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Supporting Information

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Controlled Cell Adhesion on PEG-based Switchable Surfaces

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A. Polymer synthesis and surface modifications:

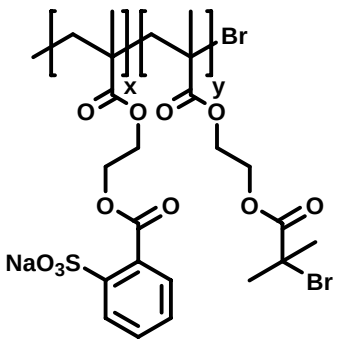
Chemicals. 2-(2-Methoxyethoxy)ethyl methacrylate (Aldrich, 95%), oligo(ethylene glycol) methyl ether methacrylate (Aldrich, M_n = 475 g·mol^{-1}), 2-hydroxyethyl methacrylate (Aldrich, 98%), copper(II) bromide (Aldrich, 99%), L-ascorbic acid (Aldrich, 99%), 2,2' bipyridyl (Fluka, 98%), sodium 3-mercapto-1-propanesulfonate (Aldrich 90%), phosphate buffered saline (PBS, BioChemika tablets, Fluka), poly(styrene sulfonate) (Aldrich, M_w = 70000), and poly(allylamine hydrochloride) (Aldrich, M_w = 70000) were used as received. The disulfide ATRP initiator **1** was synthesized according to published procedures.[1] Copper(I) bromide (Acros, 95%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol and dried. Prior to use, all surfaces were cleaned by immersion in a solution of 0.5 g of potassium permanganate in 100 ml of concentrated H_2SO_4 for 1 h.

Atom Transfer Radical Copolymerization of MEO_2MA and OEGMA in solution.[2] Copper bromide (13 mg, 0.09 mmol), 2,2' bipyridyl (28,3 mg, 0.18 mmol), 2-(2-methoxyethoxy)ethyl methacrylate (1.53 g, 8.1 mmol), oligo(ethylene glycol) methyl ether methacrylate (431 mg, 0.9 mmol), disulfide initiator **1** (20.4 mg, 0.045 mmol) and 2.4 ml of ethanol were added to a Schlenk tube sealed with a septum. The tube was purged with dry argon for 15 minutes. Then, the mixture was heated to 60 °C in an oil bath for 3 h. After this time, the reaction was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted in ethanol and passed through a short silica column (60-200 mesh) in order to remove the copper catalyst. Then, ethanol was removed on the rotavap, the filtered solution was diluted with deionized water and subsequently purified by dialysis in water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000-6000). Yield: 1.55 g.

Preparation of P(MEO_2MA-*co*-OEGMA)-modified gold surfaces via a grafting-onto strategy. The adsorption of disulfide-functionalized P(OEGMA-*co*-MEO_2MA) **2** on gold surfaces was adapted from our previous reports.[3] The 20 x 20 mm glass cover slips (Menzel, Germany) were coated with 2 nm chromium and 47 nm gold by an Auto 500 Electron Beam Evaporation System (Edwards, UK). P(OEGMA-*co*-MEO_2MA) **2** was dissolved in ethanol and adsorbed on the freshly prepared gold surfaces for 3 hours at room temperature. Unbound macromolecules were carefully removed by washing of the surfaces with ethanol for 1 hour.

Adsorption of the disulfide ATRP initiator 1 on gold surfaces. The 20 x 20 mm glass cover slips (Menzel, Germany) were coated with 2 nm chromium and 47 nm gold by an Auto 500 Electron Beam Evaporation System (Edwards, UK) and were subsequently immersed in a 1 $mmol \cdot ml^{-1}$ solution of disulfide initiator **1** for 1 h. After this, the surfaces were washed with ethanol, two times H_2O and again ethanol, before being immersed into the solution for surface-initiated ATRP.

Synthesis of the negatively charged ATRP macroinitiator 3. Macroinitiator **3** was synthesized in three steps. Firstly, a well-defined poly(2-hydroxyethyl methacrylate) precursor was synthesized by atom transfer radical polymerization in solution as previously described.[4] The precursor was subsequently transformed into the statistical copolymer **3** via sequential esterification of the hydroxy-groups analogous to a procedure described in the literature.[5] The formed polymer was purified by dialysis in water (Roth, ZelluTrans membrane, molecular weight cut-off: 4000-6000) and appeared as a yellowish solid after freeze-drying. The ratio x:y was determined to be 50:50 within the limits of accuracy by ^{1}H-NMR.



