

Wear Protection Without Surface Modification Using a Synergistic Mixture of Molecular Brushes and Linear Polymers

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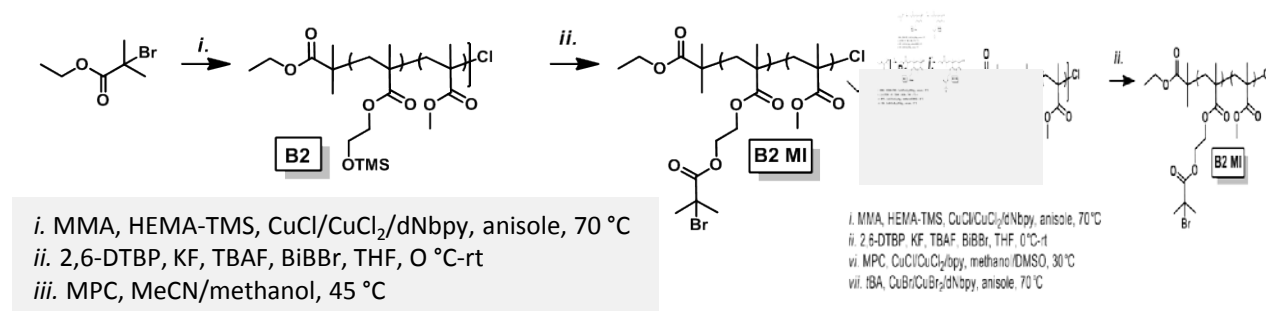
I. Synthesis of the Bottle-brush polymer

Materials: Methyl methacrylate (MMA, purity = 99%, Sigma-Aldrich, USA) and 2-(trimethylsilyloxy)ethyl methacrylate (HEMA-TMS, purity > 96%, Scientific Polymer Products Inc., USA) were passed through a column filled with basic alumina prior to use. 2-Methacryloyloxyethyl phosphorylcholine (MPC, purity \geq 97%, Sigma-Aldrich, USA) was recrystallized from acetonitrile and dried under vacuum overnight at room temperature before polymerization. Tetrahydrofuran (THF) was used after it was purified by tapping off from a solvent purification column right. Ethyl α -bromoisobutyrate (EBiB, purity \geq 98%, Sigma-Aldrich, USA), copper(I) chloride ($\text{Cu}^{\text{I}}\text{Cl}$, purity \geq 99.995% trace metals basis, Sigma-Aldrich, USA), copper(II) chloride ($\text{Cu}^{\text{II}}\text{Cl}_2$, purity \geq 99.995% trace metals basis, anhydrous, Sigma-Aldrich, USA), 2,2'-bipyridine (bpy, purity \geq 99%, Sigma-Aldrich, USA), 4,4'-dinonyl-2,2'-bipyridine (dNbpy, purity \geq 97%, Sigma-Aldrich, USA), potassium fluoride (KF, purity \geq 99%, spray-dried, Sigma-Aldrich, USA), tetrabutylammonium fluoride (TBAF, 1M solution in THF, Sigma-Aldrich, USA) and α -bromoisobutyryl bromide (purity = 98%, Sigma-Aldrich, USA) were used without any additional purification. Ruby mica-sheets were purchased from S&J Trading Inc. (Glen Oaks, NY, USA). Milli-Q quality water was obtained from a Millipore Gradient A10 S10 purification system (resistance = 18.2 M Ω .cm, TOC \leq 4 ppb). Phosphate buffer saline (10mM Phosphate, 150mM NaCl and pH 7.4) was prepared in our laboratory. Hyaluronic acids of different molecular weights were obtained from Lifecore biomedical (Minneapolis, USA). Solvents were purchased from Aldrich and used as received without further purification.

