

Free-Standing Thin Hydrogels: Effects of Composition and pH-Dependent Hydration on Mechanical Properties

Maksim Dolmat,^{||} Veronika Kozlovskaya,^{||} Donald Cropek, and Eugenia Kharlampieva*Cite This: *ACS Appl. Polym. Mater.* 2021, 3, 3960–3971

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ABSTRACT: We developed an approach to obtain nanothin free-floating poly(methacrylic acid) (PMAA) hydrogel films through the dissolution of a sacrificial SiO₂ layer. The hydrogel films were produced by chemical crosslinking of PMAA in PMAA/poly(*N*-vinylpyrrolidone) (PVPON) hydrogen-bonded precursors assembled through spin-assisted (SA) multilayer assembly. Surface-anchored precursor films and hydrogels were released from silicon templates into an aqueous solution via etching the thick sacrificial SiO₂ layer from the templates. We studied the impact of the release method on the surface morphology, thickness, and elasticity of the released (PMAA/PVPON)₆₀ and (PMAA)₆₀ films using atomic force microscopy (AFM) and demonstrated that these properties were affected neither by the film release nor by their following transfer onto Si wafers. We found that 16 h crosslinked (PMAA)₆₀ hydrogels and their more swollen 8 h crosslinked counterparts (76% hydration at pH = 5) produced free-floating films with good mechanical integrity and strength. The elastic moduli of a 16 h crosslinked (PMAA)₆₀ film decreased from 1.9 ± 0.1 GPa in the dry state to 77 ± 13 MPa at pH = 5 and then to 14 ± 3 MPa at pH = 6.5. We also showed that incorporating Zr(IV) into twice thinner (PMAA)₃₀ hydrogels (~60 nm dry thickness) improved film mechanical robustness and allowed their successful release and transfer onto Si wafers. In contrast, Zr-free (PMAA)₃₀ were less tolerant to the release/transfer procedure due to mechanical fragility. The temporary coordination links between Zr(IV) and the hydrogel could be removed from the network by an EDTA chelator. The lift-off approach developed here is simple, versatile, and applicable to a wide range of polymer films. The free-floating hydrogels obtained by this method can be used as transferrable platforms to develop lab-on-a-chip systems, nanocomposite pressure sensing platforms, rapid optical biosensors, and responsive platforms for regulating cell adhesion and more effective cell sheet recoveries.

KEYWORDS: poly(methacrylic acid), thin hydrogels, free-floating films, film release, mechanical properties

INTRODUCTION

Stimuli-sensitive hydrogels can have dramatic and reversible changes in volume under external stimuli, a characteristic crucial in drug delivery, tissue engineering, and sensing applications.^{1–3} Networks produced by crosslinking layer-by-layer (LbL) films offer compositional control at nanoscale precision, allowing control of properties such as thickness, composition, swelling/shrinkage, and crosslink density for robustness.^{4–6} LbL (multilayer) films are assembled through sequential deposition of polymer solutions onto solid substrates and are attractive in sensing, controlled delivery, separation, food packaging, and optoelectronic applications.^{7–12} Multilayer hydrogels can be produced on any substrate shape, size, or morphology while maintaining fast volume changes in response to external stimuli.^{13–18} Free-standing nanothin hydrogels would be desirable for those applications where substrate-free materials are needed. Indeed, free-standing films can be used as membranes, sensors, wound dressings, catalytic films, micromechanical devices, or even

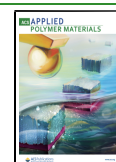
artificial organs.^{19–23} For instance, a spin-cast poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde] membrane crosslinked with polyethyleneimine was used for selective separation of proteins and other nanometer-sized compounds.¹⁹ The free-standing nanothin films of spin-cast poly(vinyl formal), polystyrene, and poly(methyl methacrylate) could hold up to 70 000 times their mass.²⁴ In addition, floating films allowed for bulk-scale analysis and higher surface-area-to-volume ratios not possible for surface-attached films confined to two dimensions.^{19,25,26}

In contrast to substrate-free spin-cast films, the synthesis of free-standing multilayer films has been much less investigated. The fabrication methods of free-standing multilayer coatings

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can include the use of a sacrificial polymeric layer soluble in organic solvents, such as polyhydroxystyrene, poly(vinyl acetate), poly(methyl methacrylate), polystyrene, and cellulose acetate (CA) that lies between a supporting substrate and the film.^{19,27–30} Alternatively, varying solution pH or ionic strength results in the disruption of interactions between a sacrificial stimuli-responsive layer and a multilayer film producing free-standing films, tubes, and hydrogels.^{31–33} For example, poly(styrene sulfonate)/poly(allylamine hydrochloride) (PAH/PSS) nanotubes were released in 0.15 M NaCl.³⁴ Thermally crosslinked ionic multilayers of $\sim 0.8\ \mu\text{m}$ thick were released from a substrate coated with poly(diallyl dimethyl ammonium chloride) (PDDA) when immersed in a solution of copper chloride at pH 3.6.³⁵ Varying the solution temperature was also shown to be useful for the release of poly(*N*-vinylpyrrolidone)/poly(methacrylic acid) (PVPON/PMAA)₁₀₀ multilayers through the disruption of hydrogen bonds within an underlying thermoresponsive multilayer at 10 °C.³⁶