Functionalization of the gold surfaces via layer by layer (LbL) deposition.[6] Initial functionalization was achieved by deposition of sodium 3-mercapto-1-propanesulfonate on Au. As a first polyelectrolyte layer, poly(allylamine hydrochloride) was deposited on this negatively charged surface from a solution with a concentration of 1 mg/ml (pH 1). Then, three deposition cycles were performed, by sequentially exposing the surfaces to solutions of poly(styrene sulfonate) (1 mg/ml, pH 1) and poly(allylamine hydrochloride) for 20 minutes. The macroinitiator **3** was deposited as the last step from a 1 mg/ml aqueous solution (pH 1). After each deposition step, the surfaces were rinsed with ultra-pure water with a flow rate of 20 $\mu l \cdot min^{-1}$, until the signal was stable.

Surface-initiated ATRP of MEO_2MA and OEGMA. In a round flask, 8.2 mg of $CuBr_2$ (0.037 mmol), 53 mg of CuBr (0.37 mmol), 145.5 mg of 2,2'-Bipyridyl (0.93 mmol), 615 mg of OEGMA 475 (1.29 mmol) and 3234 mg of MEO_2MA (17.2 mmol) were dissolved in 5 ml of Ethanol and 5 ml of H_2O. Then, a functionalized gold substrate (coated with either low molecular weight initiator **1** or macroinitiator **3**) was immersed in this mixture. The flask was sealed with a septum and degassed by bubbling argon through the solution for ca. 10 minutes. After this, the surface-initiated polymerisation was performed at ambient temperature for 30 minutes. The reaction was stopped by opening the flask, removing the substrate from the mixture, and rinsing it with ethanol and three times with ultra-pure H_2O.

Surface-initiated AGET ATRP of MEO_2MA and OEGMA. In a round flask, 123 mg of $CuBr_2$ (0.55 mmol), 145.5 mg of 2,2'-Bipyridyl (0.93 mmol), 615 mg of OEGMA 475 (1.29 mmol) and 3234 mg of MEO_2MA (17.2 mmol) were dissolved in 5 ml of Ethanol and 4 ml of H_2O. Then, a functionalized gold substrate (coated with either low molecular weight initiator **1** or macroinitiator **3**) was immersed in this mixture. The flask was sealed with a septum and degassed by bubbling argon through the solution for ca. 10 minutes. Then, a degassed solution of 65.2 mg of ascorbic acid (0.37 mmol) in 1 ml of H_2O was added with a syringe, the flask was shaken to mix and the argon bubbling was continued for several minutes. After the addition of ascorbic acid, the surface-initiated polymerisation was performed at ambient temperature for 30 minutes. The reaction was stopped by opening the flask, removing the substrate from the mixture, and rinsing it with ethanol and three times with ultra-pure H_2O.

B- Measurements and analysis:

Size Exclusion Chromatography (SEC). Molecular weights and molecular weight distributions were determined by SEC performed at 25°C in tetrahydrofuran (THF) as eluent, using three 5 μ-MZ-SDV columns with pore sizes of 10^3, 10^5, and 10^6 Å (flow rate 1 $mL \cdot min^{-1}$). The detection was performed with a RI- (Shodex RI-71) and a UV-Detector (TSP UV 1000; 260 nm). For calibration, linear polystyrene standards (PSS, Germany) were used.

Cloud point measurements. The cloud points of the polymer solutions in water were measured on a Tepper TP1 photometer (Mainz, Germany). Transmittance of polymer solutions in pure deionized water or buffer solutions was monitored at 670 nm as a function of temperature (cell path length: 12 mm; one heating/cooling cycle at rate of $1°C \cdot min^{-1}$).

Nuclear Magnetic Resonance (NMR). ^{1}H NMR Spectra were recorded at room temperature in $CDCl_3$ on a Bruker DPX-400 operating at 400.1 MHz.

