Electronic Supplementary Information

ATRP-Mediated Continuous Assembly of Polymers for the Preparation of Nanoscale Films


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Materials

4,4′-Azobis(4-cyanvaleric acid) (≥ 75%), α-bromoiso-butyryl bromide (98%), calcium hydride (CaH), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), N,N′-dicyclohexylcarbodiimide (DCC, 99%), 4-(dimethylamino)pyridine (DMAP, ≥ 99%), fluorescein 5(6)-isothiocyanate (FITC, ≥ 90%), N,N,N′,N,N″-pentamethyldiethylenetriamine (PMDETA, 99%), methacryloyl chloride (97%), poly(ethylene imine) (PEI) (Mw ~ 10 kDa), propargyl bromide (80% in toluene), copper(I) bromide (CuBr, ≥ 98%), copper(II) bromide (CuBr2, 99%), copper sulfate (≥ 99%), sodium ascorbate (NaAsc, ≥ 98%), sodium hydride (60% dispersion in mineral oil), tetra-n-butyl ammonium fluoride (1.0 M in THF), hydrofluoric acid (HF, 48 wt% in H2O), ammonium fluoride (8 M in H2O), silver chloride (AgCl), chlorotrimethylsilane (TMSCl), methacrylic acid, hydroquinone, neutral alumina, and triethylene glycol (99%) were obtained from Aldrich and used as received. 2-Hydroxyethyl acrylate (HEA, 96%) and oligo(ethylene glycol) methyl ether methacrylate (OEGMA, Mn ~ 475) were obtained from Aldrich and passed over plugs of inhibitor remover (Aldrich) and basic alumina (Scharlau) twice to remove any inhibitors present and stored below 4 °C prior to use. Triethylamine and 2,2′-azobis(2-methylpropionitrile) (AIBN, 98%) were obtained from Scharlau and Acros, respectively. Sodium bicarbonate (NaHCO3), anhydrous magnesium sulphate (MgSO4), silica gel (200-300 mesh), hydrochloric acid (HCl), dioxane, n-hexane, ethyl acetate (EtOAc), 1-butanol and diethyl ether (DEE) were obtained from Chem-Supply and used as received. N,N-dimethylformamide (DMF) (Aldrich) and N,N-dimethylacetamide (DMAc) (Aldrich) were distilled from CaH in vacuo. Dichloromethane (DCM) was distilled from CaH under argon. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. Deuterated chloroform (CDCl3) and dimethylsulfoxide (d6-DMSO) were obtained from Cambridge Isotope Laboratories. High-purity water with a resistivity greater than 18 MΩ-cm was obtained from an in-line Millipore RiOs/Origin water purification system. The chain transfer agent 2-cyanoprop-2-yl dithiobenzoate (CPDB),1 atom transfer radical polymerisation (ATRP) initiator azidopropyl 2-bromoiso-butyrate (AzPBIB)2 and azide functionalised poly(acrylic acid)3 were prepared according to literature procedures.

Nonporous silica (SiO2) particles (5 wt% suspensions, average diameter 5.35 ± 0.25 μm) were obtained from Microparticles GmbH (Berlin, Germany). Mesoporous silica (MS) particles (Separon SGX 1000, diameter 4.5 μm, pore size 100 nm) were obtained from Tessek Ltd (Czech Republic). Silicon wafers (MMRC Pty. Ltd., Melbourne, Australia) were cut to approximately 1 cm × 1 cm slides and cleaned with Piranha solution (sulfuric acid:hydrogen peroxide (7:3)) – Caution! Piranha solution is highly corrosive and extreme care should be taken during preparation and use. The slides were then
sonicated in isopropanol:water (1:1) solution for 15 min and finally heated to 60 °C for 20 min in RCA solution (water:ammonia:hydrogen peroxide (5:1:1)). The slides were washed thoroughly with Milli Q water between each step. Unmodified FB80 chips were purchased from Farfield Scientific Ltd., Manchester, U.K. The chips were cleaned in Piranha solution, rinsed thoroughly in Milli Q water, and dried under a nitrogen stream prior to use. A recyclable Kalrez gasket was sonicated in 1% Micro-90 cleaning solution for 10 min, rinsed thoroughly with Milli Q then isopropanol, and dried under a nitrogen stream before use.