However, the use of sacrificial polymeric films can result in intermixing of sacrificial polymers with the released multilayer coatings which can lead to their undesirable chemical and physical modification. Moreover, the use of organic solvents for the dissolution of a sacrificial layer can lead to morphology changes in the released film manifested in increased porosity and film wrinkling.^{30,32,35,37} For instance, CA sublayer dissolution in acetone resulted in wrinkling of poly(methacrylic acid) (PMAA) hydrogels embedded with gold nanorods due to localized stresses.³⁷

Free-standing LbL films can also be prepared without sacrificial layers using dry peeling or releasing the film directly from a substrate under pH or temperature change.^{19,32,38} For example, 8 μm poly(ethylene oxide)(PEO)/poly(acrylic acid) (PAA) films were peeled off from Teflon and polypropylene substrates.¹⁹ The 5 μm thick azlactone-containing multilayers were lifted off from silicon templates at mildly acidic conditions,³⁹ while 15 μm thick poly(L-lysine)/hyaluronic acid (PLL/HA) multilayer coatings were released from silica substrates at extremely basic conditions³² due to disruption of ionic interactions of the film and the substrate. Heating a 15 μm thick (PDDA/PSS) coacervate coating to 60 °C led to the film removal induced by the film shrinkage.³⁸ Although those studies demonstrated a fast film release without possible contamination by the sacrificial layer, they are limited to micrometer-thick films. Suitable, approaches for obtaining contamination-free, substrate-free, stimuli-sensitive nanothin hydrogels with their properties unaffected by the release procedure have yet to be developed.

In this regard, a direct comparison of a hydrogel film property before and after lifting would provide additional knowledge about the impact of the release method on film behavior. A significant limitation of releasing ultrathin hydrogels from a substrate is associated with their fragile nature. In this respect, temporarily enhancing mechanical rigidity would be an essential feature to obtain free-standing or free-floating multilayer hydrogels. To improve the mechanical properties of bulk hydrogels, one can increase crosslink density and physical dimensions (thickness), as well as modify hydrogel composition.⁴⁰ Coordination interactions between metal ions and functional groups of the polymer have been shown to increase the stiffness and toughness of metal-containing networks.⁴¹ For example, Ca^{2+} -crosslinking of an alginate film increased the tensile strength of the dry film from

40.2 to 134.6 MPa.⁴² Similarly, poly(acrylamide-*co*-acrylic acid) hydrogels were reinforced from 5 to 80 MPa through the coordination of Fe^{3+} with the hydrogel carboxylic groups.⁴³ By oscillating between oxidized Fe^{3+} and reduced Fe^{2+} physical crosslinks in poly(acrylate sodium salt)/PSS hydrogels, the hydrogel rigidity was changed from 2.1 to 0.06 MPa, respectively.⁴⁴ Implementing Zn^{2+} ions into hydroxyethyl acrylate hydrogels drastically increased the initial storage modulus of a hydrated hydrogel from 70.1 to 254 kPa.⁴⁵ The surface-anchored PMAA networks obtained by chemical crosslinking of PMAA in spin-assisted (SA) hydrogen-bonded PMAA/PVPON multilayers⁴⁶ and loaded with Cu(II) ions demonstrated selective sensing of amino acids.⁴⁷ In earlier studies, we showed that surface-attached PMAA hydrogels obtained from SA multilayers had a more organized internal structure and much higher pH-triggered swelling than dipped multilayer hydrogels.^{10,48}

In the current work, we report the synthesis of free-floating ultrathin ($\sim 100\ \text{nm}$ dry thickness) nanostructured coatings made from SA hydrogen-bonded PMAA/PVPON multilayers and PMAA multilayer hydrogels. The coatings assembled through SA multilayer assembly were transferred into an aqueous solution via etching the $\sim 300\ \text{nm}$ thick sacrificial SiO_2 layer from a Si wafer template. We studied the effects of the release method on the surface morphology, thickness, and elasticity of the released (PMAA/PVPON)₆₀ and (PMAA)₆₀ films using atomic force microscopy (AFM) imaging and nanoindentation. To explore how the release procedure affects the properties of these films, surface roughness, thickness, and mechanical properties are analyzed before and after release using AFM. To explore the effect of metal ions on film mechanical properties and swelling and to improve the mechanical rigidity of PMAA hydrogels with low crosslink density and small thickness, Zr(IV) ions are incorporated into the hydrogel through coordination with hydrogel carboxylic groups at low pH. The Zr(IV) ions have been previously demonstrated to be biocompatible⁴⁹ and have a high coordination number that was shown crucial for increasing hydrogel toughness while providing reversible hydrogel mechanics.^{50,51} We show that metal ions can serve not only as secondary crosslinks to improve film mechanical robustness but they can be easily removed from the hydrogel to recover the original network properties. Our work provides new knowledge about the controlling properties of thin films through pH-triggered hydration and incorporation of ionic species. In addition, the release approach developed here overcomes challenges associated with current methods to obtain substrate-free nanothin films and hydrogels, and it can be further extended to composite and protein films.