Equipment and Analysis: Proton nuclear magnetic resonance (^1H NMR) spectroscopy was performed using Bruker 300 MHz spectrometer. In all cases deuterated chloroform (CDCl_3) was used as a solvent, except for bottle-brush polymer which was analyzed using deuterated methanol (CD_3OD). ^1H chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Apparent molecular weights and molecular weight distributions measurements of polymers except bottle-brush polymer were measured by size exclusion chromatography (SEC) using Polymer Standards Services (PSS) columns (guard, 10^5 , 10^3 , and 500 \AA), with THF or DMF as eluent at 35 $^\circ\text{C}$ at a constant flow rate of 1.00 mL/min, and differential refractive index (RI) detector (Waters). The apparent number-average molecular weights (M_n) and molecular weight distributions (M_w/M_n) were determined with a calibration based on linear poly(methyl methacrylate) (PMMA) standards and diphenyl ether as an internal standard.

Bottlebrush polymer synthesis:

A molecular bottlebrush with 50% grafting density of hydrophilic (phosphorylcholine-, **PMPC B2**) grafts was prepared *via* ‘grafting from’ approach. The backbone for the brush was synthesized through equimolar copolymerization of HEMA-TMS and MMA, resulting in the polymer (**B2**) with DP~1200. GPC characterization of **B2** showed the signal with $M_n=132,000$ and low dispersity $M_w/M_n=1.16$ (Figure S2, black). The subsequent functionalization of **B2** with ATRP functionalities yielded the macroinitiator (**B2 MI**) with $M_n=163,000$ and low dispersity $M_w/M_n=1.15$ (Figure S2, red). ^1H NMR analysis of **B2 MI** was used to determine the ratio of MMA and HEMA-TMS incorporated into the polymer (Figure S1), confirming incorporation of 50 mol % of HEMA-TMS into the backbone. The spectra showed incomplete functionalization of HEMA-TMS resulting in 40 mol % of ATRP initiator sites in **B2 MI**. **B2 MI** was later used to graft hydrophilic side chains *via* ATRP, as shown on Scheme S1.



Scheme S1: Synthetic pathways for the preparation of ABA bottlebrush copolymers with PMPC (**PMPC B2**) side chains.

The grafting of hydrophilic PMPC side chains (**PMPC B2**) was performed in methanol/acetonitrile mixture (70/30, v./v. %) at 45 °C, yielding **PMPC B2** brush with the composition of (PBiBEM_{540-g}-PMPC₂₈)-*stat*-PHEMA₆₀-*stat*-PMMA₆₀₀.

