Electronic Supplementary Information

Light-Responsive Polymer Brushes: Active Topographic Cues For Cell Culture Applications

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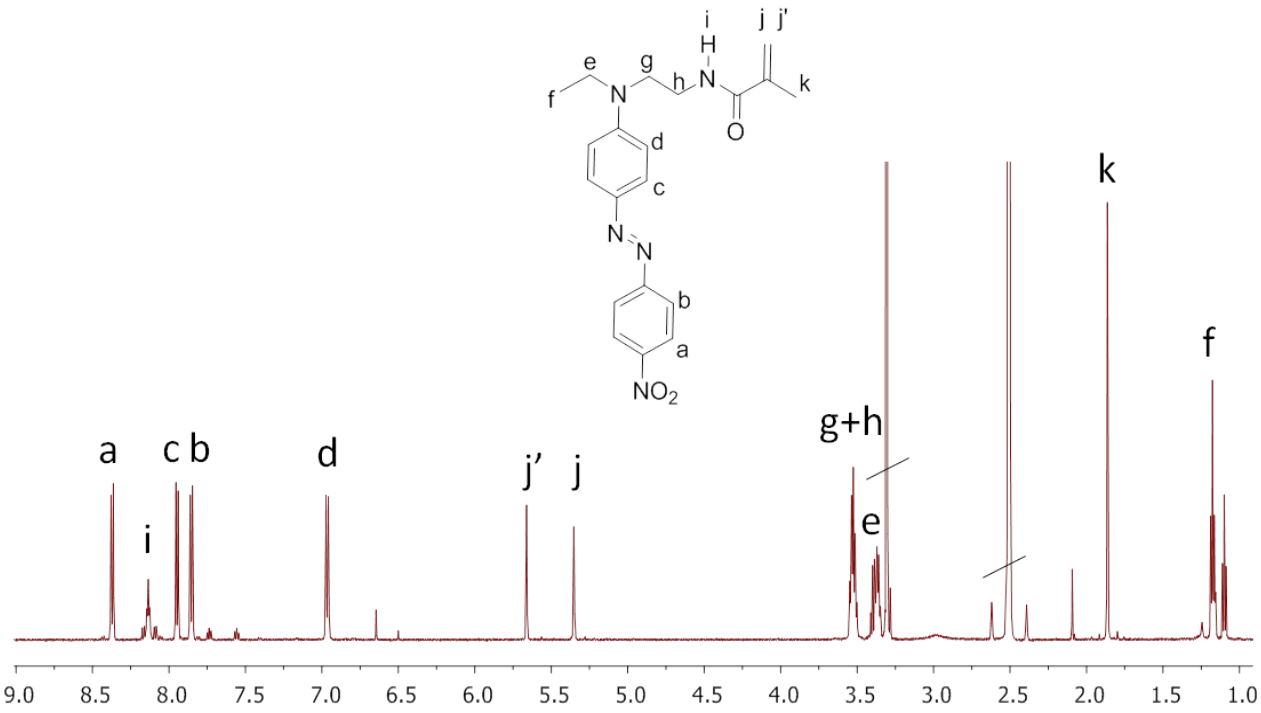


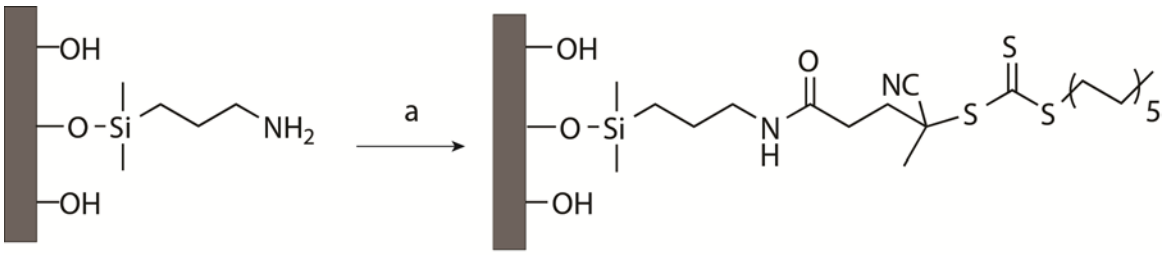
Figure S1. ^{1}H-NMR of DR1-monomer in DMSO-d_6 (600 MHz): δ ppm 8.37 (d, 2H, a); 8.14 (t, 1H, i); 7.94 (d, 2H, c); 7.85 (d, 2H); 6.97 (d, 2H, d); 5.66(s, 1H, j'); 5.35(s, 1H, j);3.55-3.50 (m, 4H, g+h), 3.39-3.35 (m, 2H, e); 1.86 (s, 3H, k); 1.18 (t,3H, f).

Silanisation and RAFT immobilisation on glass.

Synthesis of sample 1. A cleaned glass coverslip (22 x 22 mm) was activated by oxygen plasma irradiation (intensity 400 W, oxygen pressure 0.1 mmHg) for 3 minutes in a plasma dry cleaner. Immediately after plasma oxidation, a (3-aminopropyl)triethoxysilane (APTES) solution (0.213 mmol of APTES in 10 ml of absolute ethanol and 300 µl of 10% vol. aqueous solution of acetic acid) was added on the glass coverslip for 5 minutes at room temperature. Glass substrate was washed with ethyl alcohol and dried in a vacuum oven for 2 hours at room temperature.