■ EXPERIMENTAL SECTION

Materials. Poly(methacrylic acid) (PMAA, average M_w 100 000 Da) was purchased from Polysciences. Ammonium fluoride (NH_4F), ethylenediaminetetraacetic acid disodium salt (EDTA), and hydrofluoric acid (48–51%) were purchased from Sigma-Aldrich. Monobasic and dibasic sodium phosphate were obtained from Fisher Scientific. Poly(*N*-vinylpyrrolidone) (PVPON, M_w 58 000 Da), zirconium sulfate ($\text{Zr}(\text{SO}_4)_2$), and ethylenediamine (>99%, EDA) were purchased from Alfa Aesar. 3-(3-Dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (>99%, EDC) was acquired from Chem-Impex International. Poly(glycidylmethacrylate) (PGMA) was produced from glycidylmethacrylate by radical polymerization with azobisisobutyronitrile in 2-butanone as published before.⁵² All experiments were accomplished using ultrapure de-ionized (DI)

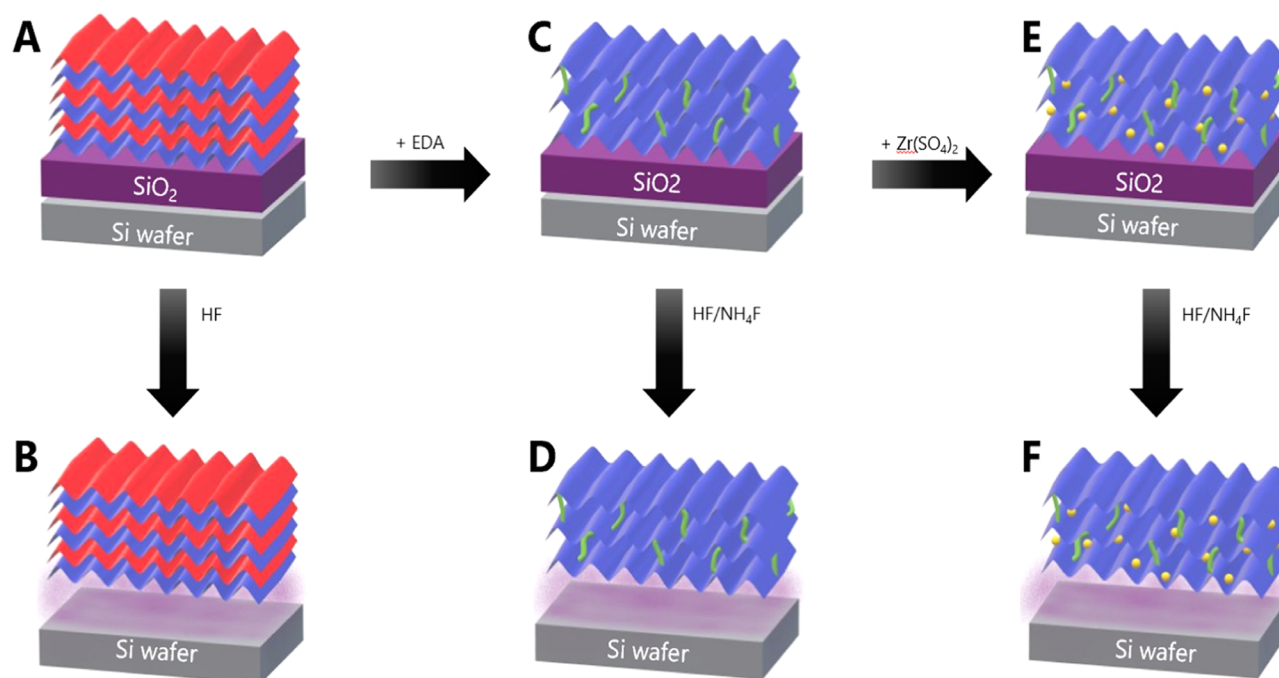


Figure 1. Synthesis of pH-sensitive free-standing hydrogen-bonded multilayers and multilayer hydrogels. (A) Hydrogen-bonded (PMAA/PVPON)_n multilayers are released into an aqueous solution as a free-standing coating (B) by etching the underlying SiO₂ layer using HF. A (PMAA)_n multilayer hydrogel (C) is obtained by crosslinking of (A) and removal of PVPON and is released into the solution after SiO₂ etching in NH₄F/HF (D). The (PMAA)_n hydrogel is additionally crosslinked with Zr (IV) by temporary links between –COOH groups and the Zr ions (E). The (PMAA)_n-Zr(IV) hydrogel coating (E) is released into an aqueous solution as a free-standing film (F).

water with a resistivity of 0.055 $\mu\text{S}/\text{cm}$ (18.2 $\text{M}\Omega\text{ cm}$) (Evoqua). Polished 2 in. diameter silicon wafers with 300 nm of a silicon oxide layer were purchased from University Wafer. 0.1 M HCl and 0.1 M NaOH were used in experiments to control pH and ionic strength.

Multilayer Film Construction. Polymers were dissolved in phosphate buffer (0.01 M, pH = 2.5) at a concentration of 1 mg mL^{-1} and deposited using a SA LbL technique (spin-coater acquired from Laurell Technologies) on silicon wafers with a thick (300 nm) SiO₂ layer. Prior to the deposition of hydrogen-bonded multilayers, silicon wafers were thoroughly cleaned as described elsewhere.⁴⁸ An initial PGMA layer was spin-cast from a 0.089 mg mL^{-1} chloroform solution onto the cleaned silicon wafer, followed by heating at 110 °C for 1 h and washing with chloroform, resulting in a 2 nm PGMA monolayer when dry. A PMAA layer was then spin-cast onto the PGMA-primed wafers from a 1 mg mL^{-1} methanol solution and crosslinked at 80 °C for 40 min, followed by rinsing with DI water. (PMAA/PVPON)_n LbL films were spin-casted onto PGMA-PMAA primed wafers as described previously.⁴⁸ Briefly, PVPON ($M_w = 58\text{ kDa}$) or PMAA ($M_w = 100\text{ kDa}$) 1 mg mL^{-1} solutions in 0.01 M phosphate buffer at pH = 2.5 were alternately deposited in 3 mL aliquots onto the wafers and rotated for 30 s at 3000 rpm on a spin-coater; then rinsed twice for 30 s with the phosphate buffer solution before the deposition of the next layer.