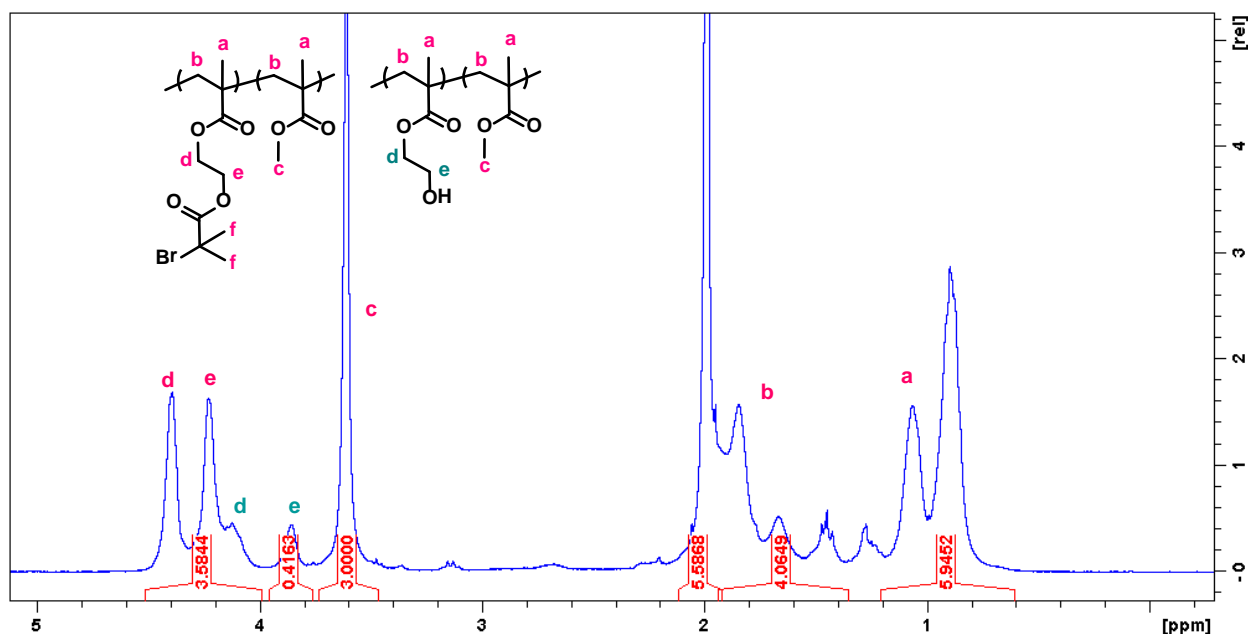


Figure S1: ¹H NMR spectra of PMMA₅₄₀-stat-PHEMA₆₀-stat-PBiBEM₆₀₀ (**B2 MI**).

Synthesis of P(HEMA-TMS)₆₀₀-stat-MMA₆₀₀ (B2). A dry 25 mL Schlenk flask was charged with ethyl α -bromoisobutyrate (EBiB) (5.8 mg, 4.4 μ L 0.030 mmol), Cu^{II}Cl₂ (3.1 mg, 0.023 mmol), dNbpv (0.113g, 0.276 mmol), HEMA-TMS (9.28g, 10.0 mL, 45.9 mmol), MMA (4.59 g, 4.9 mL, 45.9 mmol) and anisole (3.2 mL). The solution was degassed by three freeze-pump-thaw cycles. During the final cycle, the flask was filled with nitrogen and Cu^ICl (11.4 mg, 0.115 mmol) was quickly added to the frozen reaction mixture. The flask was sealed, evacuated and back-filled with nitrogen five times, and then immersed in an oil bath at 70 °C. Reaction was stopped after 67 h *via* exposure to air, reaching the degree of polymerization 1200 for the final polymer. The monomers consumption was calculated by the integration of MMA and HEMA-TMS vinyl groups signal (CHH=C-CH₃, 6.11 ppm or 5.56 ppm) against the internal standard (anisole, *o,p*-Ar-H, 6.91 ppm). The product was purified by three precipitations from hexanes, dried under vacuum for 16 h at room temperature, and analyzed by ¹H NMR spectroscopy. The ratio of PMMA (*s*, broad, CO-O-CH₃, 3.54-3.68 ppm) to P(HEMA-TMS) (*s*, broad, OCO-CH₂-, 3.90-4.17 ppm) peaks resulted in the polymer composition: P(HEMA-TMS)₆₀₀-stat-PMMA₆₀₀. Apparent molecular weights were determined using PMMA standards in THF GPC: $M_n = 132,000$ and $M_w/M_n = 1.16$ (black, Figure S2).

Synthesis of PBiBEM₅₄₀-stat-PHEMA₆₀-stat-PMMA₆₀₀ (B2 MI). The polymer, **B2**, (3.00 g, 0.017 mmol of polymer; 9.90 mmol of HEMA-TMS units), potassium fluoride (0.701 g, 11.9 mmol) and 2,6-di-*tert*-butylphenol (0.204 g, 0.99 mmol) were placed in a 100 ml round bottom flask. The flask was sealed, flushed with nitrogen, and then dry THF (30 mL) was added. The mixture was cooled in an ice bath to 0 °C, tetrabutylammonium fluoride solution in THF (1M, 0.05 mL, 0.05 mmol) was injected into the flask, followed by the drop-wise addition of α -bromoisobutyryl bromide (2.73 g, 1.50 mL, 11.9 mmol). After the addition the reaction mixture was allowed to reach room temperature and stirring was continued for 16 h. Next, triethylamine (1.0 mL) and another portion of α -bromoisobutyryl bromide (0.4 mL) were added, and the mixture was stirred for another hour. The solids were filtered off, and the solution was precipitated into methanol:water (70:30, v/v%). The precipitate was re-dissolved in chloroform and passed through a short column filled with basic alumina. The filtrate was re-precipitated three times from chloroform into hexanes and dried under vacuum overnight at room temperature. Apparent molecular weights were determined using PMMA standards in THF SEC: $M_n=163,000$ and $M_w/M_n=1.15$ (red, Figure S2).