**Characterisation methods**

Polymer molecular weight characterisation was carried out via gel permeation chromatography (GPC) using either HPLC grade THF (RCI Labscan) or DMF (Chem-Supply) as the mobile phase. GPC (THF) was conducted on a Shimadzu liquid chromatography system equipped with a Wyatt DAWN EOS MALLS detector (690 nm, 30 mW) and Wyatt OPTILAB DSP interferometric refractometer (690 nm) using three Phenomenex Phenogel columns in series (500, 10⁴ and 10⁶ Å porosity; 5 µm bead size) operating at 30 °C and a flow rate of 1 mL.min⁻¹. GPC (DMF) was conducted on a Shimadzu liquid chromatography system equipped with a Wyatt DAWN HELEOS detector (λ = 658 nm), Shimadzu RID-10 refractometer (λ = 633 nm) and Shimadzu SPD-20A UV-Vis detector using three identical Polymer Laboratories PLgel columns (5 µm bead size, MIXED-C) in series operating at 70 °C. DMF with 0.05 M LiBr (> 99%, Aldrich) was employed as the mobile phase at a flow rate of 1 mL.min⁻¹. Astra software (Wyatt Technology Corp.) was used to determine the molecular weight characteristics using known d⁰/dc values.⁴

¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy was conducted on a Varian Unity 400 MHz spectrometer at 400 and 100 MHz, respectively, using the deuterated solvent as reference and a sample concentration of approximately 20 mg/mL.

Ellipsometry measurements of the CAP ATRP coated films were performed on a UVISEL spectroscopic ellipsometer from Jobin Yvon. Spectroscopic data were acquired between 400 and 800 nm with a 2 nm increment, and thicknesses were extracted with the integrated software by fitting with a classical wavelength dispersion model.⁵

Atomic force microscopy (AFM) images of air-dried CAP ATRP films on silicon wafers were acquired with a JPK NanoWizard2 Bio-AFM or a MFP-3D Asylum Research instrument. Typical scans were conducted in intermittent contact mode with silicon cantilevers (NSC/CSC) (MicroMesh, Bulgaria).
Image processing and surface roughness analysis were performed using the JPK image processing software and Nanoscope software programs, respectively. CAPATRP film thicknesses were estimated by film scratching (mechanical removal) and by tracing a profile along the film and the scratched zone. The thickness measurements reported represent mean values over 3 different analysis sites per substrate and physical analysis of scratched films by AFM showed good agreement with ellipsometry data. AFM images of air-dried CAPATRP capsules on silicon wafers were acquired with a MFP-3D Asylum Research instrument. The capsule solution (1 μL) was deposited on a silicon wafer and air dried. Typical scans were conducted in AC mode with ultrasharp SiN gold-coated cantilevers (MicroMesh, Bulgaria).

Optical waveguide lightmode spectroscopy (OWLS) was used to follow the kinetics of CAPATRP film growth in situ. OWLS sensors are sensitive to the penetration depth of an evanescent wave travelling through the film near the waveguide surface (over 200-300 nm) and give access to the optical properties of the films (the transverse electric and magnetic refractive indices, NTE and NTM, respectively). Details about the experimental setup and the procedure can be found elsewhere. A solution (100 μL) of CAPATRP components (Cu catalyst and macrocross-linker) was injected into the OWLS cell and after 12 h was rinsed with sodium ascorbate buffer at a constant flow rate (20 μL/min). OWLS directly measures the effective NTE and NTM, from which adsorbed mass and thickness data of the CAP film are calculated using the integrated software by fitting with models of mass and thicknesses.

Dual polarisation interferometry (DPI) measurements were carried out on an Analight Bio200 dual polarization interferometer (Farfield Scientific Ltd., Manchester, U.K.) and provided the thickness, mass and density of the CAPATRP film growth in situ. DPI monitors the phase change of two perpendicularly polarized laser beams to obtain a unique thickness and refractive index of the adsorbed layer, which can be used to calculate the mass and density using de Feijter’s equation. A detailed explanation of the instrumentation and technique can be found in the literature. Briefly, a layer of PEI was first deposited onto the chip by passing 200 μL of PEI solution (1 mg.mL⁻¹ in 0.5 M NaCl) followed by PAA-azide solution (1 mg.mL⁻¹ in pH 3.5 acetate buffer), and P(OEGMA-co-PgTEGMA-co-BIBTEGMA) solution (1 mg.mL⁻¹, 1.8 mg.mL⁻¹ copper sulphate, 4.4 mg.mL⁻¹ sodium ascorbate). Each solution was flowed over the chip for 15 min (13.3 μL.min⁻¹) and rinsed in water for 10 min (20 μL.min⁻¹) after each layer deposition. The CAPATRP film was formed by incubating the chip in P(HEA-co-MOEA) macrocross-linker (1 mM), CuBr₂ (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM) over 12 h. Note that the fluidics was turned off over this period. At the end of the incubation period, the fluidics was resumed and the film was washed in water (5 μL.min⁻¹, 1 h). To
reinitiate the film, the coated FB80 chip was removed from the instrument and regeneration of the initiating moieties was performed ex-situ, as described in Section 4.1. The chip was reinserted into the instrument, and recalibrated using 80% w/w ethanol/water mixture. Note that recalibration with 80% w/w ethanol/water mixture resulted in negligible loss of film mass from the surface. The second layer of CAP_{ATRP} film was formed by incubating the chip (with the fluidics turned off) in a solution containing P(HEA-co-MOEA) macrocross-linker (1 mM), CuBr$_2$ (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM) over 12 h, followed by washing with water (5 µL.min$^{-1}$, 1 h). A refractive index increment value of 0.17 was used to calculate the mass ($M$) and density using de Feijter’s equation:

$$M = d_A \frac{n_A - n_C}{d n/dc}$$

where $d_A$ is the thickness of the film, $n_A$ and $n_C$ are the refractive index of the film and buffer, respectively, and $dn/dc$ is the refractive index increment of the adsorbed layer. The density ($D$) of the film (g cm$^{-3}$) is calculated from:

$$D = \frac{M}{d_A}$$

Flow cytometry was performed on CAP_{ATRP} films coated onto silica particles composed of fluorescently FITC-labelled polymers. Fluorescence intensity histograms were acquired with a Partec CyFlow Space instrument using an excitation wavelength of 488 nm. Data analysis was performed with Partec CyflowMax software and mean fluorescence intensities were obtained from the fluorescence intensity histograms.

Differential interference contrast (DIC) and fluorescence microscopy images of the CAP particles, capsules and replica were taken on an inverted Olympus IX71 microscope equipped with a DIC slider (U-DICT, Olympus), a UF1032 fluorescence filter cube, and a 60 oil immersion objective (Olympus UPFL20/0.5NA, W.D. 1.6).

For scanning electron microscopy (SEM), 1 µL of concentrated CAP_{ATRP} capsule solution was deposited onto a gold-coated silicon wafer slide and allowed to air-dry prior to gold sputter-coating onto silicon wafers. SEM imaging was achieved with a FEI Quanta 200 FEG scanning electron microscope operated at 5 kV (FEI Company, The Netherlands).
Experimental Methods

1. Macrocross-linker preparation

1.1 Synthesis of poly((2-hydroxyethyl)acrylate) (PHEA)

HEA (23 mL, 0.20 mol), 4,4’-azobis(4-cyanvaleric acid) (1.5 g, 4.0 mmol) and 1-butanol (58 mL) were added to a Schlenk tube, degassed via 3 freeze-pump-thaw cycles and back-filled with argon. The reaction solution was stirred for 2 h at 100 °C, cooled to room temperature and then added dropwise to DEE (600 mL). The resulting oily residue was isolated via centrifugation and dried in vacuo to yield PHEA as a clear tacky solid, 18.4 g (80%); GPC-MALLS (DMF): \( M_w = 65.4 \text{ kDa}, \frac{M_w}{M_n} = 2.8 \).

1.2 Synthesis of poly((2-hydroxyethyl)acrylate-co-(2-methacryloyloxyethyl)acrylate) (P(HEA-co-MOEA))

PHEA (\( M_w = 65.4 \text{ kDa}, 7.0 \text{ g}, 0.11 \text{ mmol} \)) was dissolved in DMAc (50 mL). Separately, DCC (4.1 g, 20 mmol) was dissolved in DMAc (50 mL) and added to the polymer solution, followed by DMAP (0.24 g, 2.0 mmol) and methacrylic acid (0.90 mL, 10 mmol). The reaction solution was stirred at room temperature for 12 h and then precipitated into DEE (500 mL). The resulting oily residue was isolated via centrifugation and dried in vacuo to yield P(HEA-co-MOEA) as a clear tacky solid, 6.8 g (88%); GPC-MALLS (DMF): \( M_w = 69.0 \text{ kDa}, \frac{M_w}{M_n} = 2.9 \); \(^1\)H NMR (400 MHz, \( d_6\)-DMSO) \( \delta \) 6.20 (br s, –CH), 5.60 (br s, –CH), 4.72 (br s, OH), 3.97 (br s, CH\(_2\)O), 3.51 (br s, CH\(_2\)OH), 2.23 (br s, ...)
CH$_3$), 1.90 (br s, $=CCCH_3$), 1.76 (br s, CH$_2$), 1.56 (br s, CH$_2$) ppm. Pendant methacrylate functionality = 9%.

1.3 Fluorescent labelling of P(HEA-co-MOE)

P(HEA-co-MOE) (1.0 g, 0.014 mmol), FITC (30 mg, 76 mmol) and hydroquinone (2.0 mg, 0.018 mmol) were dissolved in DMAc (20 mL). The reaction solution was stirred at 80 °C for 2 h and then precipitated into DEE (250 mL). The resulting oily residue was isolated via centrifugation and dried in vacuo to yield P(HEA-co-MOE)-FITC as a light yellow tacky solid.
2. Surface initiator preparation

For CAP\textsubscript{ATRP}, the substrate was initially primed with three compositionally different layers. The first layer of positively charged poly(ethylene imine) (PEI) was electrostatically assembled with a second layer of negatively charged poly(acrylic acid) (PAA) functionalised with azide groups. A third layer of poly(ethylene glycol) (PEG) functionalised with alkyne groups and ATRP initiating bromoester moieties was then covalently linked to the previous bilayer through 1,3-dipolar cycloaddition (click chemistry). This strategy was adopted for several reasons: (i) the polymer layering and click chemistry reaction were experimentally straightforward to conduct (45 min. total for the 3-layer assembly, as opposed to grafting bromoamide or bromoester groups directly to the surface, which is usually achieved via time consuming multistep synthesis; and (ii) this strategy led to a more efficient CAP\textsubscript{ATRP} process with thicker and more homogeneous films.