Synthesis of sample 2. 50 ml corning flask was washed and dried. All the chemicals, syringes and corning flask were placed inside a glove box for 30 minutes with nitrogen flow. Starting from the hypothesis that silanisation reaction goes to 50% completion and that we are interested in the functionalisation of only one face of the glass slide, 0.027 mmol (10.78 mg, 0.5 equiv. with respect to free amines of the grafted APTES) of 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (RAFT agent), 0.049 mmol (7 µl) of 1,3-diisopropylcarbodiimide (DIC) and 0.048 mmol (6.55 mg) of 1-hydroxybenzotriazole hydrate (HOBt) were added to the corning flask and stirred for 1 hour at room temperature. Silanised glass

substrate (sample 1) was set in the corning flask containing the reagent solution and reaction was conducted at room temperature on an oscillating plate. After 24 hours the reaction was stopped and the unimmobilised RAFT agent as well as excess of all other reagents were washed with *N*,*N*-dimethylformamide (DMF). The washing process was repeated three times with DMF and then a fourth wash was given with acetone, finally the sample was dried for 2 hours at room temperature. The entire process was performed inside the glove box. Also for this reaction we hypothesize 50% of conversion.



1 2

Scheme S1. Synthesis of sample 2. Reaction and conditions: (a) 0.027 mmol (10.78 mg, 0.5 equiv. with respect to free amines of the grafted APTES) of 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (RAFT agent), 0.049 mmol (7 µl) of 1,3-diisopropylcarbodiimide (DIC) and 0.048 mmol (6.55 mg) of 1-hydroxybenzotriazole hydrate (HOBt) for 24 hours. The entire process was performed inside the glove box.

X-Ray Photoelectron Spectroscopy (XPS). XPS data were recorded on a VersaProbe I (PHI) System and high-resolution spectra were acquired applying a pass energy of 69 eV. The PHI MultiPak software was used for spectra analysis. All the deconvolution procedures have been performed after having shifted the C_{1s} peak at 284.5 eV (in order to compensate for surface charging phenomena). Only organic compounds were taken into account for further examination in high resolution. Survey scan and high-resolution XPS elemental scans of C_{1s}, O_{1s}, Si_{2p} and N_{1s} are both reported for bare glass, immobilised RAFT agent and azo-pb for validation of each synthetic step (Figure S2-S4).

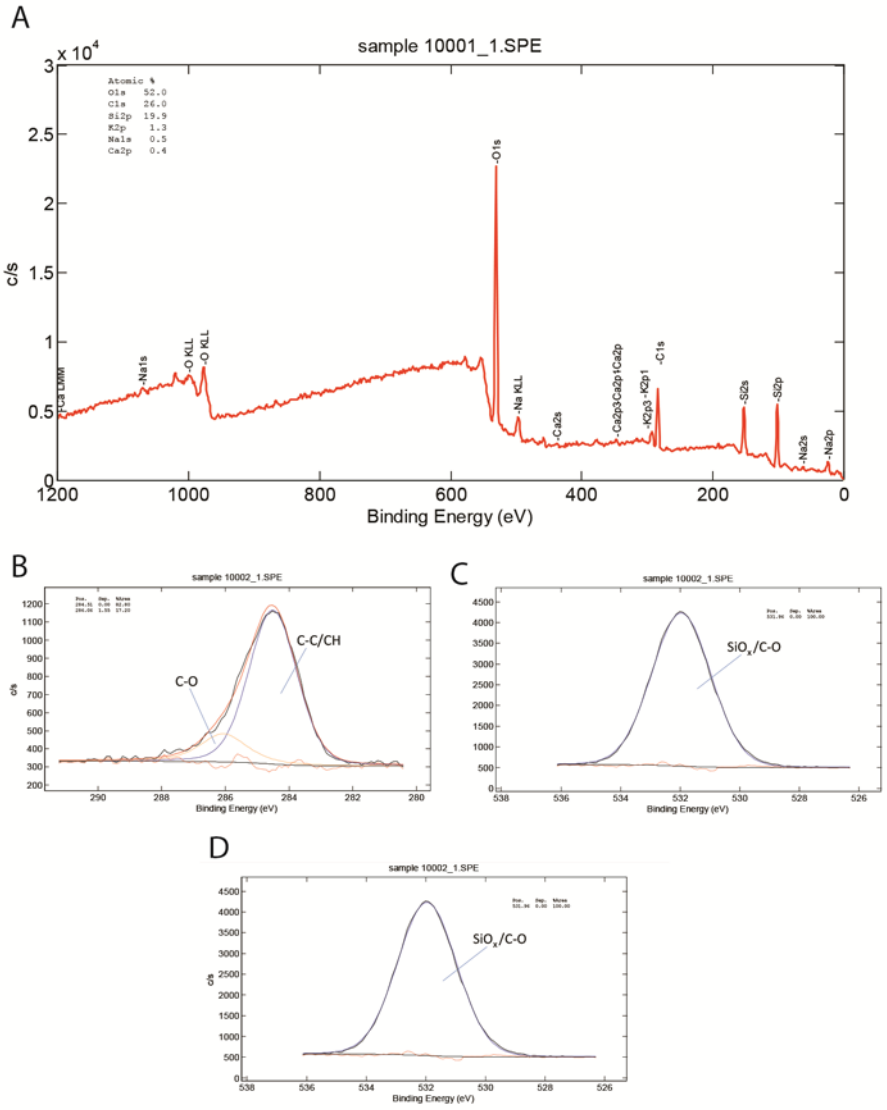


Figure S2. XPS survey scan of bare glass and relative high resolution scans of C_{1s}, O_{1s}, and Si_{2p}.

