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Instability of Surface-Grafted Weak Polyacid Brushes on Flat Substrates

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Supporting Information

ABSTRACT: We study the stability of weak polyacid brush (WPAB) gradients in aqueous media covering a range in grafting density (σ) spanning 0.05–0.5 chains/nm² using two analogous surface-anchored bromoisobuty-rate-based initiators for atom transfer radical polymerization (ATRP) bearing either an ester or amide linker. Variations in dry thickness of ester-based WPABs as a function of time and pH are consistent with WPAB degrafting via linker hydrolysis catalyzed by mechanical tension in the grafted chains. Sources of tension considered include high σ , as well as swelling and here the transfer and the presented degrafting and the stability of the stabil



electrostatic repulsion associated with increasing degree of deprotonation (α) of repeat units in the WPAB. Normalized thickness of the WPAB decreases by a maximum amount at intermediate σ between ~0.05–0.15 chains/nm², implying that contributions to tension by α are counterbalanced by charge regulation in the WPAB at high σ . Amide-based WPABs are more stable up to 264 h incubation, suggesting that commonly used ester-bearing ATRP initiators are more susceptible to hydrolysis over the time scales examined.

■ INTRODUCTION

Polymer brushes represent a specific class of macromolecules that are attached to a substrate. Polymer brushes have received extensive attention in the literature due to their relevance in a number of research fields and applications.^{1–3} Since the polymer chains are typically grafted covalently to a solid substrate, there has been particular interest in applying polymer brush systems as coatings in aqueous environments, in particular the biomedical field⁴ to direct cell adhesion,⁵ lubricate artificial joints,⁶ extend microarrays into a third dimension⁷ and prevent biofouling.⁸ This latter use is also an attractive route toward solving the outstanding issue of nonspecific biological fouling of man-made substrates.⁹ In all of these cases, the envisioned purpose of the polymer brush coating requires the coating to survive on the time scale of years, even decades.

While many brush-solvent systems are thought to be stable, i.e., the chains remain grafted to the surface during their operation, some systems have demonstrated undesired instability, which resulted in the removal of chains from the surface.^{10,11} Examples include polyelectrolytes in water^{12–16} and branched macromolecules,^{13,17} as well as polyacrylamide in methanol with a strong base.¹⁸ A proposed mechanism¹² for the instability of polyelectrolyte brushes in water, illustrated in Figure 1a, suggests that strong swelling of the brush due to electrostatic charging in the brush generates increased tension along the grafted chain backbone. This tension is ultimately

focused at the bottom-most section of the polymer brush close to the substrate.^{10,17–19} This action lowers the activation energy for breaking labile chemical bonds either in the initiator or the headgroup chemistry of the initiator that links the initiator to the substrate. For the surface-grafted initiator commonly employed with silicon substrates, [11-(2-bromo-2-methyl)propionyloxy] undecyltrichlorosilane (eBMPUS), shown in Figure 1b, two possible functional groups are liable to breakage due to hydrolysis: (1) the siloxane networks at the initiator/ substrate interface and/or (2) the ester group contained within the initiator. While some researchers have identified siloxane linkage cleavage in replacement reactions in silane monolayers²⁰ and as the point of scission in brush systems,^{13,15} there is a possibility that the ester in the initiator can also be activated for and undergo hydrolysis.^{12,18,21,22}

Regardless of the bond that undergoes hydrolysis and leads to degrafting of the polymer from the surface, one testable hypothesis that arises from this mechanism is that a weak polyacid (e.g., poly(methacrylic acid), PMAA) will exhibit increasing levels of instability with increasing pH. Since increasing pH will result in a higher degree of dissociation (α) within the polyacid brush, and thus increased solubility as well as electrostatic repulsion, the tension along the polymer

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Figure 1. (a) Strong swelling due to increased solubility and electrostatic repulsion among neighboring chains generates tension along the polymer backbone, which is focused at the grafting chemistry of the linker (green circle). This tension activates chemical bonds in the grafting chemistry for hydrolysis by hydroxide ions. When hydrolysis occurs, the chains are degrafted from the surface. (b) Possible reaction mechanisms of hydrolysis in the grafting chemistry of an ester-based, chlorosilane ATRP surface-bound initiator. (c) Increasing σ of a polymer brush increases the inherent tension of the system.

backbone will also increase. This increased tension will result ultimately in higher susceptibility of the grafting chemistry to hydrolysis. The rate of hydrolysis depends also on the concentration of hydroxide ions in solution.²³ Thus, higher pH levels should result in greater instability in a weak polyacid brush.

An additional source of tension exists in dense polymer brush systems. Because of the proximity of neighboring chains, the grafted polymer chains stretch away from the substrate.²⁴ This perturbation from the Gaussian coil conformation induces tension along the polymer chain backbone.¹⁷ Such a phenomenon is illustrated in Figure 1c, where chains in the farthest left region of the brush exhibit the least extent of stretching. As the grafting density (σ) of the polymer chains on the surface increases (i.e., moving right along the sample in the figure), the chains stretch away from the substrate due to increased excluded volume effect from neighboring chains.²⁴ When the distance between chains is significantly less than the gyration radius of the grafted chains, the chains will adopt a highly extended conformation, thus increasing tension along the chain backbone, which is supported by a single grafting point to the substrate.²⁵

In the absence of any other effects, one might expect that the densest region of the brush would show the highest levels of instability, which has been observed in a nonionic polymer brush system.¹⁸ However, σ and α are not mutually independent parameters in surface-grafted weak polyelectrolytes; σ modulates α at different regions within the brush through charge regulation.^{26–30} Briefly, charge regulation occurs when a densely grafted polymer brush does not attain the bulk α value predicted from solution pH due to strong crowding and stretching near the substrate. As a result, the degree of dissociation in weak polyelectrolyte grafts close to the substrate is lower than that in the upper portion of the brush. Therefore, in a weak polyelectrolyte brush, σ and α may influence the swelling behavior due to increased polymer solubility and electrostatic repulsion, and thus increased tension

leading to potential brush degrafting from the substrate, in ways that are very challenging to predict.