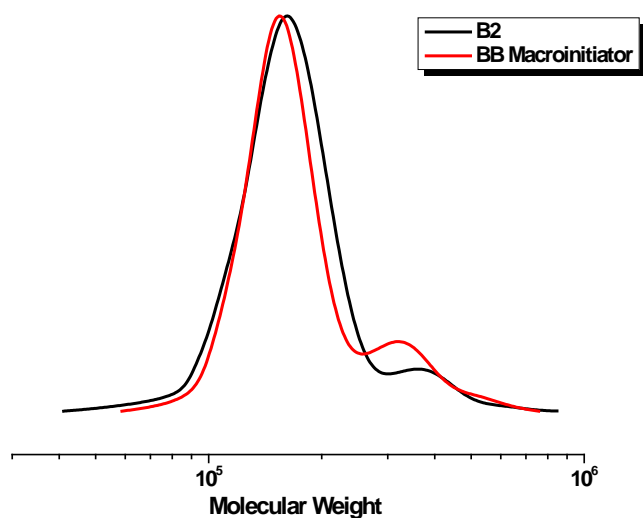


Figure S2: GPC traces recorded for (black) **B2** and (red) **B2 MI**.

Synthesis of (PBiBEM₅₄₀-g-PMPC₂₈)-stat-PHEMA₆₀-stat-PMMA₆₀₀ (PMPC B2). A dry 10 mL Schlenk flask was charged with polymer macroinitiator (**B2 MI**) (0.0059, 0.0124 mmol of BiBEM groups), 2-methacryloyloxyethyl phosphorylcholine (MPC) (1.10

g, 3.73 mmol), bpy (0.0066 g, 0.0422 mmol), Cu^{II}Cl₂ (0.33 mg, 2.5 μmol), and acetonitrile/methanol (1.0 mL/2.5 mL). The solution was degassed by three freeze-pump-thaw cycles. The flask was sealed, evacuated and back-filled with nitrogen and then immersed in an oil bath thermostated 45 °C. Then the degassed Cu^ICl solution in methanol (18.4 mg, 18.6 μmol in 1.0 mL methanol) was added to the reaction mixture. The polymerization was stopped after 1h15 min. by exposing the solution to air, achieving the brush with DP~28 of PMPC side chains as determined by ¹H NMR. The brush was purified by dialysis against methanol using a 25,000 MWCO membrane. **PMPC B2** brush was obtained as white powder. GPC characterization was not performed due to the unavailability of water GPC system. PMPC B2 brush was obtained as white powder.

II. Interaction forces in the presence of HA alone

**Properties of HA macromolecules*

The properties of the different HA samples used in this study are summarized in Table S1. Hyaluronic acid molecular weight and dispersity were assessed by aqueous SEC in 10mM PBS, pH 7.4, 150mM NaCl buffer using TSKgel columns (TSKgel G6000PW, particle size 17μm, and TSKgel G2500PW, particle size 12μm, Tosoh Bioscience LLC) at a constant flow rate of 0.5 mL/min, Multi-Angle static Light Scattering (DAWN HELEOS-II, Wyatt), and Refractometer (Optilab T-rEX, Wyatt). The absolute number-averaged molecular weights (M_n) and molecular weight dispersity (M_w/M_n) were determined with a dn/dc set at 0.16 mL/g.