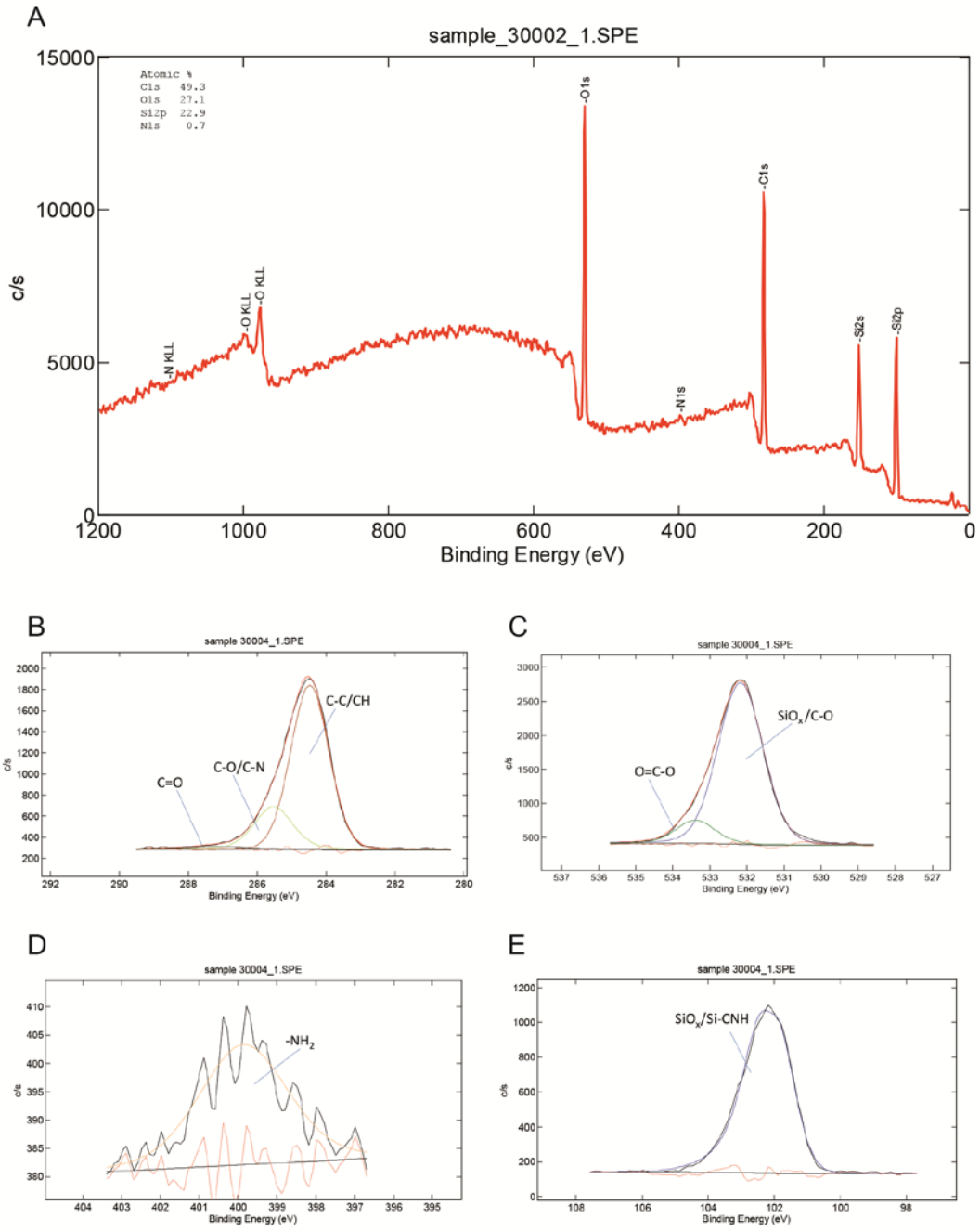


Figure S3. XPS survey scan of RAFT agent immobilised on silanised glass and relative high resolution scans of C_{1s}, O_{1s}, N_{1s} and Si_{2p}.