In this article, we examine the stability of weak polyelectrolyte brushes with a gradient in σ at different pH levels. Employing a sample design that facilitates smooth variation of σ , i.e., polymer assemblies whose areal density ranges from densely to sparsely grafted, enables systematic investigation of a broad parameter space with a minimum of materials, effort, and sample-to-sample variations. We use these samples to address the aforementioned hypothesis for densely grafted weak polyacid brushes and examine simultaneously the interplay between σ and α . Furthermore, we make use of two analogous atom transfer radical polymerization (ATRP) initiators possessing either an ester or amide functional group in order to provide insight on the bond primarily responsible for degrafting.

EXPERIMENTAL SECTION

General Methods and Materials. Ethanol, dimethyl sulfoxide (DMSO), diethyl ether (Et₂O), *tert*-butyl methacrylate (tBMA), N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA), CuCl, CuBr, trifluoroacetic acid (TFA), dichloromethane (DCM), magnesium sulfate (MgSO₄), triethylamine (Et₃N), undecyl bromide, 2-bromo-2-methylpropionyl bromide, lithium aluminum hydride (LiAlH), sodium methacrylate (NaMA), bipyridine (BiPy), and inhibitor remover packing were purchased from Sigma-Aldrich and used as received. Monosodium phosphate and disodium phosphate were purchased from Fisher Scientific and used as received. *n*-octyltrichlorosilane (OTS) was purchased from Gelest and used as received. The 0.5 mm thick, 100 mm diameter silicon wafers (orientation [100]) were purchased from Silicon Valley Microelectronics.

Sample Preparation. *Initiator Synthesis.* Ester BMPUS (eBM-PUS), [11-(2-Bromo-2-methyl)propionyloxy] undecyltrichlorosilane (eBMPUS) was synthesized according to the method of Matyjaszewski and co-workers.³¹ Amide BMPUS (aBMPUS) was synthesized by following the procedure outlined below (see also Scheme 1). 10-

Scheme 1. Synthesis of aBMPUS



undecenyl azide (compound 1 in Scheme 1) was prepared by dissolving/suspending NaN₃ (1.63 g, 25.08 mmol) in DMSO (50 mL) at 25 °C. Undecylbromide (5 mL, 22.80 mmol) was added and the resulting reaction mixture was stirred at room temperature until starting material was fully consumed (6 h). Reaction progress was followed by GC analysis. The reaction was quenched with H₂O (100 mL) [slightly exothermic] and stirred until it cooled to room temperature. The mixture was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with water $(2 \times 40 \text{ mL})$, brine $(1 \times 40 \text{ mL})$, and dried over MgSO₄. After removing solvent in vacuo, the crude product 1 in Scheme 1 was obtained as a colorless oil and was used in next step without further purification. To produce 1amino-10-undecene (compound 2 in Scheme 1), a solution of the crude azide 1 in dry diethyl ether (30 mL) was added drop-wise to a stirred mixture of LiAlH₄ (1.45 g, 38.20 mmol) in dry diethyl ether (70 mL) at 0 °C. The mixture was allowed to attain room temperature, stirred for 3 h, and then carefully quenched by the addition of H_2O (4 mL), 15%, NaOH (4 mL), and H₂O (10 mL), followed by filtration. The filtrate was washed with H_2O (2 × 20 mL), brine (1 × 20 mL), and dried over MgSO4. After removing solvent in vacuo, the crude product 2 in Scheme 1 (4.3 g) was obtained as a yellowish oil (used in next step without further purification). N-(10-undecenyl) 2-bromo-2methylpropanamide (compound 3 in Scheme 1) was prepared as follows. A solution of the crude amine 2 (4.3 g) and Et₃N (4.2 mL, 29.60 mmol) in dichloromethane (80 mL) was cooled down to 0 °C under atmosphere of argon. 2 bromo-2-methylpropionyl bromide (2.82 mL, 22.80 mmol) was added drop-wise to this solution. Resulting reaction mixture was stirred 30 mins at 0 °C. The reaction was then quenched with water (10 mL), and after separation, the organic layer was washed with 3% HCl (20 mL), 1:1 H₂O and brine mixture (30 mL), brine (20 mL) and dried over MgSO4. After removing solvent in vacuo, the crude product was purified by column chromatography (hexane: Et_2O :acetone = 10:1:2) to afford 6.65 g amide 3 in Scheme 1 as a colorless oil (92 % overall yield after 3 steps). ¹H NMR (400 MHz, CDCl₃) δ 6.70 (bs, 1H), 5.78 (ddt, I =16.9, 10.2, 6.7 Hz, 1H), 4.97 (ddt, J = 17.1, 3.7, 1.6 Hz, 1H), 4.90 (ddt, J = 10.2, 2.3, 1.2 Hz, 1H), 3.23 (td, J = 7.1, 5.8 Hz, 2H), 2.05 - 1.97 (m, 2H), 1.93 (s, 6H), 1.51 (p, J = 7.3 Hz, 2H), 1.41 - 1.20 (m, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 172.0, 139.4, 114.3, 63.9, 40.7, 34.0, 32.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 27.0. HR HR-MS (ESI) m/z calculated for C15H28ONBrNa: 340.1247, found 340.1246. Finally, [11-(2-bromo-2-methyl)propanamide] undecyltrichlorosilane (aBM-PUS) was obtained by hydrosilylation of compound 3 in Scheme 1. To a solution of 0.5 g of 3 in 10 mL trichlorosilane was added 1 or 2 drops of Karstedt catalyst (platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex, 3 wt% solution in xylenes). The solution was heated to reflux for 5 hours. The excess silane was then removed by distillation. Anhydrous dichloromethane (distilled over activated molecular sieves) was added, and the product solution was filtered through a plug of anhydrous sodium sulfate under nitrogen. Dichloromethane was removed by vacuum, and the product was dried under vacuum and weighed. Anhydrous toluene (stood over activated molecular sieves) was added to make a 5 wt% solution. The solution was transferred to vials and stored in a freezer until use.