2.1 Synthesis of 2-(2-(2-(3-(trimethylsilyl)prop-2ynyloxy)ethoxy)ethoxy)ethyl methacrylate (TMSPgTEGMA)

![Reaction Scheme](image)

Sodium hydride (2.0 g, 50 mmol) was slowly added at 0 °C to triethylene glycol (12 g, 76 mmol) dissolved in anhydrous THF (50 mL). The mixture was stirred for 20 min and then propargyl bromide (4.2 mL, 38 mmol) was added dropwise. After 20 h at room temperature the volatiles were removed \textit{in vacuo}. The resulting residue was dissolved in DCM (150 mL), washed with saturated NaHCO\textsubscript{3} (2 x 50 mL) and water (50 mL), dried (MgSO\textsubscript{4}), filtered and concentrated \textit{in vacuo}. The crude product was purified by column chromatography on silica, eluting with 2:3 \textit{n}-hexane:EtOAc to afford 2-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)ethanol as a light yellow oil, 4.5 g (63%); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, TMS): δ\textsubscript{H} 4.13 (s, 2H, OCH\textsubscript{2}C≡CH), 3.61–3.58 (m, 10H, CH\textsubscript{2}O), 3.50 (t, 2H, CH\textsubscript{2}OH), 2.75 (s 1H, CH≡C), 2.38 (br s, 1H, OH) ppm; \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, TMS) δ\textsubscript{C} 77.2 (C≡CH), 75.6 (C≡CH), 70.4 (CH\textsubscript{2}O), 70.3 (CH\textsubscript{2}O), 70.1 (CH\textsubscript{2}O), 69.6 (CH\textsubscript{2}O), 69.2 (CH\textsubscript{2}O), 61.5 (CH\textsubscript{2}OH), 60.1 (OCH\textsubscript{2}C≡CH) ppm.
The alkyne triethylene glycol (3.4 g, 18 mmol) was added to silver chloride (0.24 g, 1.8 mmol) suspended in anhydrous DCM (25 mL), followed by the addition of DBU (3.5 g, 23 mmol). The reaction mixture was heated to 40 °C and TMSCl (2.8 g, 26 mmol) was added dropwise. After 24 h the mixture was cooled to room temperature and diluted with n-hexane (200 mL). The resulting mixture was washed with NaHCO₃ (2 × 50 mL), 0.1 M HCl (2 × 50 mL) and water (50 mL), dried (MgSO₄), filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica, eluting with 8:1 n-hexane:EtOAc to afford the TMS-alkyne triethylene glycol, 2-(2-(2-(3-(trimethylsilyl)prop-2-ynyloxy)ethoxy)ethoxy)ethanol, as a light yellow oil, 2.11 g (45%); ¹H NMR (400 MHz, CDCl₃, TMS): δH 4.13 (s, 2H, OCH₂C≡CH), 3.61–3.58 (m, 10H, CH₂O), 3.50 (t, 2H, CH₂OH), 2.15 (br s, 1H, OH), 0.21 (s, 9H, Si(CH₃)₃) ppm; ¹³C NMR (100 MHz, CDCl₃, TMS) δC 105.5 (CH₂C≡C), 91.2 (C≡CSi), 70.8 (CH₂O), 70.7 (CH₂O), 70.3 (CH₂O), 69.1 (CH₂O), 69.0 (CH₂O), 61.1 (CH₂OH), 60.0 (OCH₂C≡CH), 5.2 (SiCH₃) ppm.