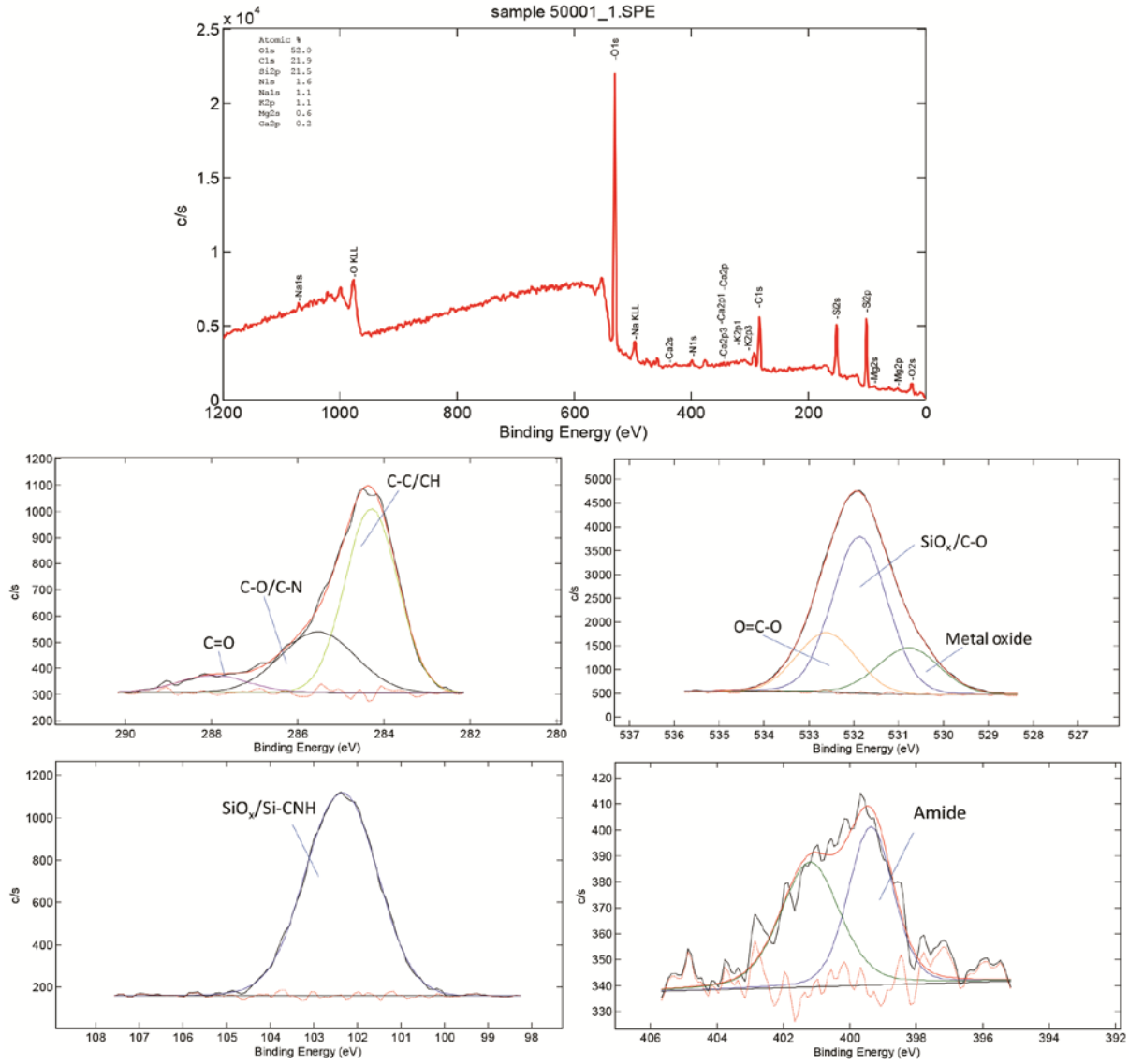


Figure S4. XPS survey scan of azo-pb and high resolution scans of C_{1s}, O_{1s}, Si_{2p} and N_{1s}.

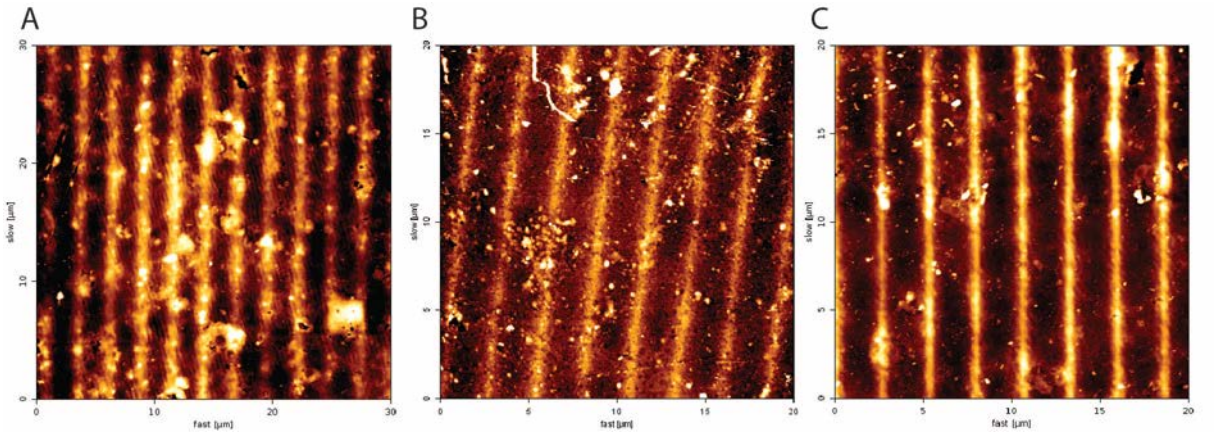


Figure S5. AFM micrographs showing the pattern obtained with different polarisation of laser beam of 1 h. (A) Vertical polarisation pattern. (B) Horizontal polarisation pattern. (C) 45° polarisation pattern.