Initiator Deposition. Silicon wafers were cut into 4.5 cm × 5 cm rectangles and were sonicated in methanol, dried with a stream of N2 gas, and treated in a UV-ozone apparatus for 20 min. For initiator gradient samples, wafers were then placed horizontally next to a well containing a mixture of OTS in mineral oil (1:4) for 7 min. After OTS deposition, the wafer was immediately placed into a solution of 30 μ L of 5 vol % eBMPUS in anhydrous toluene and 30 mL of anhydrous toluene and incubated at -20 °C overnight. An identical approach was used for aBMPUS. Our experience suggests it is important to use ethanol for the sonication of aBMPUS-modified wafers (as opposed to methanol or toluene, for example) to remove properly physisorbed material. For homogeneous initiator substrates used to form molecular weight gradients, no OTS deposition step was performed. The wafer was then removed from solution, rinsed with ethanol, dried with a stream of N2 gas, then sonicated in ethanol for 20 min and dried with a stream of N2 gas. The wafer was then immediately analyzed by contact angle using deionized (DI) water as a probing liquid, then dried with a stream of N₂ gas before polymerization.

PMAA Brush Synthesis from Sodium Methacrylate (NaMA). Silicon wafer samples functionalized with either eBMPUS or aBMPUS were inserted in solutions containing CuBr (80 mM), CuBr₂ (16 mM), bipyridine (200 mM), and 6 M sodium methacrylate in DI water, which had been titrated to pH 9 using HCl and degassed by bubbling with N₂ gas for 30 min. For grafting density gradient samples, the samples were removed after 24 h, rinsed copiously with DI water, and dried under a stream of dry N₂ gas. To produce molecular weight gradient samples, substrates were immersed into the polymerization solution at a controlled speed using a dipping apparatus described previously.³² The polymerization followed the surface-initiated (SI) ATRP reaction scheme. The polymerization time at a given point on the wafer depends on the speed of the immersion process. The polymerization was run such that the longest period of immersion was 30 min.

PMAA Brush Synthesis from tert-Butyl Methacrylate (tBMA). The polymerization solution comprised 45 mL of tBMA (purified by passing through a column containing inhibitor remover), 45 mL of DMSO, 40 μ L of PMDETA, and 0.058 g of CuCl ([tBMA]:[CuCl]: [PMDETA] = 470:1:0.33). This solution was degassed by bubbling with N₂ gas for 30 min, then charged to a custom-built glass reactor containing an initiator-modified wafer using a degassed glass syringe. The SI-ATRP proceeded for 1 h, at which point the reactor was opened and the wafer removed. The wafer was rinsed thoroughly with ethanol, then sonicated in ethanol for 20 min, followed by drying with a stream of N₂ gas. The PtBMA brush was then characterized using ellipsometry, followed by hydrolysis for a total of 40 min by a 50 vol % solution of TFA in DCM to yield the PMAA brush.

Incubation Experiments. Buffer solutions with strengths of 10 mM were prepared using sodium phosphate salts and adjusted to pH 4, 7.4, and 9 using minute quantities of HCl and KOH. The pH of each buffer was measured to ± 0.02 using an Accumet AB15 pH meter (Fisher Scientific) equipped with a platinum pH electrode. Periodic measurement of the buffer solutions were done to ensure no pH drift occurred. The parent polymer brush samples were segmented into 1 cm wide specimens and placed individually into glass vials containing buffer solution that had been filtered using 0.2 μ m syringe filters. The vials were then sealed and stored in the dark at room temperature for the duration of the incubation. After a certain incubation time (i.e., 24 or 120 h), the samples were removed, rinsed briefly with DI water and dried with a stream of N₂ gas and stored for further characterization.

Characterization Techniques. Spectroscopic Ellipsometry. Measurements were performed on a variable angle spectroscopic ellipsometer (J.A. Woollam Co.) controlled by WVASE32 software (J.A. Woollam Co.). For brush thicknesses >30 nm, data were collected at incidence angles of 65, 70, and 75° over wavelengths ranging from 400 to 1000 nm. These data were fit to a model comprising a Si substrate, SiO_x layer (thickness 1.5 nm), and a Cauchy layer. The Si and SiO_x layers used material files supplied with the WVASE32 software. The Cauchy layer was fit using thickness, and the Cauchy parameters A_n and B_n . For thicknesses <30 nm, the thickness and Cauchy parameters cannot be independently fit. Thus, data were collected at 632.8 nm over a range of incidence angles from 60 to 80° in 1° increments. These data were fit to a model comprising a Si substrate, SiO_x layer (thickness 1.5 nm) and a Cauchy layer. The Cauchy parameters, A_n and B_n , were held constant using values obtained at the thickest part of the brush. Only thickness was used as a fitting parameter.

Infrared Variable Angle Spectroscopic Ellipsometry (IR-VASE). Measurements were performed on an IR-VASE (J.A. Woollam Co.) controlled by WVASE-IR software (J.A. Woollam Co.) at a 50° angle of incidence with a resolution of 4 cm⁻¹.

Atomic Force Microscopy. Surface topography measurements were conducted in air using an Asylum MFP-3D system (Asylum Research) in tapping mode using Si tips (Model AC160TS; Asylum Research) with a resonance frequency of 300 kHz and spring constant of 42 N/m. Scans were collected over an area of 4 μ m × 4 μ m at a scan rate of 1.0 Hz and resolution S12 lines/scan. Data were analyzed in the Gwyddion software package.³³

Contact Angle Goniometry. Contact angles of DI water were measured using a Ramé-Hart goniometer. A water droplet of volume $6-8 \ \mu L$ was dispensed on the surface, and the contact angle of the droplet determined using automated computer software. Measurements were collected at various points along the Si substrate, and the location of these measurements were determined using a rule placed adjacent to the substrate during measurements.

