

SUPPORTING INFORMATION

Direct Printing of Trichlorosilanes for Selective Protein Adsorption and Cell

Growth

Dawn M. Yanker and Joshua A. Maurer*

Department of Chemistry, Washington University, St. Louis, MO, 63130

Email: maurer@wustl.edu

General

All reactions were carried out under an atmosphere of dry argon and commercial solvents and reagents were used without further purification, unless otherwise indicated. Dry tetrahydrofuran (THF) was obtained from J. T. Baker in CYCLE-TAINERS. NMR spectra were collected on a Varian UNITY 300 spectrometer. Photolithography was carried out using a HTG-3HR mask aligner with near UV optics and a CEE 100cb spin/bake system. Fluorescent and phase contrast images were obtained using a Nikon TE2000-PFS microscope running NIS-Elements imaging software and equipped with a Prior XY stage, EXFO X-Cite series 120PC UV illuminator, Photometrics CoolSNAP monochrome camera, and In Vivo Scientific incubation system.

PDMS Stamp Preparation

Master Formation. A virgin silicon wafer (50mm, Montco Silicon) was treated with piranha solution (7:4 concentrated sulfuric acid:30% hydrogen peroxide) for 2h, rinsed thoroughly with deionized water, and dried with nitrogen. SU-8 2015

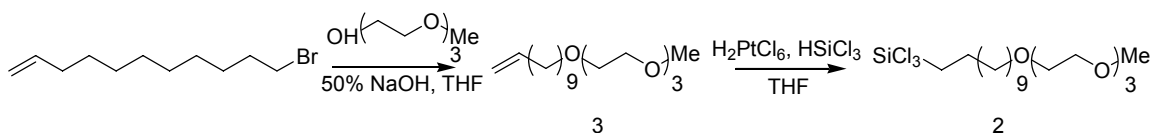
(1-2 mL, MicroChem) was applied to the wafer and an even coating of resist (nominally 20 μ m) was achieved using the six cycle spin-coater program shown in Table S1. Edge bead remover (7:3 THF:propylene glycol monomethyl ether acetate) was applied during the third and fourth cycles. The wafer was soft baked at 95°C for 5m. Patterning was achieved by exposure on a mask aligner in hard contact mode for 180.0s using a soda lime/chrome photomask (PhotoPlot Store). The wafer was post exposure baked for 6m at 95°C and developed in propylene glycol monomethyl ether acetate (Sigma-Aldrich) for 20s. The resulting master was hard baked at 180°C for 3h prior to stamp formation.

Cycle	Speed (RPM)	Ramp (RPM/s)	Time (s)
1	500	100	10
2	1000	300	30
3	800	300	3
4	1500	5000	5
5	2500	1000	10
6	0	1000	1

Table S1. Spin-coater parameters for SU-8 photoresist.

PDMS Stamp Formation. Sylgard 182 (Dow Corning) was mixed 10:1 (resin:hardener) and poured over the patterned silicon master. The polymer was degassed using a vacuum dessicator and cured at 70°C for 2h. The final stamp was separated from the master and cut to size.

Synthesis of Oligo-PEG Terminated Trichloroalkanesilane



11-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)undec-1-ene 3: A solution of 11-Bromo-1-undecene (1.90mL, 8.576mmol, Sigma-Aldrich), triethylene glycol monomethyl ether (1.60mL, 10.29mmol, 1.2eq, Fluka), 50% sodium hydroxide (1.30mL, 17.15mmol, 2eq, Sigma-Aldrich) in THF (25 mL) was heated to reflux overnight. The reaction was diluted with deionized water (5mL), extracted with hexanes (2 x 20mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude compound was subjected to flash column chromatography (3:1 hexanes:ethyl acetate) to give pure **3** (0.9033g, 49.55%) as a colorless oil. ^1H NMR (CDCl_3) δ 1.25 (m, 10), 1.40 (m, 2), 1.60 (m, 2), 2.08 (m, 2), 3.40 (s, 3), 3.44 (t, 2), 3.65 (m, 12), 4.95 (d, 1), 5.00 (d, 1), 5.85 (m, 1).