Table S1: Properties of the different HA polymers used in this study

	M_w [g/mol]	Dispersity	R_g [nm] ^a	
			10 mM ^b	150 mM
10kDa HA	1.16×10^4	1.26	-	16 ± 2
60kDa HA	5.97×10^4	1.43	-	33 ± 1
300kDa HA	3.25×10^5	1.54	71	69 ± 4
1.5MDa HA	1.32×10^6	1.44	220	154 ± 12

^aMeasured by static light scattering

^bfrom ref. ^{1,2}

**Interaction forces between mica surfaces across HA solutions*

Interaction forces measured in the SFA were obtained in presence of HA solutions in pure water and in salines (Figure S3). Each interaction force profile was measured at least three times on the same contact point and repeated over multiple contact points. Prior to the first measurement, and equilibration time of one hour was set for all tested conditions.

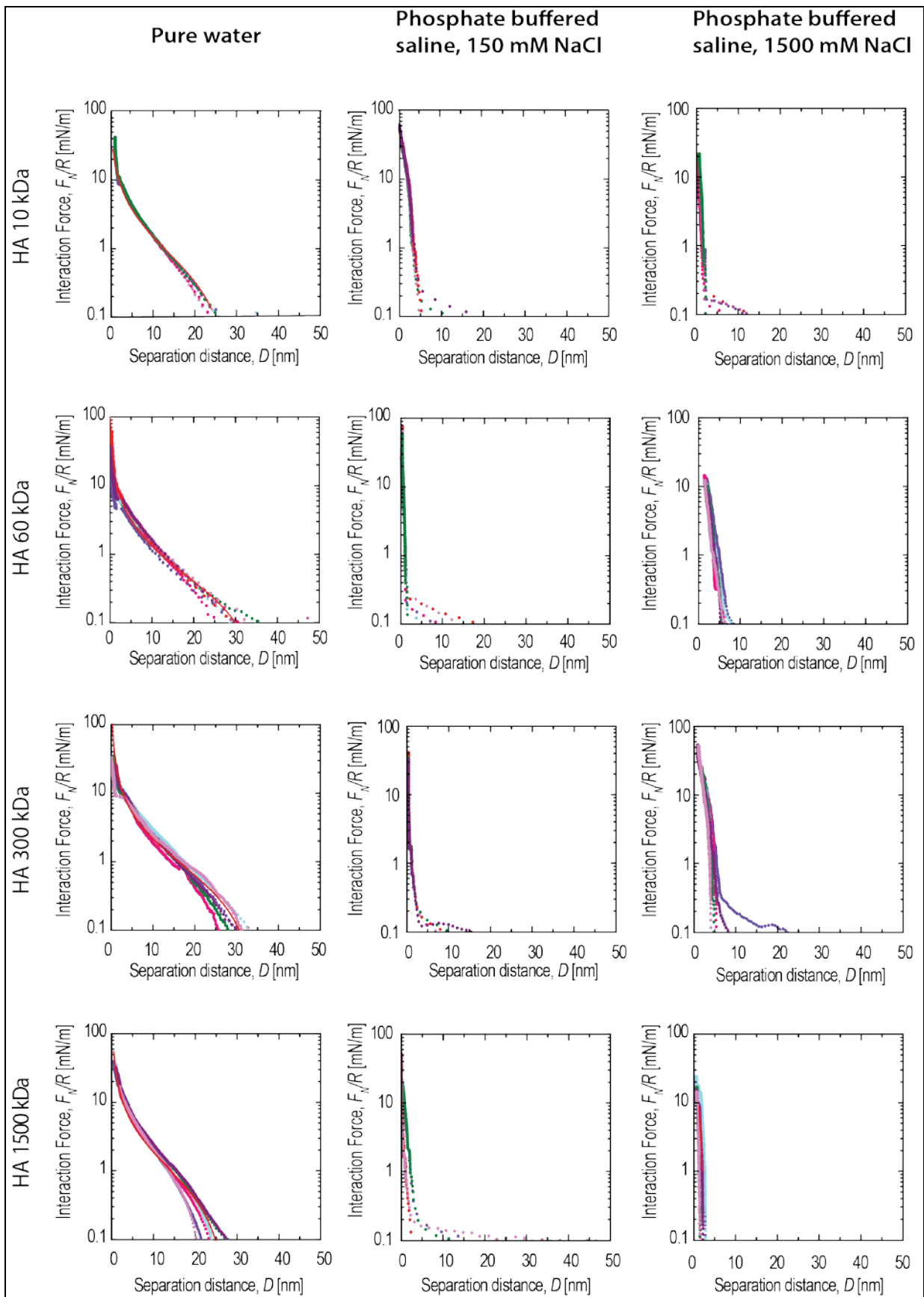


Figure S3: Interaction forces measured in presence of HA (1 mg/mL) in different media. Out run (forces measured upon separation) are not represented for clarity but presented systematically weak adhesion.

III. Interaction forces in presence of HA and BB polymer

Force profiles of the different polymer mixtures are presented in Figure S4. Each HA:BB polymer mixture was tested in three different media as described in the manuscript. Force profiles were recorded on three different contact points to ensure good reproducibility.