Methacryloyl chloride (0.78 mL, 8.0 mmol) dissolved in anhydrous DCM (10 mL) was added dropwise over 1 h to a solution of the TMS-alkyne triethylene glycol (2.0 g, 7.7 mmol) and triethylamine (0.81 g, 8.0 mmol) in anhydrous DCM (50 mL) at 0 °C. The mixture was stirred at room temperature for 18 h and then filtered to remove triethylamine hydrochloride. The filtrate was washed with saturated NaHCO₃ (2 × 50 mL) and water (50 mL), dried (MgSO₄), filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica, eluting with DCM to afford 2-(2-(2-(3-(trimethylsilyl)prop-2-ynyloxy)ethoxy)ethoxy)ethyl methacrylate (TMSPgTEGMA) as a light yellow oil, 2.1 g (88%); ¹H NMR (400 MHz, CDCl₃, TMS) δH 6.15 (s, 1H, CHH=C(CH₃)), 5.51 (s, 1H, CHH=C(CH₃)), 4.13 (s, 2H, OCH₂C≡CH), 4.05 (t, 2H, CH₂OOC), 3.61–3.58 (m, 10H, CH₂O), 3.50 (t, 2H, CH₂OH), 2.00 (s, 3H, CH₂=C(CH₃)), 0.21 (s, 9H, Si(CH₃)₃) ppm; ¹³C NMR (100 MHz, CDCl₃, TMS) δC 168.3 (CO), 133.8 (=CCH₃), 131.5 (=CH₂), 106.1 (CH₂C≡C), 91.6 (=CSi), 70.3 (CH₂O), 70.2 (CH₂O), 70.0 (CH₂O), 69.4 (CH₂O), 69.2 (CH₂O), 61.8 (CH₂OH), 60.2 (OCH₂C≡CH), 18.8 (=CCH₃), 4.1 (SiCH₃) ppm.

2.2 Synthesis of poly(oligo(ethylene glycol) methacrylate-co-propargyltriethylene glycol methacrylate) (P(OEGMA-co-PgTEGMA))
OEGMA (1.0 g, 2.1 mmol), TMSPgTEGMA (0.46 g, 1.4 mmol), CPDB (7.7 mg, 0.035 mmol) and AIBN (0.70 mg, 0.004 mmol) were dissolved in dioxane (2 mL). The mixture was degassed via three freeze-pump-thaw cycles, backfilled with argon and heated at 70 °C for 20 h. The mixture was quenched in liquid nitrogen, diluted with THF (10 mL) and precipitated into DEE (100 mL). This precipitation procedure was repeated three times. The resulting residue was isolated by centrifugation and dried in vacuo to afford P(OEGMA-co-TMSPgTEGMA) as a red tacky solid, 1.1 g (75%); GPC-MALLS (THF): \( M_w = 42.9 \text{ kDa}, \frac{M_w}{M_n} = 1.13; \) \(^1\text{H} \text{NMR} \) (400 MHz, CDCl\(_3\), TMS) \( \delta_{\text{H}} 7.53-7.30 \) (m, ArH end group), 4.25 (br s, OCH\(_2\)=C), 4.05 (br s, CH\(_2\)OOC), 3.60 (br s, CH\(_2\)O), 3.10 (br s, CH\(_3\)O), 1.95-1.80 (m, CH\(_2\)), 1.4-0.85 (m, CH\(_3\)), 0.21 (br s, Si(CH\(_3\))\(_3\)) ppm. From \(^1\text{H} \text{NMR} \) spectroscopic analysis the degree of polymerisation (DP) of the copolymer was determined to be 80, with 35% alkyne repeat units.

P(OEGMA-co-TMSPgTEGMA) (0.50 g, 0.43 mmol based upon alkyne groups) and acetic acid (37 \( \mu \text{L}, 0.65 \text{ mmol} \)) were dissolved in THF (10 mL) at 0 °C and argon was bubbled through the solution for 10 min. A 0.2 M tetra-\( n \)-butyl ammonium fluoride solution in THF (3.2 mL, 0.65 mmol) was added slowly via syringe with vigorous stirring. After 12 h at room temperature the reaction mixture was passed through a neutral alumina column, concentrated in vacuo to ca. 5 mL and precipitated into \( n \)-hexane (50 mL). The resulting residue was isolated by centrifugation and dried in vacuo to yield P(OEGMA-co-PgTEGMA) as a light red tacky, 0.40 g (80%); GPC-MALLS (THF): \( M_w = 39.3 \text{ kDa}, \frac{M_w}{M_n} = 1.18; \) \(^1\text{H} \text{NMR} \) (400 MHz, CDCl\(_3\), TMS) \( \delta_{\text{H}} 7.55-7.30 \) (m, ArH end group),
4.35 (br s, OCH$_2$C≡C), 4.10 (br s, CH$_2$OOC), 3.65 (br s, CH$_2$O), 3.10 (br s, CH$_3$O), 2.65 (br s, CH≡C), 1.95-1.80 (m, CH$_2$), 1.30-0.85 (m, CH$_3$) ppm. Quantitative removal of the TMS-protecting groups was confirmed by $^1$H NMR spectroscopic analysis.
2.3 Synthesis of alkyne-functionalised poly(oligo(ethylene glycol) methacrylate) ATRP initiator (poly(OEGMA-co-PgTEGMA-co-BIBTEGMA))