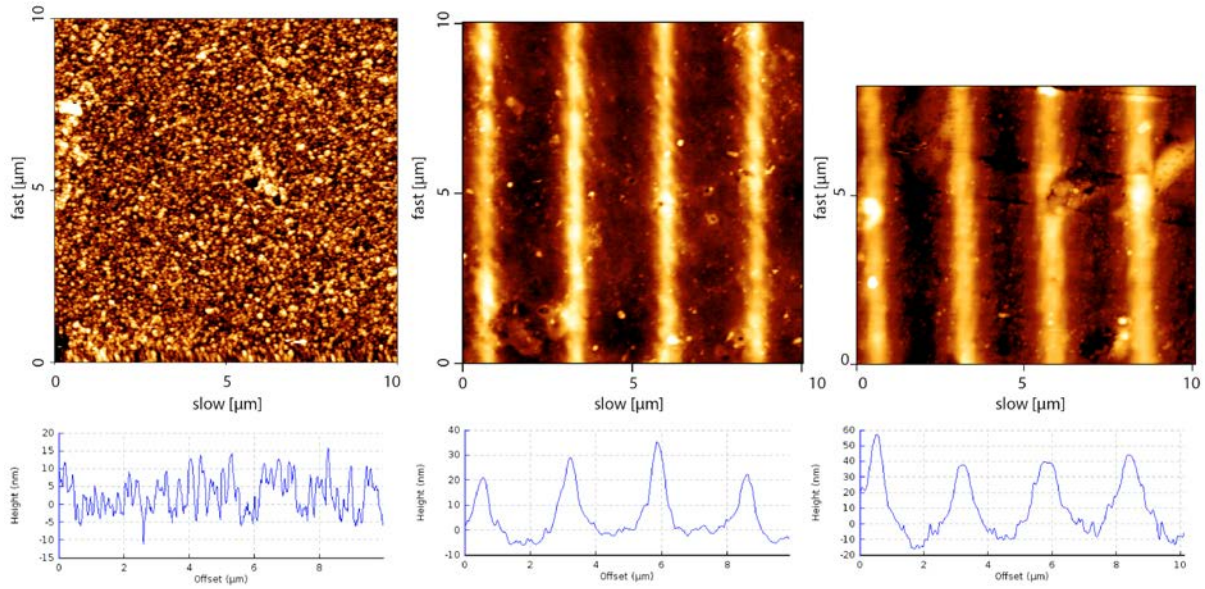


Figure S6. AFM images of azopolymer brushes. (A) Azopolymer brushes before illumination (B) 1 hour illumination by Lloyd's mirror, 30 nm pattern height (45° polarisation) and (c) 2 hours illumination by Lloyd's mirror, 60 nm pattern height (45° polarisation).

	Average Roughness
Linear Pol Θ=45° 1 h (Figure S4B)	8.369 nm
Linear Pol Θ=45° 2 h (Figure S4C)	13.40 nm
Linear s-polarisation (Figure S3A)	8.022 nm
Linear p-olarisation (Figure S3B)	5.073 nm
Non illuminated sample (Figure S4A)	3.90 nm

Table S1. Average roughness measured by AFM in differently illuminated azo-pb. Scanning area was 10 µm x 10 µm for roughness measurement.

UV/Vis Spectrophotometry. UV/Vis absorption was measured on polymer brushes with a UV CARY 100 scan spectra photometer (VARIAN, Australia). Polymer brush sample was mounted with the help of a custom-made 3 cm-long paper holder. Data intervals of 1 nm and a scan of 600 nm/min were used. The base line was corrected by using bare glass. Data were recorded from 200 to 800 nm (glass absorption was until 350 nm). The maximum UV absorption peak for the polymer brushes was observed at 482 nm. After Lloyd's mirror illumination for 3 hours a broadening in the absorption peak was observed.

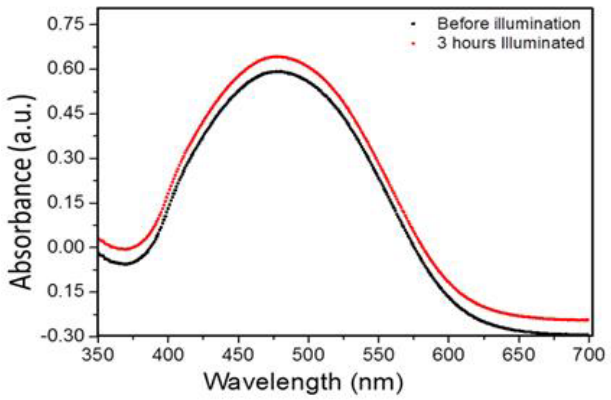


Figure S7. UV/Vis spectra of azo-pb (black line) before and (red line) after Lloyd's mirror illumination (3 hrs).