Contact angle. The dynamic contact angles of uncoated, cleaned gold and polymer coated gold were measured at different temperatures ranging from 25 °C to 45 °C with a contact angle measuring system G10 (Krüss Surface Science, Germany) including a microscopy heating plate Linkam MS100 (Linkam Scientific Instruments, UK). Double deionized water was gently placed on the sample surfaces using a syringe. Each sample was measured four times and the results were averaged. The standard error of mean was calculated for each sample.

Surface Plasmon Resonance (SPR). Measurements were performed on a Reichert SR 7000 DC, using a flow of 20 µl·min^{-1} for the rinsing steps. Ultra-pure H_2O was used as rinsing medium. The surface functionalization steps were performed by injecting the active species, stopping the flow to ensure exposure of the surface to the solution for a defined time, and switching back to a flow of H_2O after this time.

Elipsometry. Film thicknesses were measured on dry samples by elipsometry on a Multiscope from Optrel GbR, Kleinmachnow, Germany. The instrument was used in a null ellipsometer configuration, with an angle of incidence of 70°C.

X-Ray Photoelectron Spectroscopy (XPS). XPS measurements were performed on an Axis 165 instrument (Kratos Analytical, UK) with monochromatic Al Ka radiation (anode: 15 kV, 20 mA) in hybrid mode (i.e. with electrostatic and with magnetic lenses). Thermal electrons from a filament were used to compensate the charging of the sample. The survey spectra for the determination of the elemental composition were recorded with a pass energy of 80 eV, while for the high resolution spectra we used 20 eV. The data were processed using the Kratos Vision2 software. For the quantification of the elemental concentrations and the binding states a linear background was subtracted. The high resolution spectra were fitted with Gaussian functions in order to determine the concentrations of the atoms in the various binding states.

C- Cell cultures and cell-adhesion assays:

L929 mouse fibroblasts (No.: ACC 2, DSMZ, Germany) were cultivated in DMEM containing 25 mM HEPES supplemented with 10% FCS, 100 µg/ml Penicillin-Streptomycin solution and 4 mM L-glutamine at 37 °C and 5% CO_2. The 90% confluent cell layer was passaged every 3 days using trypsin/EDTA (0.25%/0.05% w/v). 1 x 10^5 of dissociated cells were resuspended in cell medium and seeded onto the coated gold substrates inside a 3.5 cm

Petri dish and allowed to adhere. Proper cell spreading on the surface was assumed by microscopic control (DMIL, Leica, Germany). Phase contrast images were taken every 2 min for 28 min using a Nikon Digital Sight DS-L1 (Nikon, Germany). The thermoresponsive behavior of the surfaces (switch from 37°c to 25°C) was first investigated after 2 days of cultivation. Afterwards, the surfaces were incubated for one additional day at 37°C and tested again.

D- Additional Figures:

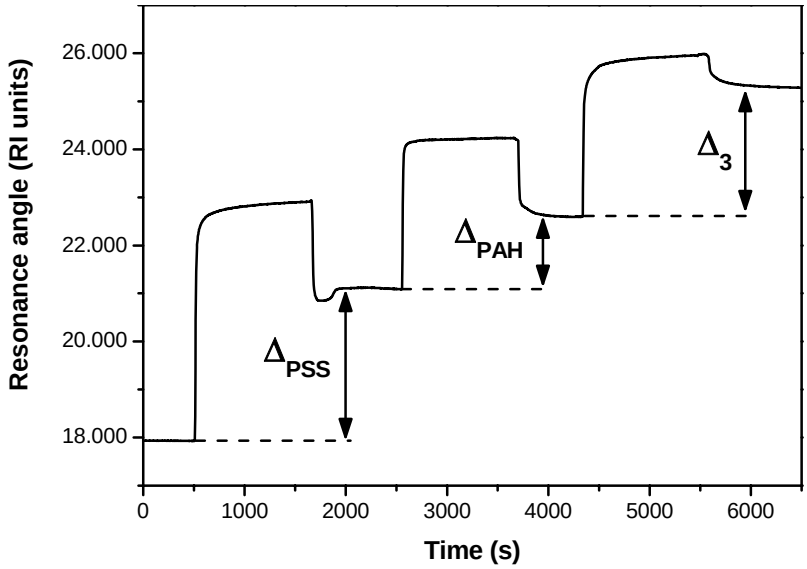


Figure S1. SPR characterization of the layer-by-layer modification of a pristine gold surface (one deposition cycle). In this approach, the under-layers are made of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH). The last layer is the polyanionic ATRP macroinitiator **3**.

