Supplementary Information

Use of pooled blood plasmas in the assessment of fouling resistance

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Materials

$CuBr_2$ (99%), CuBr (99.99%), CuCl (99.99%), 2,2'-bipyridyl (99%), 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam, 98%), 2-hydroxyethyl methacrylate (HEMA, 99%), oligo(ethylene glycol) methacrylate (HOEGMA, M_n = 526 g mol^{-1}), oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA, M_n = 300 g mol^{-1}), 11-mercapto-1-ol, α-bromoisobutyryl bromide, sodium carbonate, and methacryloyl chloride were purchased from Sigma-Aldrich. β-propiolactone was purchased from SERVA Electrophoresis. 3-[*N*-(dimethylamino)propyl] acrylamide (98%) and DL-1-aminopropan-2-ol (98%) were acquired from TCI. Di(ethylene glycol) undecanethiol (EG2) and carboxy-capped tri(ethylene glycol) undecanethiol (COEG3) for SAM preparation were purchased from Prochimia, Poland. ATRP initiator (ω-mercaptoundecyl bromoisobutyrate) was synthesised by the procedure reported earlier.[1]

Monomer synthesis

(3-Acryloylamino-propyl)-(2-carboxy-ethyl)-dimethyl-ammonium (CBAA)

CBAA was synthesised by a modification of the procedure reported earlier.[2,3] Dimethyl aminopropyl acrylamide (7.8 g, 55 mmol) was dissolved in 100 mL of anhydrous THF and cooled to -20 °C. Subsequently, β-propiolactone (5.0 g, 69 mmol) was dissolved in 40 mL of THF and added dropwise under nitrogen atmosphere. The reaction was allowed to proceed for 24 h at 4 ºC. The white precipitate was washed with dry THF and ether and dried under vacuum to yield 19.4 g (yield: 80 %) ^{1}H NMR (Bruker 250 MHz, D_2O, δ): 6.32 (t, 1H, CHH=CH), 6.2 (t, 1H, CHH=CH), 5.87 (t, 1H, CHH=CH), 3.66 (t, 2H, N-CH_2-CH_2-COO), 3.48 (m, 4H, NH-CH_2-CH_2-CH_2), 3.17 (s, 6H, N-$(CH_3)_2$), 2.75 (t, 2H, CH_2-COO).

N-(2-hydroxypropyl) methacrylamide (HPMA)

To a mixture of 1-aminopropan-2-ol (66.3 g, 0.88 mol) and anhydrous sodium carbonate (106 g, 1 mol) in 250 mL of freshly distilled dichloromethane was added dropwise methacryloyl chloride (89.1 g, 0.85mol) in 120 mL of dichloromethane while cooling to 0 ºC for 1 h with intense stirring. The mixture was left to react for a further 30 min, after which 30 g of anhydrous sodium sulfate were added and then filtered off. The filtrate was concentrated to half of the volume and the product was left to crystallise at -20 ºC and the recrystallised from acetone. Yield: 80%.

Preparation of surfaces

Self-Assembled Monolayers (SAM)

The substrates used were gold-coated silicon wafer for ellipsometry and gold-coated glass chips for SPR. The substrates were rinsed with ethanol and deionised water, blow dried with nitrogen, and cleaned with UV-ozone cleaner (Jelight) for 20 min. The substrates were immediately immersed in a solution of di(ethylene glycol) undecanethiol (0.7 mM) and carboxy-capped tri(ethylene glycol) undecanethiol (0.3 mM) in ethanol and kept at room temperature in the dark for 1 day. The preparation of ATRP initiator SAM was analogous but placing the chips in a 1 mM solution of ω-mercaptoundecyl bromoisobutyrate at room temperature.

Polymerisation of MeOEGMA and HOEGMA

A solution of $CuBr_2$ (8.1 mg, 36.4 μmol), 2,2'-bipyridyl (145 mg, 930 μmol) and HOEGMA (M_n = 526 g mol^{-1}, 10 g, 19 mmol) or MeOEGMA (M_n = 300 g mol^{-1}, 5.7 g, 19 mmol) in 10 mL of water was degassed by flow of Ar under stirring and CuCl (37 mg, 374 μmol) was added. The mixture was transferred under Ar protection to the reactor containing the substrate coated with an initiator SAM. After 30 min of reaction at 30 ºC, the polymerisation was stopped by removal of the substrate and rinsing it with ethanol and deionised water, and the chips were stored in water. The obtained polymer layers were 30-nm-thick.

Polymerisation of HEMA

The polymerisation was performed identically as for HOEGMA and MeOEGMA, except that the solvent used was 10 mL of water:ethanol 1:1 and the monomer was HEMA (11.5 g, 89 mmol). After 45 min of reaction at 30 ºC, the polymerisation was stopped by removal of the substrate and rinsing it with ethanol and deionised water, and the chips were stored in water. The obtained polymer layer was 30-nm-thick.