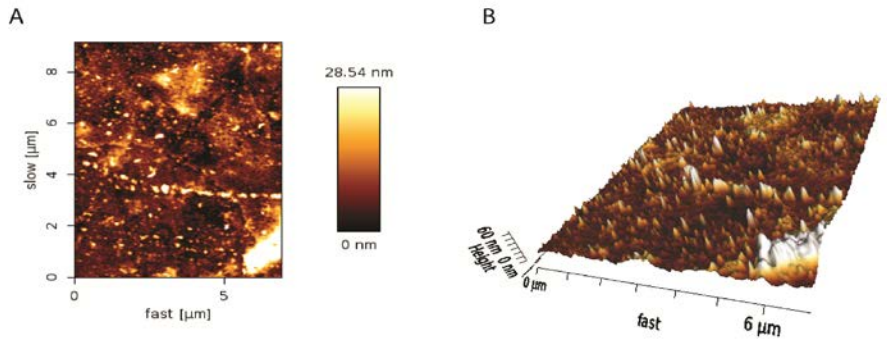


Figure S8. A) AFM image of azo-pb after rigeneration in DMF and B) its 3D projection.

NIH-3T3 fibroblasts on patterned azo-pb. NIH-3T3 fibroblasts (ATCC CRL-1658) were cultured in high-glucose DMEM and incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Prior to cell seeding, azo-pb substrates were sterilized under UV light for 30 min. After 24 hours, cells were observed with an Olympus CKX41 microscope and images were taken with a Manta GigE Vision camera (Allied Vision Technologies).

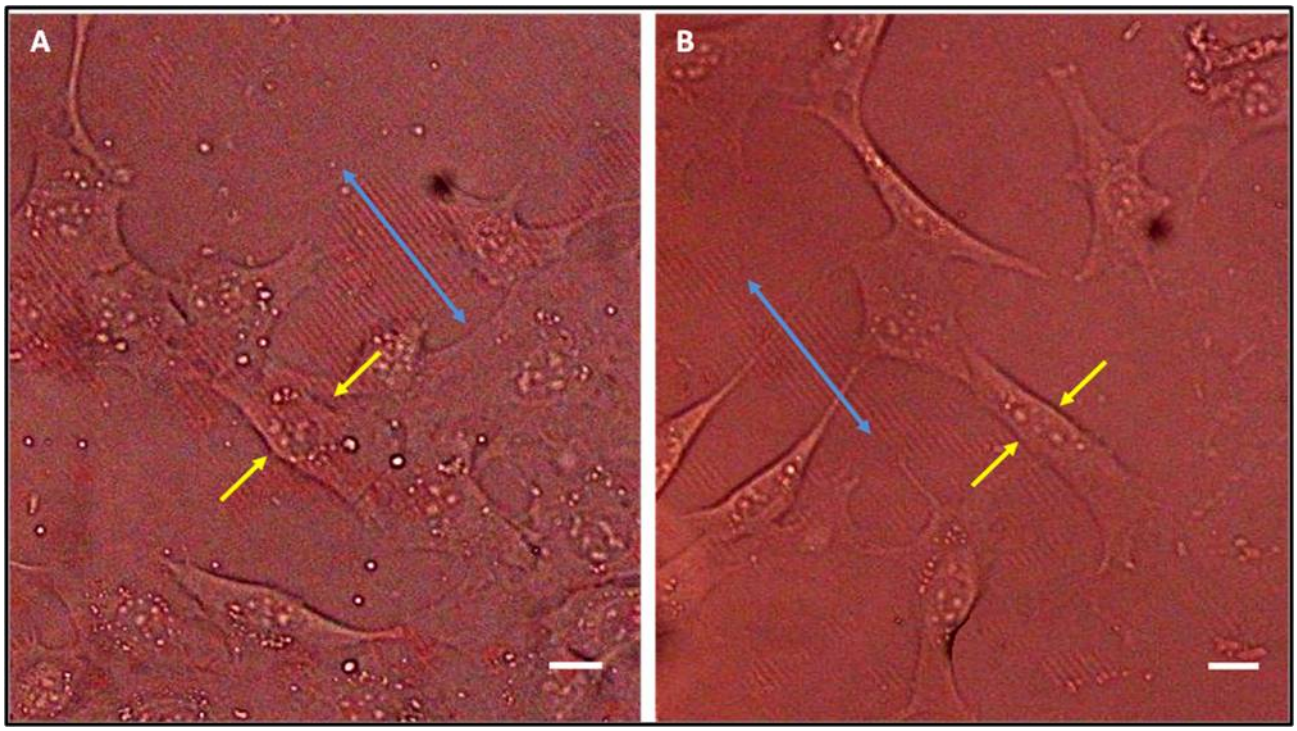


Figure S9. (A) and (B) represent phase contrast images of NIH-3T3 cells on patterned azo-pb after 24 hours. Light blue arrows represent pattern direction and yellow arrows identify cells at the nuclear regions. Scale bars are 10 µm.

Ultrasonic cavitation in presence of HUVECs. Cells were stained with vital CellTracker™ CM-Dil fluorescent dye before seeding. A staining solution in PBS was made to a final concentration of 2 µg/ml diluting a 2 mg/ml stock solution of dye in DMSO. Cells were covered with 2 ml of the staining solution and incubated first at 37 °C for 5 minutes and then at 4 °C for 15 minutes. After

that, cells were washed twice with PBS and finally fresh cell culture medium was added. For imaging during ultrasonication experiment medium was supplemented with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer before the experiment, while ultrasonic cleaner bath was set at 37 °C, in order to reproduce cell culture conditions. Adherent cells were sonicated for 10 minutes at 59 kHz, observed with a TCS SP5 confocal microscope (Leica Microsystems) or with a Axio Observer Z1 (Carl Zeiss) equipped with a thermo-chamber after 1, 5 and 10 minutes and incubated overnight at 37 °C in a humidified atmosphere with 5% CO_2 before final observation.