RESULTS AND DISCUSSION

The data in Figure 2 plot the *ex situ* dry thickness of two gradient polymer assemblies, determined by spectroscopic ellipsometry, against either polymerization time for the molecular weight (MW) gradient sample (panel a) or against

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Figure 2. Thickness profiles derived from ellipsometric data for a PNaMA brush expressing either a gradient in MW (left panel) or σ (right panel). After a certain MW or σ level, there is a decrease in the dry thickness of the grafted polymer layer. The lines are intended as guides for the eye.

the expected grafting density (σ ; calculation described in Supporting Information) for the σ gradient sample (panel b). These samples were prepared using eBMPUS initiators, which contain an ester functional group as part of the initiator

molecule. These SI-ATRP were conducted at pH 9, well above the pK_a of PMAA.³⁴ Thus, the polymer brush is expected to exhibit significant charging.

The MW gradient sample exhibits an increase in thickness with increasing polymerization time through about 20 min, which is expected for ATRP reactions.³⁵ At polymerization times longer than 20 min, when the brush has reached an *ex situ* thickness in excess of 200 nm, there is first a reduction in the rate of thickness increase, followed by a decrease in thickness with increasing polymerization time. In the σ gradient sample, an analogous phenomenon occurs, wherein the thickness of the brush first increases with increasing σ . Interestingly, this value of σ appears to be at a point when the brush would exceed 200 nm in *ex situ* thickness, which is comparable to a critical thickness observed in poly(hydroxyethyl methacrylate) (PHEMA) polymer brushes.¹⁶

The dry thickness of a polymer brush (h) scales as the product of MW and σ , i.e., $h = (MW^*\sigma)/(\rho^*N_A)$, where ρ is polymer density and N_A is Avogadro's number. Thus, in order for the polymer brush to exhibit a thickness decrease, either MW or/and σ must decrease. Since the aliphatic backbone of methacrylate polymers is unlikely to break down under the current experimental conditions, the brush MW should remain constant. In contrast, the silane bonding chemistry between the initiator and silicon substrate or the ester group in the eBMPUS initiator may undergo hydrolysis. Recent work has illustrated



Figure 3. (a) Thickness values derived from VASE data show the height profile of an eBMPUS-based brush expressing a gradient in σ as it is modified from PtBMA (green) to PMAA (orange) and subsequently incubated in pH 9 buffer for 24 h then dried and measured in air (black). (b) IR-VASE data providing chemical information on the modification of PtBMA (green) to PMAA (orange) and subsequent incubation at pH 4, 7.4, and 9 for 24 h (blue, red and black, respectively). (c) "Dry" ellipsometric thicknesses after 24 h of incubation normalized by preincubation thicknesses for samples incubated at pH 4, 7.4, and 9 for 24 h (blue, red, and black, respectively). The lines are intended as guides for the eye.

that this bond can be activated for hydrolysis by mechanical tension.²¹ Furthermore, swelling of polymer brushes can generate significant tension along the polymer backbone, which focuses at the grafting point of the polymer brush.^{17,19} This tension is directly proportional to the MW of the grafted chains, and longer chains of a nonionic polymer in the presence of a strong base have been shown to cleave preferentially from a solid substrate.¹⁸ In the case of polyelectrolytes, pH-induced swelling can produce sufficient tension to activate the grafting chemistry for hydrolysis.^{12,15,17,19}

The σ of a polymer brush can also generate tension. At low σ , when the distance between neighboring chains (*d*) is much greater than the gyration radius of the grafted chains (R_g), the grafted chains will adopt a Gaussian conformation. At high σ , when $d \ll R_g$, the chains extend normal to the substrate due to excluded volume interaction. This extension will induce tension along the chains and can lead to degrafting of the tethered chains.¹⁸

Since both brushes were polymerized at pH 9, brush swelling due to electrostatic repulsion and highly solubilized chains generates tension along the chain backbone. This tension is modulated by variations in the MW and σ levels of the grafted chains. When critical values of these parameters are exceeded, sufficient tension is focused at the grafting point of the brush to catalyze hydrolysis either in the silane headgroup or the ester bond of the initiator. We hypothesize, then, that the thickness reductions observed in Figure 2 are due to a reduction in σ as a result of chains degrafting from the substrate.

The continued reduction in thickness after the critical values for both samples is worth further consideration, since one might expect the brush to obtain an equilibrium thickness at the critical values of MW and σ . In the case of the MW gradient, the continued reduction in σ (i.e., thickness) at longer polymerization times, and thus higher MWs, may be due to the longer incubation period in the pH 9 polymerization solution. The σ gradient sample requires a more subtle explanation, since all points of the sample experience identical polymerization/incubation times. However, denser regions of the sample will reach the critical stretching level sooner than less dense regions of the brush. These denser regions will experience a longer period of incubation above this critical level, and thus demonstrate a greater extent of degrafting.

To further explore the influence of σ on stability of weak polyacid brushes, we opted for a nonaqueous synthesis route using *tert*-butyl methacrylate (tBMA) and subsequent hydrolysis of PtBMA brushes using trifluoroacetic acid (TFA) in dichloromethane.³⁶ To minimize systematic errors among multiple samples, "parent" polymer brushes of PtBMA were synthesized on a large substrate, which was then cut into smaller segments and individual samples derived from this "parent" sample were subjected to hydrolysis reaction for various incubation times at different pH values.