To produce hydrogels, PMAA layers within hydrogen-bonded multilayers were chemically crosslinked as described previously.⁴⁸ Briefly, the hydrogen-bonded films were exposed to EDC solution (5 mg mL^{-1} , 0.01 M phosphate buffer, pH = 5.0,) for 40 min, followed by crosslinking with EDA (5 mg mL^{-1} , 0.01 M phosphate buffer, pH = 5.8) for 8 or 16 h. After that, the crosslinked PMAA hydrogel films were soaked in buffer solutions at pH = 8.0 (0.01 M phosphate buffer) for 2 days to release PVPON, followed by deswelling of the films in 0.01 M phosphate buffer at pH = 5 (15 min) and drying at ambient conditions.

Release of Multilayers. To release hydrogen-bonded (PMAA/PVPON)_n films, a hydrogen-bonded (PMAA/PVPON)_n film on a Si wafer was cut into small squares (5 mm^2) with a razor, and the film on the wafer was placed into 8 wt % HF solution in a plastic Petri dish

(10 cm; Fisher Scientific) for 1 min to dissolve the thick SiO₂ layer. After the film squares were released from the wafer, the HF solution was replaced with acidic DI water (pH = 3.5), and released free-floating films were transferred to a clean Si wafer (University Wafer, SiO₂ layer of 20–30 Å) and dried in a Petri dish in a desiccator cabinet. To release (PMAA)_n hydrogels, a NH₄F/HF (5:1) etching solution (pH = 5) was used for SiO₂ etching, followed by replacing the etching solution with DI water at pH = 5 to deswell the hydrogels. After that, the hydrogel films were placed onto a Si wafer and dried as described for the hydrogen-bonded films.

Incorporation of Zr(IV) within Hydrogels. Incorporation of Zr(IV) within the PMAA hydrogel was carried out by submerging surface-anchored PMAA hydrogels in a 0.01 M Zr(SO₄)₂ salt solution at pH = 1 for 10 min. After the exposure, surface-attached hydrogels were rinsed with DI water at pH = 1 using a spin-coater to remove unbound ions from the surface, submerged in 0.01 M buffer solution at pH = 5 for 10 min followed by drying in a Petri dish in a desiccator cabinet. Similarly, Zr(IV) was incorporated into the released hydrogels by placing the PMAA hydrogel film, which was prior released, transferred onto a Si wafer, and dried, into the 0.01 M Zr(SO₄)₂ salt solution (pH = 1; 10 min) followed by rinsing with DI water at pH = 1, soaking in 0.01 M buffer solution at pH = 5 for 15 min, and drying in a Petri dish in a desiccator cabinet. To chelate Zr(IV) from PMAA-Zr(VI) hydrogels, the PMAA-Zr(VI) hydrogels (either surface anchored or released and transferred to a clean Si wafer) were exposed to 0.5 M EDTA solution at pH = 7 for 7 days while replacing the EDTA solution twice a day.

Atomic Force Microscopy (AFM). For AFM analysis, at least two separate films were prepared; each film was cut into smaller squares, and each square film was analyzed in at least five places. Images of multilayers were acquired using an atomic force microscope (AFM; NT-MDT Spectrum Instruments) equipped with an SPM system controller. Probes NSG30 (tip curvature radius <10 nm, force constant 22–100 $\text{N} \times \text{m}^{-1}$), NSG03 (tip curvature radius = 10 nm, force constant 0.35–6.1 $\text{N} \times \text{m}^{-1}$), and colloidal NSG01 Bio 600/Au tip (sphere curvature radius = 300 nm, force constant 1.45–15.1 $\text{N} \times \text{m}^{-1}$) were purchased from NT-MDT Spectrum Instruments. High-