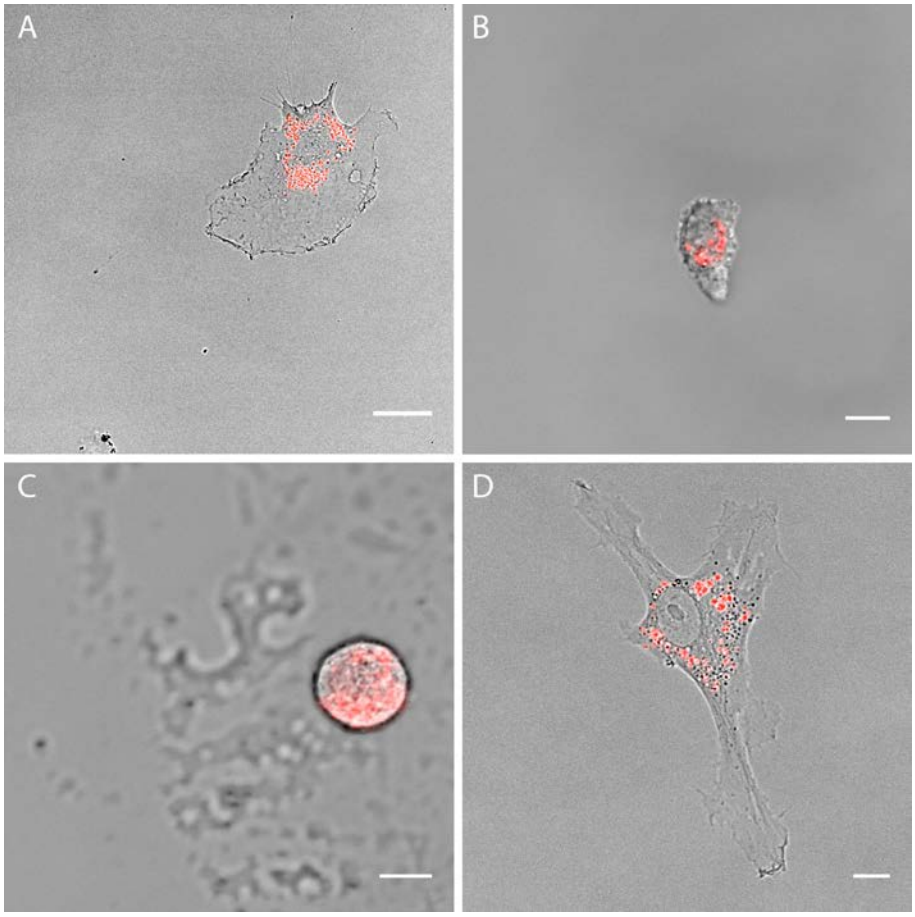


Figure S10. Ultrasonication of non patterned polymer brushes in presence of cells. (A) image of adherent HUVECs on polymer brushes, (B) image of a single cell after 1 minute and (C) after 10 minutes of sonication at 59 kHz in a sonicating bath set at 37 °C. (D) Image of a sonicated cell after an overnight incubation. Cells were stained with vital CellTracker™ CM-DiI.