The thickness of a representative, eBMPUS PtBMA brush sample plotted as a function of distance from the OTS reservoir is shown in Figure 3a (green). This specific sample is used for pH 9 incubation studies and is presented as an example, while the data for the other samples are presented in the Supporting Information. A smooth variation in thickness as a function of position on the substrate is seen in each sample, and is consistent with the variation. Assuming that the MW of the grafted polymer chains is comparable along the substrate (i.e., the polymerization rate does not depend dramatically on the density of the initiator centers), the differences in thickness of the PtBMA brush originate from differences in σ and ultimately from the variation in BMPUS fraction on the substrate. The IR-VASE data shown in Figure 3b, which plots the ellipsometric parameter Ψ , confirm the chemical identity of the grafted polymer layer as PtBMA based on the green data in the panel labeled "PtBMA". Previously, the parameter Ψ has been shown to be sensitive to the chemical functional groups on a surface.³⁷ The distinct carbonyl peak at 1720 cm⁻¹ is clearly visible and characteristic of methacrylate-based macromolecules.³⁸ The series of peaks located between 2900 and 3000 cm⁻¹ are associated with the multiple C–H bonds in the *tert*-butyl group in the polymer side chain.

The thickness maximum at 1.5 cm (abscissa) is a real feature based on visual inspection of the samples. A darker coloration, consistent with a thicker polymer brush, is visible just after the gradient region (position <1.5 cm) compared to the lighter coloration in the homogeneous portion of the brush (position >2.0 cm). Importantly, this feature appeared in the PtBMA brush, prior to any further chemical modification or exposure to water. We do not have a clear explanation for this result at this time.

The orange data in Figure 3a depict the thickness of the PMAA samples derived by hydrolyzing the PtBMA brushes with TFA. For all samples, the shape of the PMAA thickness profile replicates that of the original PtBMA brush, in which thickness of the chains increases with increasing position along the wafer (i.e., increasing σ). The reduction in thickness stems from the removal of the bulky tert-butyl group from the polymer side chain. The homogeneous portion of the samples (i.e., small variation in σ ; position >2.0 cm) shows a consistent reduction in thickness for all three samples to ≈ 0.58 of the PtBMA brush thickness, suggesting uniform modification across all samples. This level of reduction also suggests that nearly all of the PtBMA repeat units have been hydrolyzed to PMAA, and degrafting of the chains is not substantial during this modification step (cf. Supporting Information). The gradient portion of the samples (position on the abscissa <1.5 cm) follows a less consistent pattern, and does not indicate a clear trend in behavior. Note that these samples were not exposed to aqueous solution prior to the measurements from which these data are derived. While two of the samples show a smaller thickness reduction in the gradient region compared to the homogeneous region, the remaining sample shows a greater thickness reduction. The origin of these differences is not apparent from the data.

This chemical modification is confirmed by the orange data in Figure 3b, in the panel labeled "PMAA", wherein the peaks near 3000 cm⁻¹ associated with the tert-butyl group are reduced, with residual signal at this wavenumber due to the methyl group on the polymer backbone. A small, broad peak has appeared at \sim 3400 cm⁻¹, which is characteristic of hydroxyl stretches associated with water.³⁸ The continued presence of the carbonyl peak at $\sim 1700 \text{ cm}^{-1}$ confirms that the brush layer has "survived" the hydrolysis reaction, and it exists in its protonated state (vide infra). The reduction in intensity of this peak may originate from either reduced concentration of carbonyl groups at the surface due to chain degrafting, or due to a reduction in film thickness. We note these data are derived from ellipsometric measurements, and thus film thickness will influence their value. In either case, all subsequent samples were treated equally to this point, and thus start from the same initial condition prior to pH buffer incubation.

Following hydrolysis of the PtBMA brushes to PMAA brushes, the samples were incubated in phosphate buffer solutions at pH 4, 7.4, or 9 (all 10 mM of total salt) for 24 h. After incubation, the samples were removed from the buffer solutions, dried by a stream of N_2 gas, and characterized by ellipsometry. The black data in Figure 3a plots the resulting thickness of the brush incubated at pH 9 as a function of substrate position. There is a clear increase in sample thickness following 24 h incubation, which is also observed in the sample incubated at pH 7.4. The sample incubated at pH 4 shows no significant change in thickness.

The IR-VASE data presented in Figure 3b confirm residual charging within the brushes incubated at pH 7.4 and 9 following removal from incubation solution. The data for the pH 4 sample (blue) do not show any distinct difference from the PMAA brush prior to incubation (orange). In contrast, the pH 7.4 (red) and pH 9 (black) data exhibit two significant changes in the Ψ data. First, the carbonyl peak at ~1700 cm⁻¹ has diminished in intensity, while a peak at \sim 1550 cm⁻¹ has appeared. This latter peak is associated with carboxylate groups in PMAA, and has been used to track changes in situ in PMAA brushes as a result of changing pH.³⁷ The second change in the Ψ data is the increased intensity of the peak at ~3400 cm⁻¹, which is associated with the hydroxyl stretch in water. Thus, we conclude that incubation of the PMAA brushes at pH 7.4 and pH 9 leads to dissociation of the proton in the pendant carboxylic acid groups, resulting in charging along the brush. Even after removing the samples from the buffer solutions, rinsing with DI water and drying with N2 gas, these charges remain stabilized, possibly due to complexation with sodium ions from the buffer salts. These residual charges lead to the presence of water in the weak polyelectrolyte brush in ambient lab conditions.^{39–41} Whether this water is retained in the brush during the washing procedure or absorbed from the atmosphere following drying is not clear from the data.