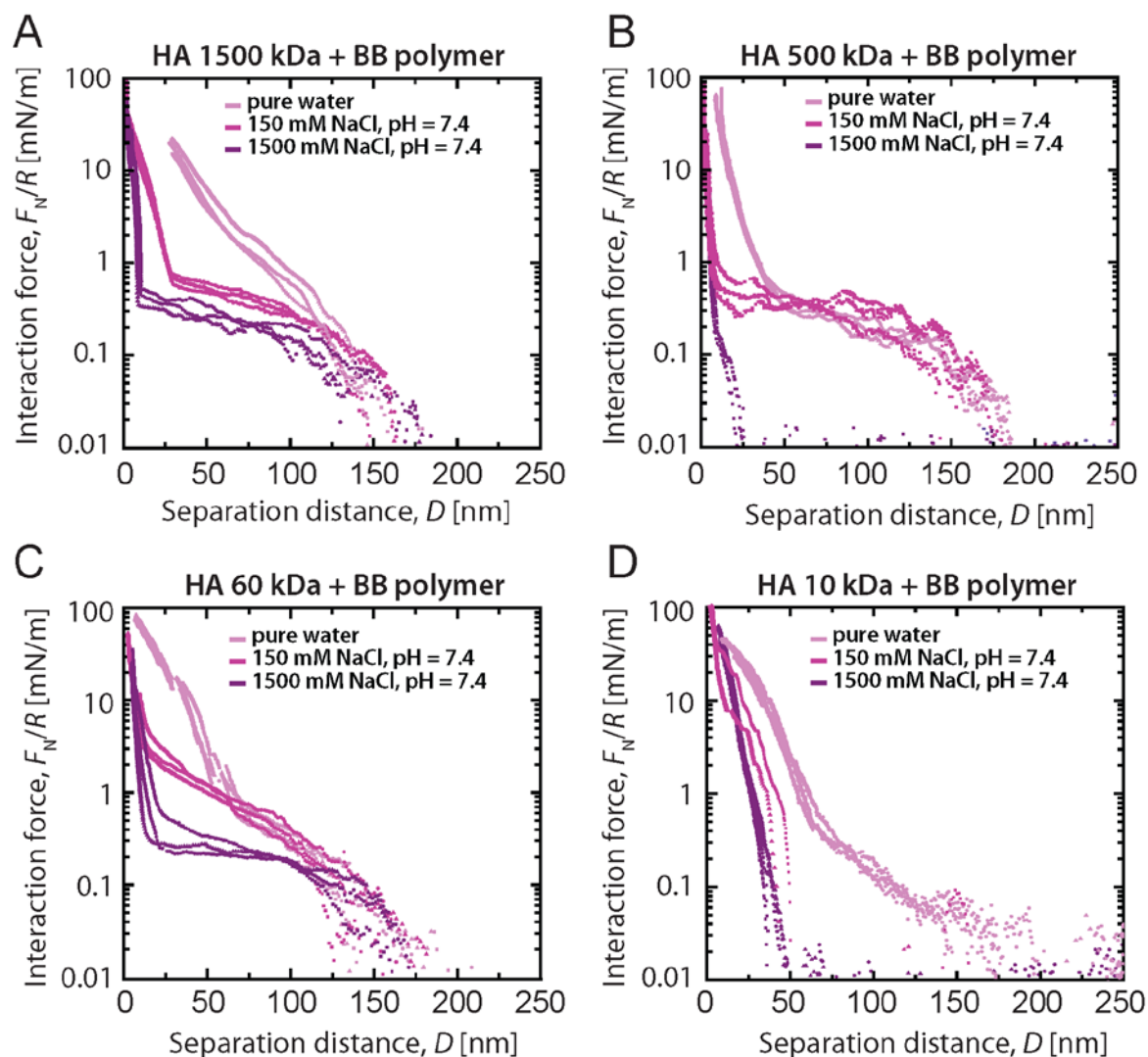


Figure S4: (A-D) Interaction forces between mica surfaces across mixtures of HA and BB polymer and fitting of the long and short range forces with a two layers (proximal-blue and distal-red) model.

IV. Evolution of the refractive index and separation distance of confined saline and HA solution during shear

To elucidate if polymer chains were still present in between the surfaces at the onset of wear, we monitored the film thickness and the refractive index of the confined film during shear (Figure S5). As the applied pressure increases (not shown in the figure) the film thickness decreases and concomitantly the