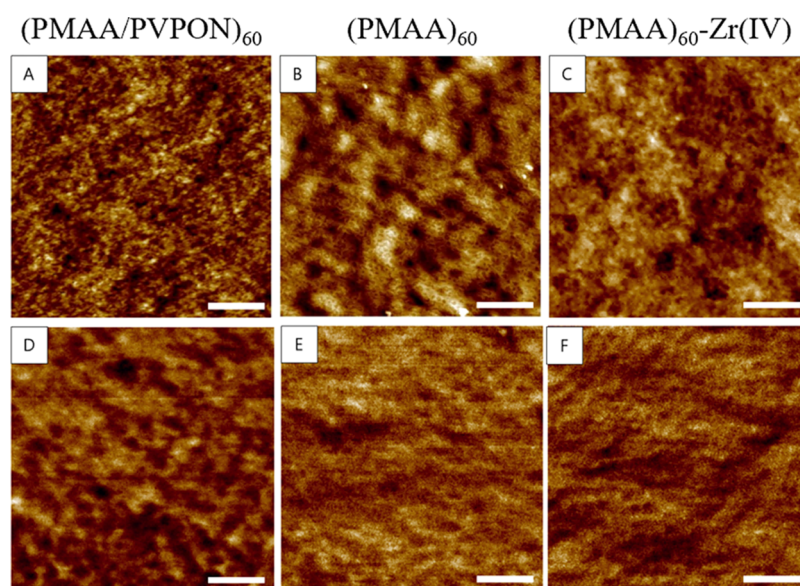


Figure 2. AFM topography images (A–C) and Young's modulus maps (D–F) obtained in the dry state from surface-anchored (PMAA/PVPON)₆₀ films (A, D), 16 h crosslinked (PMAA)₆₀ (B, E), and (PMAA)₆₀-Zr(IV) hydrogels (C, F). The scale bar is 200 nm in all images. The height (*z*)-scale is 3.6 (A), 3.1 (B), and 3.5 nm (C).

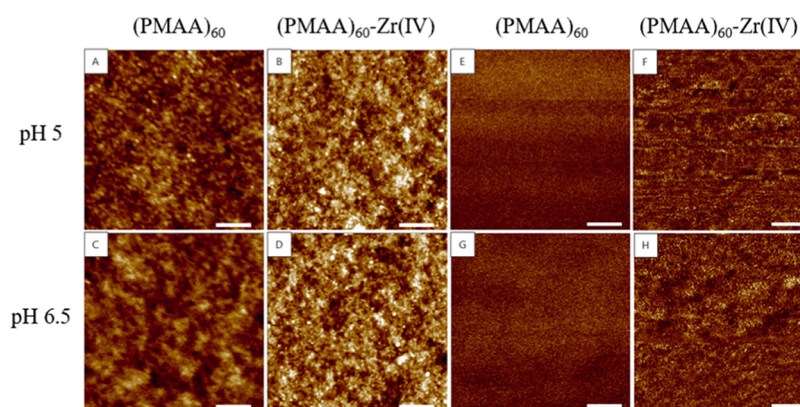


Figure 3. AFM topography (A–D) and Young's modulus images (E–H) of 16 h crosslinked (PMAA)₆₀ (A, C, E, G) and (PMAA)₆₀-Zr(IV) hydrogels (B, D, F, H) at pH = 5 (A, B, E, F), and pH = 6.5 (C, D, G, H). The scan size is 2 μm^2 (A–D) and 1 μm^2 (E–H). The scale bar is 400 nm (A–D) and 200 nm (E–H). The height (*z*)-scale is 10 nm (A), 13.8 (B), 10.0 (C), and 13.8 nm (D).

resolution AFM topography images were acquired from at least five different locations of each sample using a pyramidal sharp tip and the average root-mean-square (rms) roughness was calculated from the collected images. Roughness and mechanical properties in the dry state were obtained from 1 μm^2 regions at different positions of 10 μm^2 images using NSG30 probes at a scan rate of 1 Hz. Similarly, the roughness of the films in the liquid state was collected from 2 μm^2 regions using NSG03 probes at a scan rate of 0.8 Hz, and mechanical properties were obtained from 1 μm^2 areas using a colloid probe NSG01 Bio 600/Au (a diameter of a sphere is 600 nm) at a scan rate of 1 Hz to avoid a sharp tip penetration into the soft hydrogel network.⁵³ The independence of Young's modulus was previously demonstrated for micrometer and nanometer thick films. To avoid effects from the underlying during AFM indentation measurements, the film samples were prepared with dry thicknesses of at least 100 nm and AFM probe-induced film deformations did not exceed 10% of film thicknesses (indentation depths of 10–20 nm). Film thickness was measured using a scratch method, where a vertical cut through a dried film was made by a razor. The topography images (30 μm^2) over the cut area were taken in tapping mode in both dry and liquid states. The thickness of the film was calculated as the height between the substrate area ("cut" area) and the film surface.²⁸

RESULTS AND DISCUSSION

To obtain free-standing films, the multilayer was assembled on silicon wafers with a 300 nm thick silicon oxide, followed by the oxide dissolution to release the film into solution. First, hydrogen-bonded 60-bilayer (PMAA/PVPON) films were assembled on silicon wafers at pH = 2.5 using the SA LbL (Figure 1). Second, surface-anchored (PMAA)₆₀ hydrogels were obtained by EDA-assisted crosslinking of PMAA layers in the (PMAA/PVPON)₆₀ films, followed by the PVPON release at basic pH as previously described.⁴⁶ As demonstrated earlier, PVPON with M_w 58 kDa can be completely removed from the crosslinked hydrogel due to the disruption of hydrogen bonds between PMAA and PVPON at pH > 6, when PMAA carboxylic groups become ionized ($pK_a \approx 5.8$ –6.2).⁴⁸ The surface-anchored films were released from the silicon templates by exposing hydrogen-bonded and/or hydrogel films to etching solutions (see the Experimental Section) leading to films free floating in solution. The free-floating films were transferred onto surfaces of clean silicon wafers for characterization and were denoted as free-standing films. Finally, to

incorporate Zr(IV), the as-deposited surface-anchored and the corresponding free-standing (PMAA)₆₀ hydrogel films were soaked in Zr(SO₄)₂ solution at pH = 1 (Figure 1). To study the effect of film release and transport onto a different substrate on film properties including film thickness, roughness, and Young's modulus, these properties of free-standing hydrogen-bonded films and their corresponding hydrogels were compared with their surface-anchored counterparts.