P(OEGMA-co-PgTEGMA) (0.10 g, 0.090 mmol based upon alkyne groups), AzPBIB (9.2 mg, 0.037 mmol), CuBr (5.3 mg, 0.037 mmol), PMDETA (6.4 mg, 0.037 mmol) and DMF (0.5 mL) were added to a Schlenk tube. The mixture was degassed via three freeze-pump-thaw cycles, backfilled with argon and heated at 40 °C for 24 h. After cooling to room temperature the volatiles were removed in vacuo, the residue was dissolved in DCM (1.0 mL) and passed through a neutral alumina column. The solution was concentrated in vacuo to ca. 2 mL and precipitated into n-hexane (30 mL). The resulting residue was isolated by centrifugation and dried in vacuo to yield P(OEGMA-co-PgTEGMA-co-BIBTEGMA) as a light red tacky solid, 80 mg (73%); GPC-MALLS (THF): \( M_w = 40.3 \text{ kDa}, M_w/M_n = 1.20 \). \(^1\)H NMR (400 MHz, CDCl\(_3\), TMS) \( \delta \) 7.55-7.30 (m, ArH end group), 4.55 (br s, CH\(_2\)N), 4.20 (br s, OCH\(_2\)C≡C), 4.00 (br s, CH\(_3\)OOC), 3.70 (br s, CH\(_2\)O), 3.10 (br s, CH\(_3\)), 2.55 (br s, CH=C), 2.10 (br s, (CH\(_3\))\(_2\)CBr), 1.90-1.80 (m, CH\(_3\)), 1.30-0.85 (m, CH\(_3\)) ppm. The bromoisobutyrate modified alkyne repeat units in the final copolymer is approximately 12% as determined from \(^1\)H NMR analysis.

2.4 Formation of ester bromide initiator layer deposition on Si wafer

A Si wafer (ca. 1 cm × 1 cm) was added to an Eppendorf tube containing a PEI solution (1 mg.mL\(^{-1}\) in 0.5 M NaCl buffer, 5 mL). The Si wafer was allowed to stand for 15 min at room temperature and then washed with Milli Q water (3 × 20 mL). The PEI-coated Si wafer was then added to an azide-functionalised poly(acrylic acid)\(^3\) solution (1 mg.mL\(^{-1}\), 1 mL) and after 15 min at room temperature was washed with Milli Q water (3 × 20 mL). The azide-functionalised Si wafer was then added to a solution of P(OEGMA-co-PgTEGMA-co-BIBTEGMA) (1 mg.mL\(^{-1}\), 3 mL), copper sulfate (1.8 mg.mL\(^{-1}\), 1 mL) and sodium ascorbate (4.4 mg.mL\(^{-1}\), 1 mL). The Si wafer was allowed to stand for...
15 min at room temperature and then washed with Milli Q water (3 × 20 mL). The Si wafer bearing the initiator prelayer was dried under a flow of argon before use. All solutions were adjusted to pH 3.5 with 0.1 M HCl buffer.

2.5 Formation of ester bromide initiator layer deposition on particles

Similar to planar substrates (Section 2.4), particles were primed with the initiating prelayer. Particles (SiO$_2$ or MS particles, 5 wt% suspensions, 200 µL) were first incubated in a PEI solution (1 mg.mL$^{-1}$ in 0.5 M NaCl buffer, 1 mL) for 15 min at room temperature, isolated by centrifugation and washed for 1 min in 5 mM sodium ascorbate buffer at pH 3.5 (3 × 1 mL). The particles were then added to azide-functionalised poly(acrylic acid)$^2$ (1 mg.mL$^{-1}$, 500 µL) and after 15 min at room temperature were isolated by centrifugation and washed for 1 min in 5 mM sodium ascorbate buffer at pH 3.5 (3 × 1 mL). The particles were then added to a solution of poly(OEGMA-$co$-PgTEGMA-$co$-BIBTEGMA) (1 mg.mL$^{-1}$, 300 µL), copper sulfate (1.8 mg.mL$^{-1}$, 100 µL) and sodium ascorbate (4.4 mg.mL$^{-1}$, 100 µL) for a further 15 min at room temperature, isolated by centrifugation and washed for 1 min in 5 mM sodium ascorbate buffer at pH 3.5 (3 × 1 mL). All solutions were adjusted to pH 3.5 with 0.1 M HCl buffer.
3. One step assembly of CAP_{ATRP} films onto planar surfaces and particles

ATRP was conducted under ‘activator regenerated by electron transfer’ (ARGET) conditions in water at ambient temperature. In ARGET the use of a reducing agent added in excess allows an efficient deoxygenation of the system and prevents Cu catalyst oxidation. Compared to conventional ATRP, ARGET-ATRP presents several advantages, which considerably simplifies the film preparation and allows minimization of the amount of catalyst used.