refractive index of the confined film increases slightly from 1.33 (bulk water) up to ~ 1.47 . Experiments performed in PBS and in buffered HA present

the same trend suggesting that HA is quickly depleted from the contact as the normal pressure is applied.

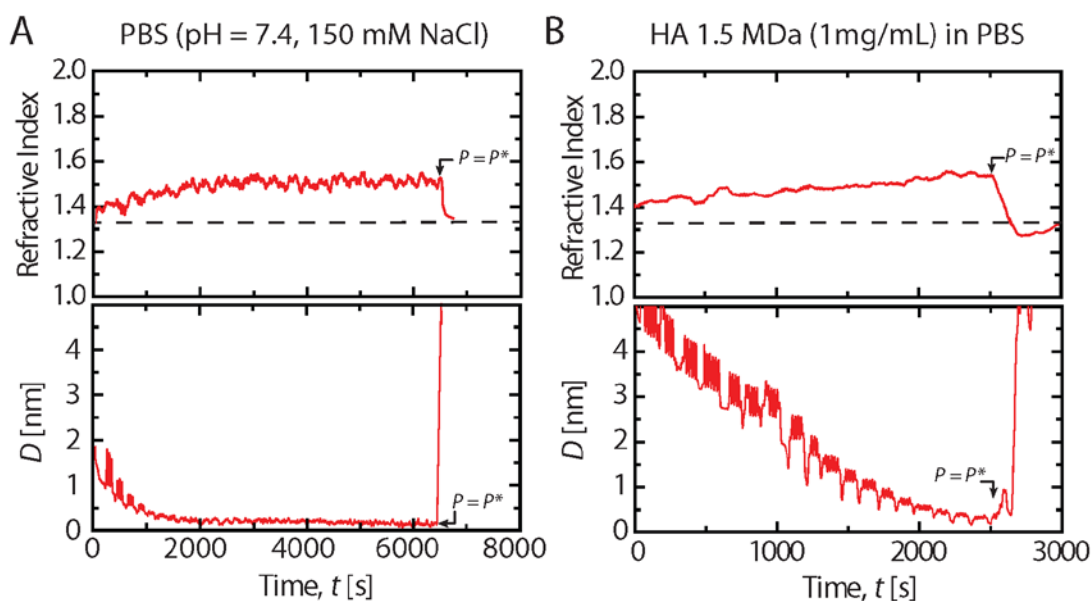


Figure S5: Measurement of the film thickness and refractive index during shear ($v_s = 3 \mu\text{m/s}$). The film thickness D decreases gradually due to the increase of the normal pressure/load during the course of the experiment. When $P = P^*$, damage of the surfaces occurs abruptly as shown by the rapid increase in D . Shearing speed $v_s = 3 \mu\text{m/s}$