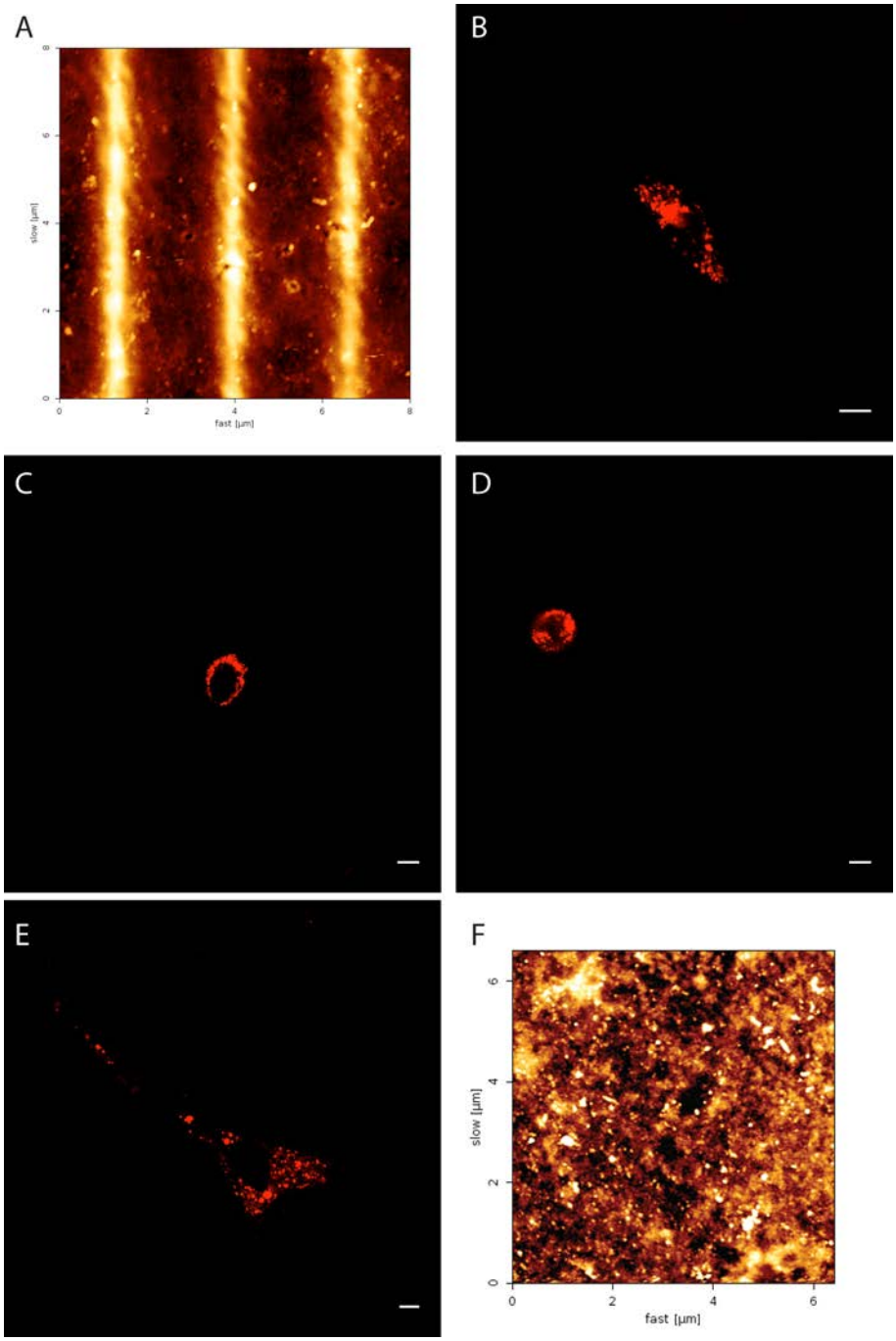


Figure S11. (A) AFM micrograph of the patterned area of azo-pb before treatment and cell seeding. Confocal images of HUVEC on azo-pb (B) before ultrasonication treatment, (C) after 1 minute and (D) after 10 minutes of ultrasonication at 59 kHz in a bath set at 37 °C. (E) Cell imaged after an overnight incubation. Scale bars are 10 µm. Images were acquired with a TCS SP5 confocal microscope (Leica Microsystems). (F) AFM micrograph of the patterned area after ultrasonication treatment and cell removal.

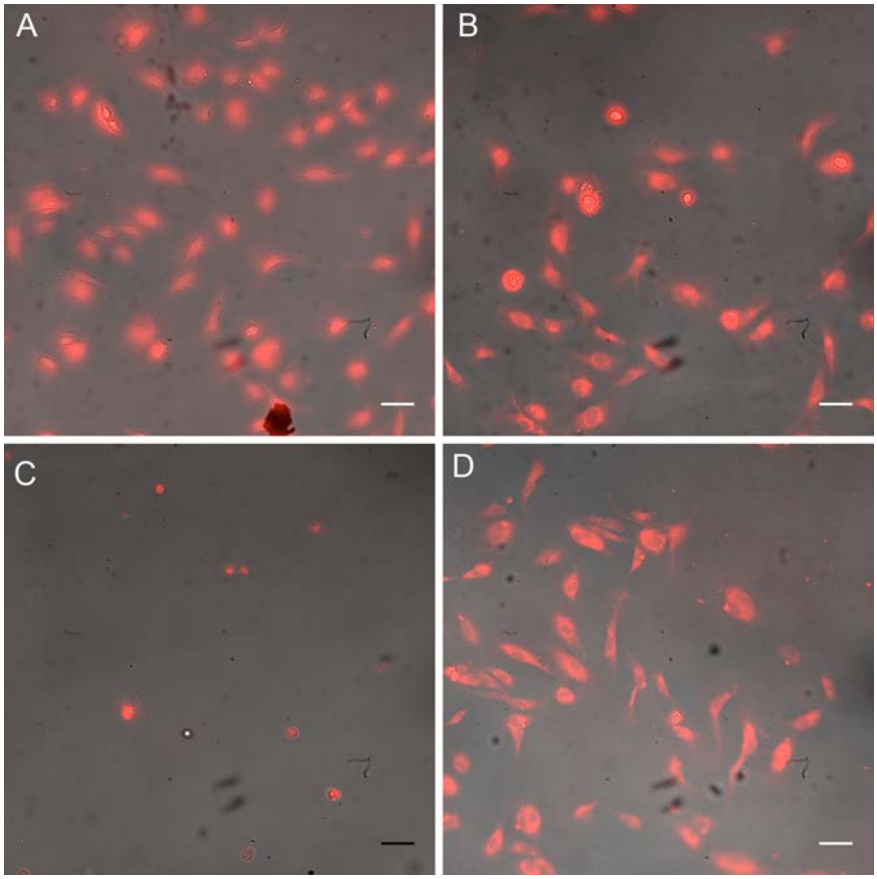


Figure S12. Image of HUVECs on a glass petri dish (A) before ultrasonication treatment, (B) after 1 minute and (C) after 10 minutes of ultrasonication at 59 kHz in a bath set at 37 °C. (D) Cells imaged after an overnight incubation. Scale bars are 50 µm. Images were acquired with an Axio Observer Z1 (Carl Zeiss).