Surface-Anchored Films. Prior to investigating free-standing films, the properties such as thickness, morphology, and Young's modulus of surface-anchored hydrogen-bonded films and their corresponding hydrogels were explored.

AFM topography images of surface-anchored dry and hydrated films are shown in Figures 2 and 3, respectively, while the main parameters of these films are shown in Figure 4A–C. The average root-mean-square (rms) roughness of a 1

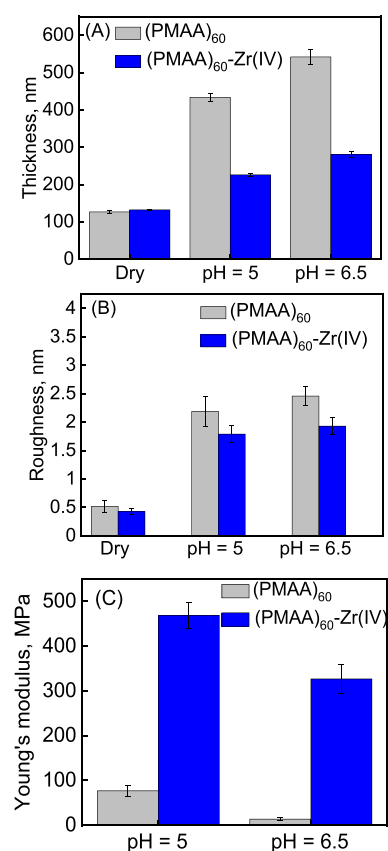


Figure 4. Thickness (A), roughness (rms) (B), and Young's modulus (C) of 16 h crosslinked (PMAA)₆₀ and (PMAA)₆₀-Zr(IV) hydrogels as analyzed by AFM. Roughness (rms) was analyzed from 2 μm^2 areas. Average Young's moduli were obtained from 1 μm^2 areas.

μm^2 area for bare SiO₂ on a Si surface was 0.22 ± 0.04 nm (Figure S1A, Supporting Information), which is within the typical range of rms roughness (0.1–0.4 nm) of silicon oxide surfaces.⁵⁴ A deposited precursor (PGMA) layer did not change the surface roughness resulting in a rms of 0.20 ± 0.05 nm (Figure S1B). However, the surface roughness increased to 0.52 ± 0.04 nm after the assembly of (PMAA/PVPON)₆₀ films (Figure 2A, Table 1; Figure S2A–C), which is in good agreement with the roughness of ~ 1 nm for SA hydrogen-bonded and ionically paired two-component multilayers in the dry state.^{46,55} Crosslinking of the (PMAA/PVPON)₆₀ film gave a (PMAA)₆₀ multilayer hydrogel with a dry rms roughness

Table 1. Dry-State Thickness (nm), Root-Mean-Square Roughness (nm), and Young's Modulus (GPa) of Surface-Anchored and Free-Standing (FS) Hydrogen-Bonded Films and 16 h Crosslinked Hydrogels^a

film, dry	thickness, nm	roughness, nm	Young's modulus, GPa
(PMAA/PVPON) ₆₀	149 ± 2	0.52 ± 0.04	1.9 ± 0.2
(PMAA) ₆₀	127 ± 5	0.52 ± 0.11	1.9 ± 0.1
(PMAA) ₆₀ -Zr(IV)	132 ± 2	0.43 ± 0.04	2.2 ± 0.2
FS-(PMAA/PVPON) ₆₀	142 ± 4	0.41 ± 0.05	1.8 ± 0.1
FS-(PMAA) ₆₀	119 ± 3	0.45 ± 0.11	1.9 ± 0.1
FS-(PMAA) ₆₀ -Zr(IV)	126 ± 3	0.46 ± 0.03	1.9 ± 0.2

^aRoughness and Young's modulus were collected from 1 μm^2 areas.

of 0.52 ± 0.11 nm (Figure 2B, Table 1), indicating no significant changes in the film morphology. Conversely, hydration of (PMAA)₆₀ hydrogels led to a significant increase in roughness by 4.2- and 4.7-fold compared to its dry thickness at pH = 5 and 6.5, respectively, (Figures 3A,C and 4B) with the corresponding rms values of 2.19 ± 0.27 and 2.46 ± 0.17 nm (Table 2). The increase in the film roughness upon hydration was also observed in our earlier work on partially deuterated (PMAA)₁₇ hydrogels obtained by the SA LbL method and was attributed to chain relaxation by swelling of chain loops upon hydration.⁴⁸

The dry thickness of a (PMAA/PVPON)₆₀ film measured by AFM using a scratch method was 149 ± 2 nm and decreased after crosslinking and the PVPON release to 127 ± 5 nm for the (PMAA)₆₀ hydrogel (Table 1). As expected, the hydrogel swelled at the pH > pK_a of PMAA due to the electrostatic repulsion between the ionized carboxylic groups.⁴⁸ The dry (PMAA)₆₀ hydrogel swells from 127 ± 5 nm in the dry state to 434 ± 7 nm at pH = 5 (3.4-fold thickness increase), and to 541 ± 10 nm at pH = 6.5 (4.3-fold swelling) (Tables 1, and 2 and Figure 4A).