V. Evaluation of BB polymer interaction with HA and PVP by microcalorimetry

Isothermal titration calorimetry of the BB polymer in different polymer solutions was performed using a VP ITC from MicroCal. running on Origin(R) 7. In the syringe, a buffered BB polymer solution was loaded at a concentration of 0.6 mg/mL and in the receptor cell, a buffered solution of HA or PVP at 1 mg/mL was loaded. All solutions have an ionic strength of 150 mM and were degassed prior use. Experiments consisted in 25 injections of 10 μL each in the receptor cell (1.42 mL) at an injection speed of 2 $\mu\text{L/s}$ and agitation speed of 300 rpm.

As a control, the BB polymer solution was also titrated in buffered saline to obtain the dilution heat of the polymer (Figure S6).

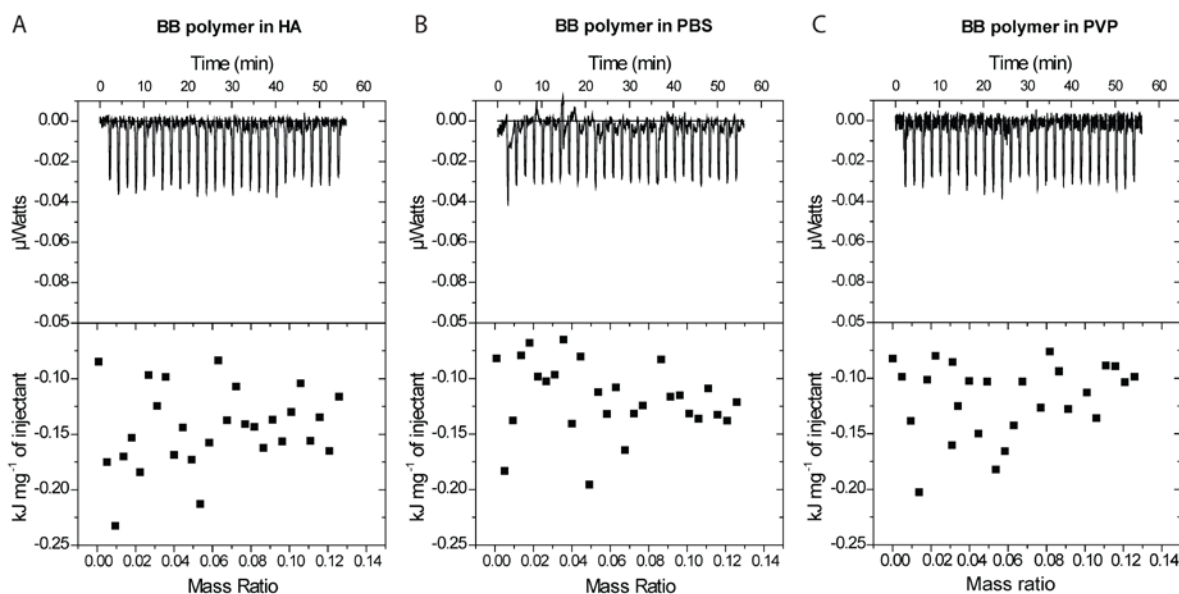


Figure S6: Differential power (top panel) and integrated released heat (lower panel) recorded during the titration of BB polymer into A) HA in buffered saline; B) buffered saline; C) PVP in buffered saline.

The experiments show no differences between the titration experiments in HA or PVP and buffered saline, which demonstrate that there is no direct interaction between the polymers.

References

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