11-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)undecyl)trichlorosilane 2: To **3** (0.1393g, 0.316mmol) and chloroplatinic acid hexahydrate (0.0138g, 0.0316mmol, 0.10eq, Sigma-Aldrich) was trichlorosilane (0.09mL, 0.632mmol, 2eq, Sigma-Aldrich) in THF (10mL). The reaction was allowed to proceed at room temperature until turning clear. The solvent was removed *in vacuo* and the crude product purified by Kugelrohr distillation (Buchi GKR-50) to give **2** (0.0307g, 38.61%) as a colorless oil. ^1H NMR (CDCl_3) δ 1.25 (m, 12), 1.40 (m, 2), 1.65 (m, 2), 1.90 (m, 2), 2.00 (m, 2), 3.40 (s, 3), 3.44 (t, 2), 3.65 (m, 12).

Patterning Adhesive and Non-Adhesive Protein Regions on Glass

The stamping method described is given as an example; variations in the procedure are described below. Glass coverslips (25mm, VWR) were cleaned by soaking in piranha solution for 2h. Coverslips were then twice rinsed with deionized water, dried under nitrogen, rinsed with ethanol, and dried under nitrogen. The stamp was coated with octadecyltrichlorosilane (**1**) (10 mM in toluene) by dropping the solution onto the stamp (5-6 drops) and drying with nitrogen. Slides were then stamped for 10s. The bare regions of the glass were then allowed to react with a 1 mM or 5mM **2** in toluene under argon for 0.5h. After soaking, coverslips were twice rinsed with ethanol and dried under nitrogen.

Both stamping parameters and the reaction of **2** with the glass surface were explored in this study. Incubation in 1 mM **2**, following stamping as described above, was carried out for varying lengths of time: 5m, 15m, 30m, 45m, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, 6h, 8h, and 10h. The concentration of **1** used for stamping was varied: 1mM, 10mM, and 100mM. A variety of stamping times were explored: 5s, 10s, 15s, 30s, 45s, and 3m (these were incubated in 5 mM **2**).

Pattern Visualization

A patterned coverslip was placed in Attofluor cell chamber (Invitrogen) and rinsed three times with Dulbecco's phosphate buffered saline (DPBS) (Invitrogen). The coverslip was then incubated with 5 μ L/mL Anti-Guinea Pig IgG (whole molecule)

FITC conjugate (Sigma) in DPBS at 37°C for 1h. Excess protein was removed by rinsing with DPBS (3x) and the coverslip was covered with fresh PBS. Protein fluorescence was visualized using a Nikon B-2E/C filter cube.

Patterned Cell Growth

Preparation of Fluorescently Labeled Fibronectin. To 20 μ L Human Plasma Fibronectin (1mg/mL in 100mM CAPS, 0.15M NaCl, 1mM calcium chloride, pH 11.5, Invitrogen) was added 1 μ L of 1M sodium bicarbonate in sterile water and 2 μ L Alexa Fluor 350 carboxylic acid, succinimidyl ester (5 mg/mL in DMF, Invitrogen) or 2 μ L of Alexa Fluor 647 carboxylic acid, succinimidyl ester (5 mg/mL in DMF, Invitrogen). The reaction was mixed and allowed to proceed at room temperature for 1h. The reaction was stopped by addition of 3 μ L 1.5M hydroxylamine in 1N sodium hydroxide and mixed with 20 μ L of unlabeled fibronectin.

Cell Culture. A patterned coverslip (stamped with 10 mM **1** and incubated in 5 mM **2** for 30 min) in either an Attofluor cell chamber or a small tissue culture dish was coated with fibronectin at 20 μ g/mL as previously described for Anti-Guinea Pig IgG FITC conjugate. CHO-K1 cells (ATCC) were separated using TrypLE Express (Invitrogen), resuspend in Dulbecco's Modified Eagle Medium (DMEM, low glucose 1X, glutamax, 1g/L D-glucose, 110mg/L sodium pyruvate, 50mL FBS, 5mL pen/strep, Invitrogen), and counted using a hemacytometer (Bright-Line, Hausser Scientific). After rinsing the patterned coverslip with DPBS,

approximately 90,000 cells were applied in 1 mL of DMEM. Plated cells were grown at 37°C, 5% CO₂, and 96% RH). Cultures were visualized by inverted microscopy in an Attotfluor cell chamber using phase contrast optics and either a Nikon UV-2E/C filter cube or a Semrock CY5-4040A filter cube.