3.1 CAP_{ATRP} onto planar surfaces

All substrate manipulations were conducted in individual 7 mL vials sealed to the atmosphere. Si wafers (ca. 1 × 1 cm) functionalised with a bromo-initiator prelayer (Section 2.4) were added to 1 mL of an aqueous stock solution (pre-filtered through a 0.45 μm filter) containing P(HEA-co-MOEA) macrocross-linker (1 mM), CuBr₂ (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM). After reaction at room temperature, the polymer-coated wafers were removed (different thickness films were obtained by variation of the exposure time), washed with water (3 × 20 mL), soaked in water (20 mL) for 12 h and then air dried prior to analysis. GPC analysis of the P(HEA-co-MOEA) macrocross-linker solution before and after the CAP process (25 h) showed negligible change in molecular weight distribution. Details of this analysis are provided in Fig. S4.

3.2 CAP_{ATRP} onto particles

A suspension of the bromo-initiator functionalised particles (SiO₂: 5 wt%, 100 μL; MS: 5 wt%, 25 μL) were combined with 400 μL of an aqueous stock solution (pre-filtered through a 0.45 μm filter) containing P(HEA-co-MOEA)-FITC macrocross-linker (1 mM), CuBr₂ (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM). The mixture was agitated with an orbital shaker at room temperature for either 10 (SiO₂) or 24 h (MS) and the particles were isolated by centrifugation, washed for 1 min in water (3 × 1 mL) and soaked in water (1 mL) for 12 h prior to analysis.
4. CAP reinitiated films

4.1 CAP\textsubscript{ATRP} reinitiated films on planar substrates

All substrate manipulations were conducted in individual 7 mL sealed to the atmosphere. The previously assembled P(HEA-co-MOEA)-based CAP\textsubscript{ATRP} films on Si wafers were added to vials, followed by DMF (1 mL) and triethylamine (0.15 mL, 1.1 mmol). The vials were cooled to 0 °C under argon and α-bromo\textsubscript{i}sobutyryl bromide (0.12 mL, 1.0 mmol) in DMF (1 mL) was slowly added. The vials were kept at 0 °C for 3 h and then at room temperature for 12 h. The initiator-functionalised substrates were removed, washed rapidly with water (20 mL) and DMF (2 × 20 mL), and then immersed in 1 mL of an aqueous stock solution (pre-filtered through a 0.45 μm filter) containing P(HEA-co-MOEA) macrocross-linker (1 mM), CuBr\textsubscript{2} (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM). After standing at room temperature for a predetermined time the polymer-coated wafers were removed, washed with water (3 × 20 mL) and soaked in water (20 mL) for 12 h. After air drying the L2 P(HEA-co-MOEA)-based CAP films were analysed and then used for subsequent layering experiments via repetition of the above procedure.

4.4 CAP\textsubscript{ATRP} reinitiated films on silica particles

Non-porous silica particles

The previously assembled P(HEA-co-MOEA)-based CAP\textsubscript{ATRP} films on particles (SiO\textsubscript{2}) were washed for 1 min in anhydrous DMF (3 × 1 mL), dispersed in DMF (0.4 mL) in an oven dried vial at 0 °C and triethylamine (40 μL, 0.25 mmol) was added. Separately, α-bromo\textsubscript{i}sobutyryl bromide (65 μL, 0.50 mmol) was dissolved in DMF (0.4 mL) and slowly added to the particles. The dispersion was kept at 0 °C for 3 h and then room temperature for 4 h. The particles were isolated by centrifugation, washed for 1 min with water (1 mL) and DMF (2 × 1 mL) and 400 μL of an aqueous stock solution (pre-filtered through a 0.45 μm filter) containing P(HEA-co-MOEA)-FITC macrocross-linker (1 mM), CuBr\textsubscript{2} (1 mM), PMDETA (3 mM) and sodium ascorbate (20 mM) was added. The mixture was agitated with an orbital shaker at room temperature for 10 h and the particles were isolated by centrifugation, washed for 1 min in water (3 × 1 mL) and soaked in water (1 mL) for 12 h. After analysis the reinitiation and layering steps were repeated using the above described procedure to afford particles with up to 4 CAP\textsubscript{ATRP} assembled P(HEA-co-MOEA)-based layers.
5. Preparation of $\text{CAP}_{\text{ATRP}}$ polymer capsules and replica spheres

*Caution! This method requires the use of HF which is highly toxic and great care must be taken when handling!*

$\text{CAP}_{\text{ATRP}}$ polymer capsules were prepared by dissolving the underlying silica core of the SiO$_2$ particles coated with CAP film. The P(HEA-co-MOEA)-coated (core-shell) particle suspension (1 wt%, 20 µL) was combined with 8 M ammonium fluoride (40 µL) buffered HF (2 M, pH 4) and mixed very gently for 10 min. The formed capsules were then centrifuged at low speed (2000 g) for 10 min and washed with Milli Q water ($3 \times 1 \text{ mL}$).