To more readily compare across pH values, Figure 3c plots the thickness for all three pH levels normalized by the initial thickness. The sparsest region of the brushes shows no significant increase in thickness for all pH values. The sample incubated at pH 4 exhibits a slight increase within the gradient, and essentially no swelling in the homogeneous portion of the substrate. In contrast, the samples incubated at pH 7.4 and pH 9 show a trend of increasing swelling with increasing wafer position. Furthermore, the sample at pH 9 exhibits a higher swelling factor than the pH 7.4 sample. This finding is consistent with expected differences in charging within the brushes incubated at these pH levels. Although free PMAA chains in aqueous solutions exhibit a p K_a of $\approx 5.0-5.5$ (i.e., well below pH 7.4 and pH 9), charge regulation shifts the pK_a of the polyacid brush to a higher value and leads to a gradient in pK_a along the length of the brush.²⁸ As a result, measured values of $pK_a \approx 7.0$ for the "bulk" PMAA brush and $pK_a \approx 4.6$ for the brush/water interface have been reported.³⁴ As α inside the brush increases with increasing pH, the brush thickness increases as well due to repulsion among charges within the brush and increased solubility of the charged polymer chains. This increased charging persists following the removal from incubation solution (vide supra) and leads to a greater normalized thickness when "dry". If Na⁺ ions are complexed with the residual, charged carboxylate groups, then an increased quantity of these ions may also contribute to the greater thickness at pH 9.

Figure 4 presents atomic force microscopy (AFM) images collected from the dense portion of the initial PtBMA and



Figure 4. AFM micrographs of the PtBMA brush and PMAA brush prior to incubation, as well as the "dry" PMAA brushes following 24 h incubation at pH 4, 7.4, and 9. Scale bars represent 1 μ m in length. The lower panel plots the RMS roughness value derived from each micrograph.

PMAA brushes, and the samples incubated at pH 4, pH 7.4, and pH 9 for 24 h. The root-mean-square (RMS) roughness calculated from each of these images is plotted in the lower panel. The morphology of the PtBMA and PMAA brushes is similar, although the PMAA brush exhibits a marked reduction in the RMS from 1.85 to ~1.0 nm for PtBMA. The sample incubated at pH 4 for 24 h shows little change from the preincubation RMS roughness value, consistent with the results seen from VASE and IR-VASE measurements, and presents a comparable morphology. The samples incubated at pH 7.4 and 9 exhibit a significant reduction in RMS roughness value to \sim 0.4 nm, accompanied by changes in the surface morphology. These findings confirm that the grafted film structure has been altered as a result of incubation in different pH buffers, and that these changes persist following standard drying procedures. Given the film swelling and residual moisture content seen in the VASE and IR-VASE data, we reason that the samples incubated at pH 7.4 and pH 9 present a flatter polymer/air interface. This smoothness may be induced by an attempt to

minimize the surface area between the partially hydrated polymer film and air, which is hydrophobic.

The samples were further incubated and examined *ex situ* using VASE at periodic time intervals. The fitted thickness from the ellipsometric data for the densest portion of the brush (position 3.0 cm along the abscissa) are plotted in Figure 5.



Figure 5. Evolution of dense brush thickness for samples incubated in pH 4, 7.4 and 9 buffer solutions (black, red and blue data, respectively). The lower illustrations present a potential explanation of the data in Figure 3 and the dense brush incubation data contained in this figure. Starting from the upper left illustration, the sequence follows the numbered arrows. See the text for a detailed discussion of each step. The lines are intended as guides for the eye.

The data are normalized to the swollen "dry" thickness observed at 24 h (cf. Figure 3c). Prolonged incubation leads to a strong dependence of *ex situ* thickness on pH. The sample at pH 4 stays essentially unchanged over a period of several weeks. In contrast, the samples at pH 7.4 and pH 9 show a reduction in normalized thickness. On an absolute basis, the thickness at the longest incubation period (554 h) at pH 9 is below the initial, preincubation PMAA thickness. This point suggests strongly a removal of chains from the substrate as the origin of this thickness reduction. Furthermore, on a normalized basis, the sample incubated at pH 9 shows a greater thickness

reduction than pH 7.4. Thus, the data indicate that the stability of a densely grafted, weak polyacid brush decreases with increasing pH. Increasing pH of the buffer solution increases α within the brush, as confirmed by the IR-VASE data, resulting in higher levels of tension at higher pH. This correlation supports the hypothesis put forth previously¹² and illustrated in Figure 1, namely, that tension generated by strong swelling due to electrostatic repulsion and increased solubility activates the surface grafting chemistry for hydrolysis, the rate of which increases with increasing pH.

In the lower panel of Figure 5, we present schematics illustrating the mechanism seen in our data. Starting with the upper left illustration and following the path of arrow 1, a PMAA brush with initial thickness h_0 is incubated in a buffer of given pH. The thickness h_0 corresponds to the orange data in Figure 3a. During incubation, the polymer chains attain a certain α , and Na⁺ ions from the buffer salt penetrate into the brush to screen the charges on deprotonated acid groups. Moving along the path of arrow 2, removal of the sample from the incubation solution and drying leads to residual charging and moisture within the brush, stabilized by the Na⁺ ions, resulting in a swollen "dry" thickness of h_1 . The thickness h_1 corresponds to the black data in Figure 3a, and the data point at 24 h in Figure 5. Continuing along arrow 3, upon further incubation of this sample, tension generated along the polymer chain by strong swelling due to charge repulsion and increased solubility leads to hydrolysis of the grafting chemistry at the substrate/polymer interface. Finally, following arrow 4, the degrafting process leads to a localized reduction in σ , resulting in relaxation of the neighboring polymer chains following removal from solution, which is observed as a reduction in thickness, as illustrated for thickness h_2 . The thickness h_2 corresponds to the various time points in Figure 5.

This mechanism raises the question of how removal of a grafted chain affects the stability of adjacent chains. The reduction in σ will result in a local reduction of tension of the remaining chains, which suggests the adjacent chains are less likely to degraft. However, in weak polyelectrolyte brushes, such as the ones studied here, α is known to be a function of σ (as well as distance along the brush's vertical direction) through charge regulation.^{26–28} As a result, due to diminished charge regulation, the reduction in σ may lead to an increase in α relative to the densest regions of the brush, leading to increased tension that promotes degrafting.