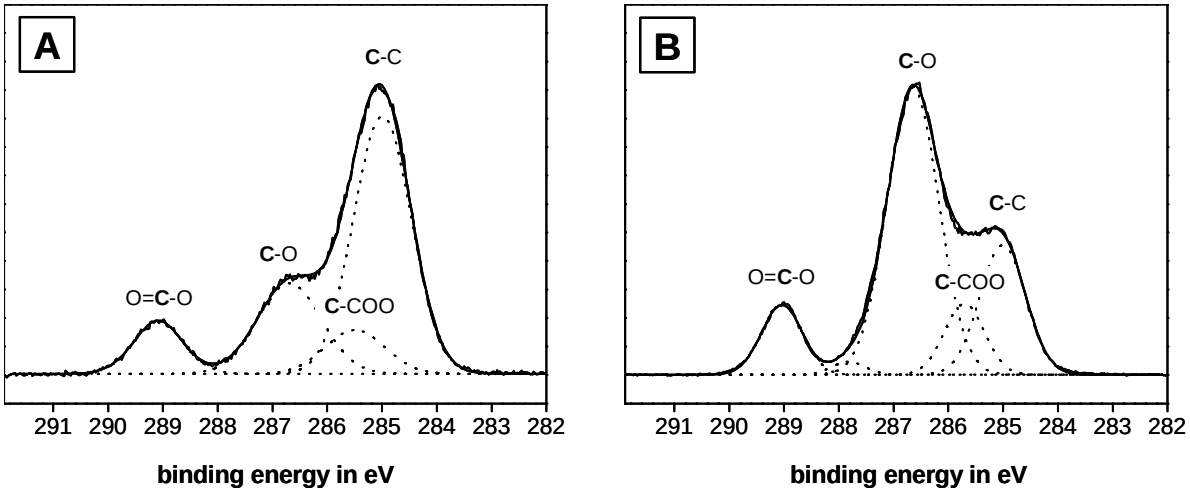


Figure S2. XPS C1s spectra recorded for a gold surface: (A) after LbL modification and adsorption of **3**; (B) after growth of P(OEGMA-*co*-MEO_2MA) brushes via AGET ATRP.

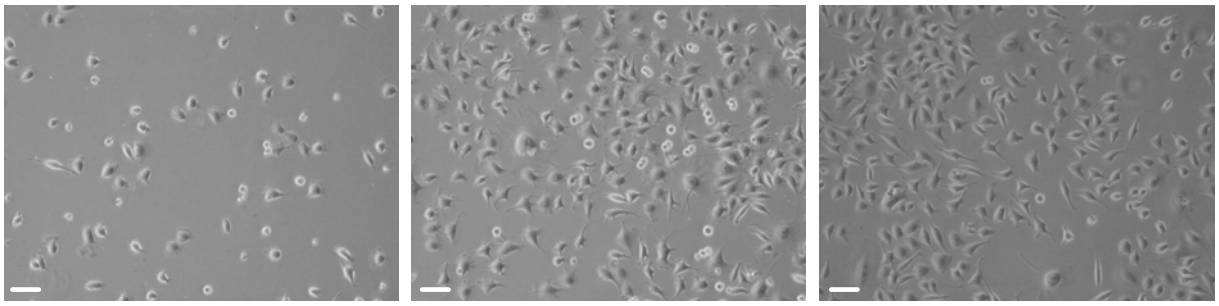


Figure S3. Images of L929 mouse fibroblasts on P(OEGMA-*co*-MEO_2MA)-modified gold substrates after 20h (left), 40h (middle) and 66h (right) of incubation at 37°C. The surfaces presented on this figure were prepared using a "grafting-from" approach. Scale bars correspond to 100 µm.

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