Polymer replicas were prepared by dissolving the underlying silica template of the MS particles coated with CAP film. The CAP film-coated particle suspension (1 wt%, 30 µL) was combined with 8 M ammonium fluoride (150 µL) buffered HF (2 M, pH 4) and mixed very gently for 30 min. The formed replicas were centrifuged at low speed (2000 g) for 10 min and washed with Milli Q water ($3 \times 1 \text{ mL}$).
Supplementary Information

S1: CAP\textsubscript{ATRP} film kinetics and control experiments by ellipsometry

![Graph](image)

**Fig. S1** Ellipsometry thickness measurements of the CAP\textsubscript{ATRP} films and control experiments with exposure time. Initiating prelayers were prepared on separate Si wafers and the variation in prelayer thickness is shown in blue, whereas the CAP\textsubscript{ATRP} films are represented in red. Control experiments (CTRL) were conducted in the absence of copper catalyst (No Cu) or bromo ester initiating groups (No Ini). All experiments were conducted in triplicate.

**Details of CAP\textsubscript{ATRP} control experiments:**

Two control experiments were investigated for CAP\textsubscript{ATRP} by removing either the initiator functionality or the catalyst. In the first control experiment, the prelayer functionalised substrate without bromo ester initiating groups was exposed to a solution of P(HEA-co-MOEA) macrocross-linker and Cu catalyst. In the second control experiment, the prelayer functionalised substrate with surface-bound initiator was exposed to a solution of P(HEA-co-MOEA) macrocross-linker without Cu catalyst. In both cases, negligible film growth was observed (see Table S1).

**Table S1** Ellipsometry data for the control experiments with no bromide initiator and no Cu catalyst.

<table>
<thead>
<tr>
<th>Control experiments</th>
<th>Film thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bromide initiator</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td>No Cu catalyst</td>
<td>1.7 ± 1.1</td>
</tr>
</tbody>
</table>
S2: \( \text{CAP}_{\text{ATRP}} \) film kinetics followed by OWLS

**Fig. S2** Kinetic profiles of the \( \text{CAP}_{\text{ATRP}} \) film formation followed by OWLS, showing evolution of (a) NTE and (b) NTM with time. Black arrows indicate injection of the \( \text{CAP}_{\text{ATRP}} \) components, whereas grey arrows indicate washing steps with sodium ascorbate buffer. Surface mass and thickness of the CAP coatings were estimated by the OWLS fitting model.

*Description of the fitting model:*
Using experimentally determined values of NTE and NTM, and known optical parameters of the waveguide layer, substrate and buffer, the thickness and the refractive index of the adsorbed layer can be calculated. Using the model and assuming that the refractive index of the adsorbed layer varies linearly with the concentration of the adsorbed material, the mass of deposited material per unit surface area can be calculated.
S3: Thickness and surface morphology of CAP<sub>ATRP</sub> films by AFM analysis

Height mode AFM images (2D (x,y) and 3D-view) of a scratched zone of the CAP<sub>ATRP</sub> film emphasise the uniform nature of the film. A z-profile analysis of this scratch (white dashed line) provided a total thickness of *ca.* 30 nm for the film which is composed of the initiating prelayer (*ca.* 15 nm) and the CAP film. Thus, AFM scratch analysis provides a CAP film thickness of *ca.* 15 nm, which is in good agreement with the thickness measured by ellipsometry (12 nm) and DPI (13.7 nm).

**Fig. S3** (a) 2D and (b) 3D height mode AFM images showing a scratched zone of a CAP<sub>ATRP</sub> film obtained after 24 h polymerisation time. (c) z-profile is associated with the white dashed line across the scratch in Fig. S3a.
S4: GPC analysis of macrocross-linker before and after CAP$_{ATRP}$ film formation

Fig. S4   GPC refractive index chromatograms of P(HEA-co-MOEA) macrocross-linker solutions before (fresh) and after the CAP process (25 h). Negligible change in macrocross-linker distribution and molecular weight characteristics demonstrates the surface-confined nature of CAP process since no polymerisation occurs in solution. Molecular weight characteristics were determined using a multi-angle laser light scattering (MALLS) detector.
S5: AFM analysis of CAP_{ATRP} films after 2 and 4 reinitiation and CAP steps

Fig. S5  Height mode AFM images showing scratched zones of CAP_{ATRP} films obtained after (a) 2 and (b) 4 reinitiation and CAP steps. (c) The z-profiles are associated with the solid red lines across the scratches. The scratched zones are located to the right of each AFM image, whereas the CAP film is located to the left.
S6: AFM z-profile analysis of CAP_{ATRP} capsules

**Fig. S6** Height mode AFM image and associated z-profile of a CAP_{ATRP} capsule. The capsule wall thickness of 15 ± 1 nm was obtained from z-profile analysis of 10 capsules.
References