Figure 6 displays the thickness derived from ellipsometric data for the entire length of the eBMPUS σ gradient samples incubated at pH 9 through 554 h. Data for pH 4 and pH 7.4 are available in Figure S6 of the Supporting Information. The data are normalized to the thickness measured after 24 h of incubation (red). The data in Figure 5 are in the cluster of data points near 52 nm PtBMA thickness. In addition, we plot the results from aBMPUS σ gradient samples incubated through 264 h. These initiator species are shown in Figure 7. We have plotted the thickness as a function of initial PtBMA thickness, which is a proxy for σ of the PMAA brush. Ideally, in order to elucidate the effect of σ on the stability of PMAA brushes, we would plot changes in normalized thickness as a function of σ of the PMAA brush. Unfortunately, there is currently no known way to measure σ nondestructively.^{42,43}

As mentioned earlier, in a grafted polymer film, the dry thickness (h) is proportional to MW* σ . Assuming that the MW of the PtBMA chains does not depend on σ , any variation in h is due primarily to variation in σ . Therefore, we plot the PMAA



Figure 6. Thickness profiles of PMAA brush samples prepared using eBMPUS and aBMPUS expressing a gradient in σ incubated at pH 9 normalized by the thickness measured for each point at 24 h (red). The initial PtBMA thickness for each sample is used as a proxy for the σ level of each sample (see text). The estimated σ level derived from the PtBMA thickness is plotted on the upper *x*-axis (see text). The left panel presents data for eBMPUS initiators and the right panel data for aBMPUS initiators. Data for pH 4 and pH 7.4 are available in the Supporting Information. The lines are intended as guides to the eye.



Figure 7. Initiator species, eBMPUS and aBMPUS, used to examine the susceptibility of the linkage group (i.e., ester vs amide) in BMPUS to hydrolysis.

thickness against PtBMA thickness, the latter of which is proportional to σ_i such that increasing PtBMA thickness corresponds to increasing σ . By plotting the data in this way, Figure 6 depends on parameters inherent to the polymer brush and serve to generalize the observations and conclusions reached through analysis of the data. Assuming that PtBMA thickness is directly proportional to σ allows us to estimate σ by considering a value of σ at the densest portion of the brush, which we have taken to be the substrate position furthest from the silane reservoir during the OTS deposition procedure. Recent measurements for PMMA brushes grown with an identical initiator and deposition procedure⁴² provide a value of ~0.5 chains/nm². We can then scale this value of σ for a given point on the substrate by the PtBMA brush thickness at that point. The upper axis of the panels in Figure 6 plots this estimated σ scaling. Note that due to the anomalous thickness observed at a position of 1.5 cm on the eBMPUS PtBMA brush, the thickest measurement point of the eBMPUS PtBMA is not in the region expected to be the densest portion of the brush based on WCA measurements (cf. Figure S2 in the Supporting Information). Interestingly, as seen below, this point behaves as though it is indeed at a σ value consistent with its position on the substrate.

Focusing first on the samples incubated at pH 4, the eBMPUS data do not show any distinct trends with incubation time or σ , and the normalized thickness remains above 0.8. The same is true for the aBMPUS data, other than there appears to be a relatively small, but consistent reduction in thickness with incubation time. The eBMPUS data for pH 7.4 and pH 9 show trends in both incubation time and σ . For both samples, there is a general reduction in thickness with increasing incubation time

from 24 h onward. These findings are consistent with the trends seen for the densest region of the brush. The samples at pH 7.4 and pH 9 also exhibit a maximum reduction in thickness at an intermediate σ value (i.e., at neither the densest nor sparsest region of the brush). This minimum first develops in the data at 264 h of incubation (green), and is located between 10 to 20 nm PtBMA thickness ($\approx 0.1-0.2$ chains/nm²). Examination of the data in Figure 3a reveals these data points are at the onset of the σ gradient. Increasing PtBMA thickness from this point (i.e., increasing σ) leads to a fairly smooth increase in normalized thickness. Likewise, decreasing σ also results in a higher normalized thickness, although these normalized thicknesses are all below the 24 h thickness. Finally, the apparent rate at which this minimum appears in the data varies between pH 9 and pH 7.4. While the sample at pH 7.4 shows a gradual reduction in thickness at each measurement time, the sample at pH 9 shows a rapid decrease in thickness at the 264 h measurement time. Further incubation at pH 9 beyond 264 h does not result in a significant change of thickness at the point of maximum decrease, but does so in the denser region of the brush. For both pH 9 and pH 7.4, the thickness at the sparsest region of the grafted film appears to reach a steady value after 264 h.

In contrast to the eBMPUS data, the aBMPUS data at pH 7.4 and pH 9 show continued swelling through 144 h of incubation (orange), followed by thickness reductions at 264 h (green). Unlike the eBMPUS data, these thickness reductions do not show an intermediate minimum value and appear consistent across all values of σ . The "shape" of the thickness profile at 144 h is largely preserved at 264 h. Interestingly, the data at 144 h of incubation does show a point of maximum swelling for both pH 7.4 and pH 9, and it occurs around a similar σ value (≈ 0.2 chains/nm²) as the point of maximum reduced thickness seen in the eBMPUS data. The difference in the evolution of the thickness profiles between eBMPUS and aBMPUS suggests there are significant differences between ester and amide functional groups in susceptibility to hydrolysis. The reduction in thickness in the aBMPUS samples suggests that hydrolysis of the siloxane network may occur in both initiator species, or that amide bonds may be partially susceptible to hydrolysis under tension.