The Young's modulus maps of dry hydrogen-bonded (PMAA/PVPON)₆₀ (Figure 2D) and hydrogel (PMAA)₆₀ (Figure 2E) films revealed a homogeneous modulus over the 1 μm^2 areas with similar average Young's moduli of 1.9 ± 0.2 and 1.9 ± 0.1 GPa for (PMAA/PVPON)₆₀ and (PMAA)₆₀ films, respectively (Table 1). As the result of hydration, the elasticity of the (PMAA)₆₀ hydrogel decreases significantly by ~ 25 times from 1.9 ± 0.1 GPa in dry state to 77 ± 13 MPa when the hydrogel is in solution at pH = 5 (Table 2, Figures 3E, G, and 4C). The elastic modulus of hydrogels lowers by 136-fold of its dry value to 14 ± 3 MPa when the solution pH is further increased to pH = 6.5.

Earlier reports showed that the elastic modulus for SA multilayer films in the dry state is typically found in the range from 0.7 to 6 GPa, depending on film composition, preparation conditions, and measuring technique.^{56,57} It was also shown that hydration of polyelectrolyte LbL films drastically decreases Young's modulus due to the plasticizing effect of water, similar to the bulk hydrogels.⁵⁸ Depending on the degree of hydration, Young's modulus of wet multilayer films was found to range from a few kilopascal to several hundreds of megapascal.^{59,60} For example, Cohen and colleagues found that the Young's modulus of ionically paired PAH/PSS films decreased by an order of magnitude from 5.8 ± 0.4 GPa to 590 ± 90 MPa upon immersion in DI water.⁵⁶ Tsukruk and co-workers showed that hydrated multilayers of hydrogen-bonded tannic acid/PVPON

Table 2. Properties of 16 h Crosslinked Surface-Anchored (PMAA)₆₀ and (PMAA)₆₀-Zr(IV) Hydrogels at pH 5 and 6.5 Measured by AFM^a

film	thickness, nm		hydration, %		Young's modulus, MPa		roughness, nm	
	pH = 5	pH = 6.5	pH = 5	pH = 6.5	pH = 5	pH = 6.5	pH = 5	pH = 6.5
(PMAA) ₆₀	434 ± 7	541 ± 10	70 ± 2	77 ± 3	77 ± 13	14 ± 3	2.19 ± 0.27	2.46 ± 0.17
(PMAA) ₆₀ -Zr(IV)	226 ± 5	281 ± 7	42 ± 3	53 ± 4	466 ± 29	327 ± 32	1.79 ± 0.15	1.93 ± 0.15

^aHydration of the hydrogels was calculated as the ratio of the thickness difference between its hydrated and dry state to the total thickness of the film at hydrated state. Roughness measurements were collected from 2 μm^2 areas and Young's modulus measurements were collected from 1 μm^2 areas.

exhibited a Young's modulus decrease from 700 ± 200 MPa in dry state to 200 ± 3 kPa in water.⁵⁷ Poly(3,4,5-trihydroxybenzyl methacrylamide)/PEO hydrogen-bonded multilayers were also reported to undergo significant plasticization with an almost 3-fold decrease in Young's modulus upon the hydration of the 0.6 μm thick film.⁶¹

Our data are consistent with a general trend of decreasing Young's modulus with hydration. Our results also indicate that the decreased elasticity is strongly dependent on the ionization of carboxylic groups and, consequently, the pH-dependent hydration of the PMAA hydrogel. Thus, even a slight increase in the hydration, from $70 \pm 2\%$ at pH = 5 to $77 \pm 3\%$ at pH = 6.5 results in a substantial decrease in Young's modulus from 77 ± 13 to 14 ± 3 MPa. The pH-dependent softening of multilayer hydrogels was previously observed for weakly crosslinked PMAA-PVPON and PMAA hydrogel capsules^{62–64} Despite the small (7%) increase in hydration, the hydrogel swelling change in this pH range is significant. Thus, the swelling ratio of the PMAA hydrogel calculated as the thickness of the hydrated hydrogel over its dry thickness increases by 90% from 3.4 ± 0.2 to 4.3 ± 0.2 when pH is increased from pH = 5 to 6.5. This behavior can be rationalized through the fact that PMAA has a pK_a value of 5.8–6.2, and the PMAA chain segments between crosslinks become deprotonated and expand,⁶⁵ which leads to softening of the network.

Incorporation of Zr(IV) ionic species into the hydrogel was performed by submerging surface-anchored (PMAA)₆₀ hydrogels in 0.01 M solution of $\text{Zr}(\text{SO}_4)_2$ for 10 min at pH = 1. The acidic pH has been selected to favor the coordination of Zr(IV) with the protonated carboxylic groups of PMAA and where Zr(IV) ions exist as neutral octameric complexes ($R_g = 5$ Å)⁶⁶ due to the hydrolysis of Zr salt in the presence of aqueous hydrochloric acid. The Zr(IV) incorporation did not significantly affect film parameters in the dry state. The roughness of (PMAA)₆₀-Zr(IV) hydrogels with the thickness of 132 ± 2 was 0.43 ± 0.04 nm, while Young's modulus was found to be 2.2 ± 0.2 GPa (Figure 2); these values were similar to the corresponding values of the (PMAA)₆₀ hydrogel before the ion loading (Table 1).