Polymerisation of CBAA-3 and HPMA

Robust surface-initiated ATRP was carried out according to our optimised procedures.[4–6] Ethanol (10 mL) was degassed by three cycles of freeze-pump-thaw and transferred to a Schlenk tube containing CuBr (19.1 mg, 133 μmol), $CuBr_2$ (5.9 mg, 26.5 μmol) and Me_4Cyclam (40.9 mg, 160 μmol). The blue solution of the catalyst was syringed to a Schlenk tube containing the monomer, *N*-(2-hydroxypropyl) methacrylamide (HPMA, 953 mg, 6.7 mmol) or carboxybetaine acrylamide (CBAA-3, 1500 mg, 6.7 mmol). Finally, the polymerisation solution was transferred to the reactors containing the substrates coated with a SAM of ω-mercaptoundecyl bromoisobutyrate and the reaction was allowed to proceed for 2 h at 30 ºC. The substrates coated with poly(HPMA) or poly(CBAA) brushes were washed with ethanol and water and stored in water. The obtained dry thickness by ellipsometry was 34 nm for poly(HPMA) and 30 nm for poly(CBAA)

Characterisations

Ellipsometry

The thickness of the polymer layers in the dry state was determined by ellipsometry using a variable angle spectroscopic imaging auto-nulling ellipsometer EP3-SE (Nanofilm Technologies GmbH, Germany) at an angle of incidence of 70 º in the wavelength range of 399-811 nm in air at room temperature and the results were fitted with multilayer models in the EP³-SE analysis software. The thickness and refractive index of the polymer layers were obtained from simultaneous fitting of the obtained ellipsometric data using a Cauchy relationship model. Due to the low penetration depth of the light, the Au layer was modelled as bulk gold using the predefined EP³-SE dispersion function.

Contact Angle

The dynamic sessile drop method was used to measure the water contact angle of the surfaces and assess their wettability, with a DataPhysics OCA 20 instrument. A 5 µL drop was placed on the surface and its volume was increased up to 15 µL and decreased at a flow rate of 0.5 µL s^{-1}. The data was evaluated with a circular fitting algorithm.

Table 1. Dynamic water contact angles of the prepared surfaces

Surface	Water contact angle [º]	
	Advancing	Receding
SAM	30	9
Poly(HEMA)	56	16
Poly(HOEGMA)	55	27
Poly(MeOEGMA)	55	22
Poly(CBAA)	23	8
Poly(HPMA)	40	21

FTIR

Grazing Angle Specular Reflectance (GASR) FTIR was used to characterise the chemical composition of the prepared brushes, using a FTIR Bruker IFS 55 spectrometer equipped with a Pike Technologies 80Spec GASR attachment and polariser under continuous purge with dry air (grazing angle 80 º, p-polarisation, MCT detector, 2 cm^{-1} resolution, 128 scans).

The spectra of the prepared surfaces on gold-covered substrates are shown in Figure 1. Spectrum (1) corresponds to the mixed SAM consisting of EG2/COEG3. For the polymer brushes, it can be seen for poly(HEMA) (2), poly(HOEGMA) (3) and poly(MeOEGMA) (3) the ester carbonyl band at 1733 cm^{-1} and the group of bands between 1300 and 900 cm^{-1} corresponding to methacrylate polymers. Poly(CBAA) and poly(HPMA) show the pair of bands around 1650 and 1550 cm^{-1} characteristic for amides.

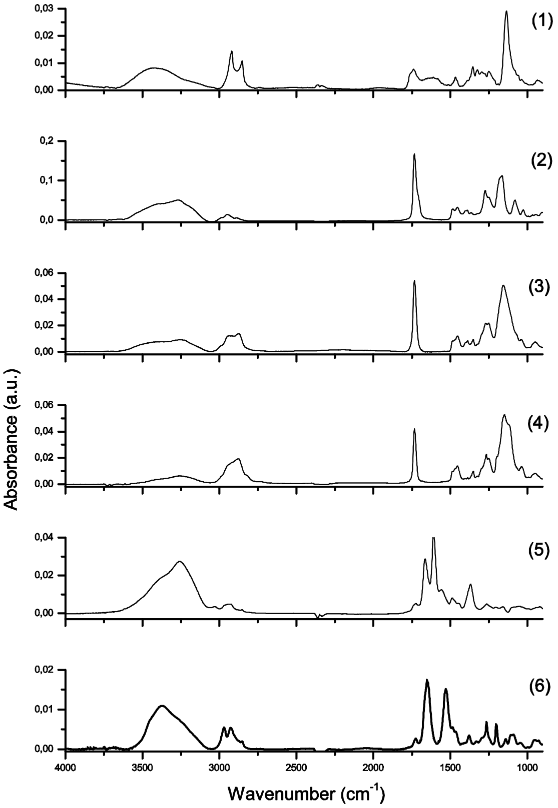

Fig. 1 FTIR spectra of layers on gold chips. (1) SAM, (2) poly(HEMA), (3) poly(HOEGMA), (4) poly(MeOEGMA), (5) poly(CBAA), and (6) poly(HPMA).

Fouling studies

The irreversible non-specific protein adsorption was measured by SPR using an instrument based on the Kretschmann geometry and spectral interrogation, built by the Institute of Photonics and Electronics, Czech Republic.[7] The temperature was set at 25 ºC and controlled within a range of ± 0.01 ºC. In a typical experiment, phosphate-buffered saline solution (PBS) was driven through the flow cell by a peristaltic pump until a stable baseline was achieved. Blood plasma was then pumped for 15 min, after which it was replaced with PBS. The sensor response is the difference in the resonance wavelength of the surface plasmons in PBS, before and after contact of the surface with blood plasma. From the resonance wavelength shift the mass deposited on the surface can be obtained by calibration. In our conditions, a shift of 1 nm in the vicinity of 750 nm corresponds to a change in adsorbed mass of 15 ng cm^{-2}.[8,7,2]

Notes and references

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