Figure 8 depicts AFM images collected at three regions of the eBMPUS samples incubated at pH 4 and pH 9 for 144 h. The



Figure 8. AFM images show portions of gradient samples incubated in pH 4 (top row) and pH 9 (bottom row) buffers for 24 h. The scale bar in each images corresponds to 1 μ m. The thickness values correspond to the PtBMA thickness of the samples prior to any modification steps. The bottom plot illustrates the evolution of the RMS of AFM images collected along the gradient samples.

initial PtBMA thickness of each point is indicated above the images. The PtBMA thicknesses of each point are comparable, allowing for comparison of morphologies across pH buffer levels. Plotted below the AFM images are RMS roughness values extracted from the images for PtBMA, PMAA, and the pH 4 and pH 9 samples plotted as a function of initial PtBMA thickness. Since the pH 4 and pH 9 samples came from different segments of the PtBMA brush, the PtBMA and PMAA data were selected from the pH 9 sample. The morphology depends strongly on σ and grows increasingly smooth with increasing σ . This point is seen in the RMS roughness data as well, which exhibit a decreasing function with increasing PtBMA thickness (i.e., increasing σ). As before, morphology also depends strongly on pH incubation level. While the morphology and RMS roughness values for the pH 4 sample do not differ appreciably from the PMAA specimen, the sample incubated at pH 9 shows markedly lower RMS roughness values. Furthermore, the morphology at 18 nm PtBMA thickness differs significantly from that of the pH 4 sample, and resembles the morphology seen for the pH 9 brush at 52.4 nm PtBMA thickness.

Taken together, the results presented in Figure 6 and Figure 8 suggest that the polymer chains within the gradient behave comparably to the dense brush regions incubated at the same pH and bear residual charges and retained moisture after removal from the incubation solution. We conclude that chains in the gradient regions are stretched more strongly compared to the dense region during incubation due to a reduction in charge regulation (i.e., increase in σ), leading to a relatively larger

reduction in σ . This concept is illustrated schematically in Figure 9.



Figure 9. Charge regulation contributes to maximum degrafting at intermediate σ . As σ decreases from the dense portion of the brush (furthest right on sample in figure), α increases, resulting in an increase in tension and activation of the grafting chemistry for hydrolysis. Further decreasing σ reduces crowding due to larger distances between neighboring chains, and the grafted chains are not forced to adopt a highly stretched configuration. Not drawn to scale.

At the highest grafting densities (right edge of the sample schematic in Figure 9), neighboring charges can suppress the formation of additional electrostatic charges in the brush layer. This suppression results from a competition between the enthalpic benefit of charge formation and the energy penalties due to stretching the polymer chain normal to the surface and increasing osmotic pressure due to an increased concentration of counterions confined in the brush.^{27,28} When the stretching and osmotic pressure penalties exceed the benefit of forming an additional electrostatic charge, the polymer achieves an α below that corresponding to a bulk chain in the same buffer solution. This reduction in α due to σ is termed charge regulation in polymer brush systems. Furthermore, the same confinement that leads to charge regulation can produce significant variations in pH and reaction equilibria in the microenvironment at the initiator/brush interface at different σ levels,²⁸ which also may influence the stability of polyelectrolyte brushes at different pH levels. It is worth emphasizing that the effect of σ , osmotic pressure and solution pH and ionic strength contribute in simultaneous and coupled ways to the local microenvironments within the brush, such that while some parameters may contribute more significantly to the free energy of the system, all the parameters are important.⁴⁴

With decreasing σ the entropic stretching penalty imposed on the grafted chains is reduced, while simultaneously the density of counterions confined in the brush decreases due to a reduction in the density of repeat units. As a result, α increases with decreasing σ . Thus, at some critical σ level, the grafted chains can achieve a maximum α . Note that this maximum α is not necessarily equal to the bulk α . At this point, the grafted chains will experience a maximum in tension generated along the backbone, which has been shown to focus at the grafting point¹⁷ and activate ester groups for hydrolysis.²¹ This tension originates from the strong swelling of the polymer chains, which is modulated by a combination of σ and α . At the sparsest regions of the grafted film, the chains cannot feel their adjacent neighboring grafts and are not subjected to extension away from the substrate due to σ . Without this additional source of tension, the grafting chemistry is not liable to breakage by hydrolysis. This behavior is in contrast to nonionic

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polymer systems, which have exhibited decreased degrafting rates with decreasing σ .¹⁸

One question that remains unanswered by the experiments in this article is whether the tension generated by strongly swelling the grafted chains with increasing pH originates with electrostatic repulsion or increased solubility or both. The presence of 10 mM of sodium ions in the incubation solution leaves the possibility for charge screening to occur in the brush, which would diminish the impact of electrostatic repulsion and possibly lower tension in the polymer brush. However, higher levels of ionic strength enable higher levels of α within the brush,²⁷ and has been observed to increase instability of dense PMAA brushes at 37 °C.¹⁴ Without *in situ* swelling data to identify screening, incubation data with varying ionic strength or data using nonionic buffering agents to prevent screening, we cannot comment on the interplay of σ and ionic strength in the PMAA gradients employed in this study. This line of inquiry has significant implications for the application of these systems, since the ocean and the body both contain significant quantities of ions.

CONCLUSION

We have demonstrated that σ and α both play a pivotal role in affecting the stability of weak polyacid brushes on flat substrates immersed in aqueous environments. Our findings support the proposed hypothesis that tension generated by strong swelling of the grafted polymer chains due to charge repulsion and increased solubility activates mechanically bonds in the grafting chemistry for hydrolysis. As a result, weak polyacid brushes demonstrate increasing instability with increasing pH. Furthermore, the phenomenon of charge regulation results in a coupling of σ and α that leads to increased instability at intermediate σ levels relative to regions of the brush with higher σ . Finally, we contributed evidence that an amide bond in the initiator chemistry is more stable than an ester bond, but that hydrolysis of the siloxane grafting network may occur for both initiators. These findings hold significant importance in the application of polyelectrolytes in aqueous environments, e.g., as antifouling and antimicrobial surfaces, and suggest that polyelectrolyte instability also may be capable of providing insight into the mechanical forces and shifts in chemical equilibria that occur in nanoscale, confined systems.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macro-mol.5b01289.

Grafting density (σ) estimation calculations, thickness data for all samples used to construct plots in the main text, plots for σ gradient samples incubated at pH 4 and pH 7.4, and hydrolysis conversion calculations (PDF)

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Notes

The authors declare no competing financial interest.

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