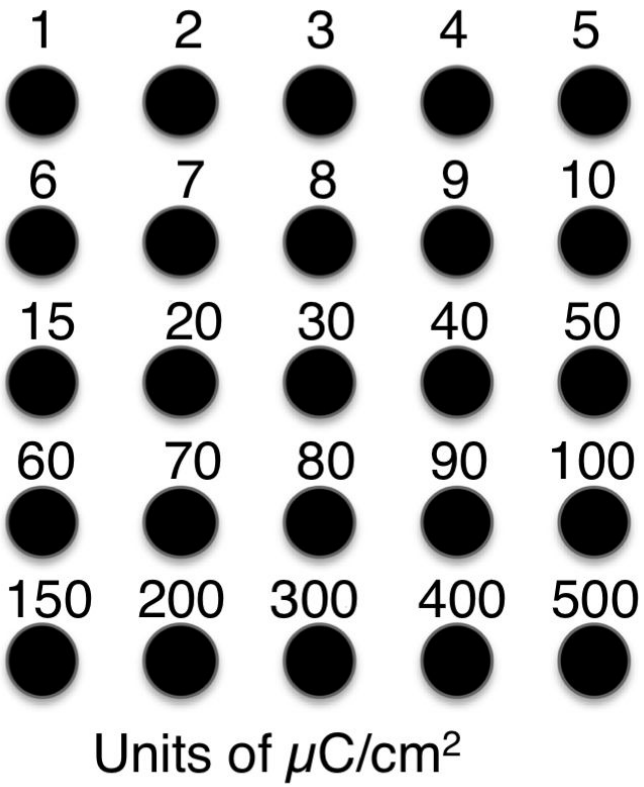

Figure S1. Schematic description of the 5x5 array of microgel pads with the dose indicated for each pad.

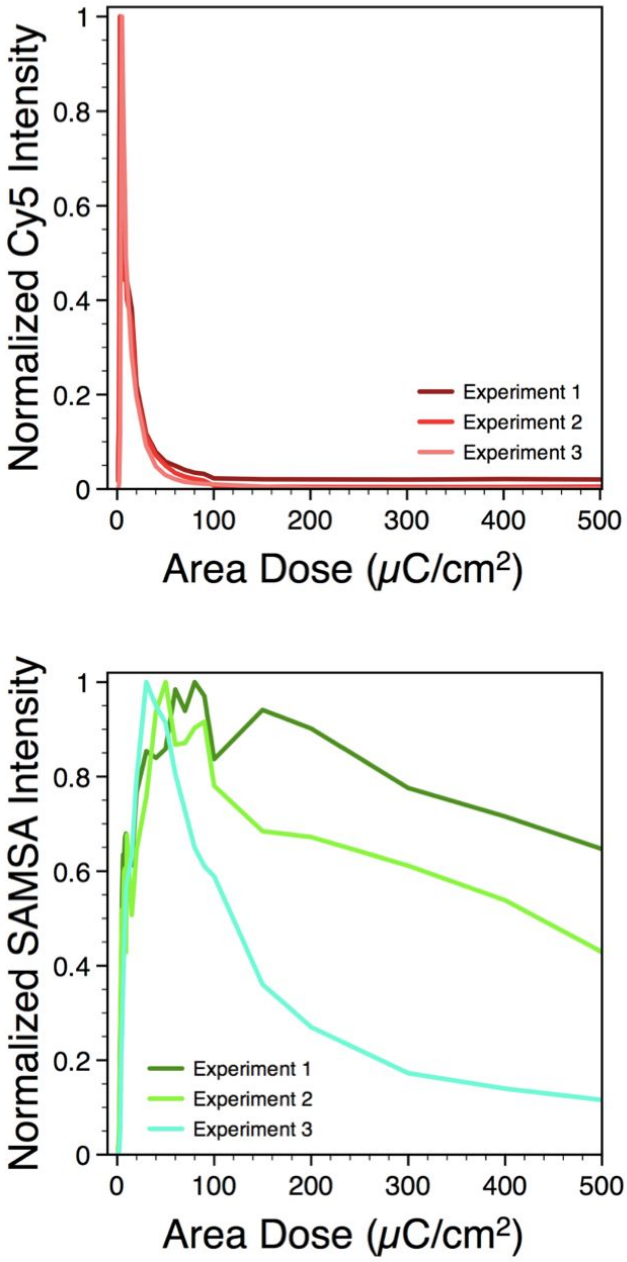

Figure S2. Normalized intensity data where SA-Cy5 (top) and SAMSA-FITC (bottom) signals were collected from each of three different patterning experiments. Note that both the SA reactivity (top) and the SAMSA reactivity (bottom) are reproducible at lower doses (< ~100 μC/cm^2).

References

1. Presolski, S. I.; Hong, V. P.; Finn, M. G., Copper-Catalyzed Azide–Alkyne Click Chemistry for Bioconjugation. *Current Protocols in Chemical Biology* **2011,** *3* (4), 153-162.

2. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W., NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **2012,** *9*, 671.