Conversely, ion incorporation significantly influenced the properties of hydrated hydrogel films. The (PMAA)₆₀-Zr(IV) hydrogel only swelled 1.7 and 2.1 times its dry thickness at pH = 5 and 6.5, respectively (Table 2 and Figure 4A). Thus, the presence of Zr(IV) led to a 1.9-fold suppression in the hydrogel swelling at both pH values as compared to ion-free films. Specifically, the hydrated thickness decreased from 434 ± 7 to 226 ± 5 nm at pH = 5 and from 541 ± 10 to 281 ± 7 nm at pH 6.5 after the ion incorporation (Table 2). The reduced swelling of (PMAA)₆₀-Zr(IV) at those pH conditions can be attributed to the presence of additional physical crosslinks by the coordination of Zr(IV) ions with carboxylic

groups and loss of pendant charged COO^- groups to reduce electrostatic repulsion. This result agrees with our previously published studies which showed that introducing copper(II) ions within the PMAA hydrogel suppresses swelling of hydrogels up to 4 times due to the coordination of Cu(II) with carboxylic groups.⁴⁷

We did not observe any thickness changes for the (PMAA)₆₀-Zr(IV) hydrogel from its initial values at pH = 5 and 6.5 when the hydrogel was alternatively exposed to solutions with these pH values indicating no release of Zr(VI) from the PMAA hydrogel at pH = 5 or 6.5. This result is attributed to the strong complexation of Zr(VI) with carboxylic groups (the complex stability constants ($\log K$) in the range of 11.5–29.7).⁶⁷

The suppressed swelling of the (PMAA)₆₀-Zr(IV) hydrogel at both pH values also resulted in a strong increase in the corresponding elastic moduli. Young's modulus increased 6-fold at pH = 5 from 77 ± 13 to 466 ± 29 MPa before and after the ion loading, respectively (Figure 4C and Table 2). An even larger 23-fold difference in Young's moduli was observed between (PMAA)₆₀ and (PMAA)₆₀-Zr(IV) at pH = 6.5, with the corresponding elasticity values of 14 ± 3 and 327 ± 32 MPa (Table 2). Exposure of ion-loaded hydrogels to both pH values also resulted in a slight decrease (~ 1.2 -fold) in the surface roughness compared to that of ion-free hydrogels, which is consistent with the decreased hydrogel hydration (Figure 4B and Table 2).

The effects of Zr(IV) incorporated into hydrogels correlate with the data obtained for hydrated ion-free hydrogels, which showed a sharp change in the elastic modulus despite a moderate increase in the hydrated thickness. Importantly, the ion-loaded hydrogel preserved its pH-responsive properties and swelled by 1.2-fold when the solution pH was changed from pH = 5 to 6.5, which was similar to the swelling ratio of Zr(IV)-free hydrogel under those conditions. Also, the elasticity modulus of the (PMAA)₆₀-Zr(IV) hydrogel responded to the pH increase from pH = 5 to 6.5 by decreasing 1.4-fold. These data indicate that besides varying the solution pH, the elastic modulus of nanonetworks can also be finely tuned by incorporating metal ions within the network.

Free-Floating Hydrogen-Bonded and Hydrogel Films.

The hydrogen-bonded (PMAA/PVPON) multilayer films were released from substrates into the solution by exposing the surface-anchored films to 8 wt % HF solution to dissolve the underlying silica layer, followed by replacing the acidic solution with DI water at pH = 3.5. An optical image in Figure 5A shows a free-floating (PMAA/PVPON)₆₀ film, which was cut into several $\sim 0.5 \times 0.5$ cm² pieces prior to release. The free-floating films are clearly seen at the water–air interface implying the good mechanical strength of the hydrogen-bonded film.

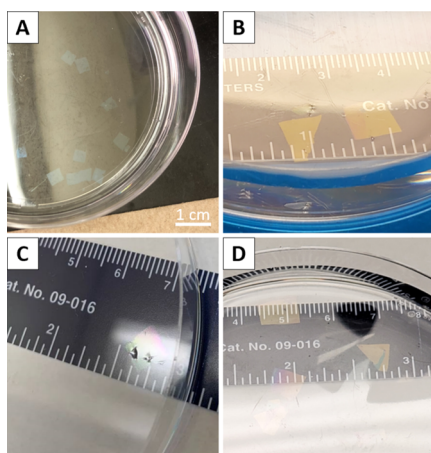


Figure 5. Optical images of free-floating (PMAA/PVPON)₆₀ films at pH = 2 (A). Optical images of free-floating 16 h crosslinked (PMAA)₆₀ hydrogels (B), (PMAA)₆₀-Zr(IV) hydrogels (C), and 8 h crosslinked (PMAA)₆₀ hydrogels at pH = 5 (D). The upper scale in (B–D) is in centimeters.

Previously, the dissolution of polystyrene substrates to obtain free-standing films was shown to lead to 30 μm diameter holes in released poly(L-lysine)/hyaluronic acid multilayer films.³² In another study, the roughness of PAA/PAH multilayers dramatically increased from 1.7 to 244 nm for the as-prepared and released free-standing films, respectively.³⁵ The extreme roughness of PAA/PAH free-standing films appeared to be due to surface wrinkles because of the disruption of ionic interactions between a sacrificial PDDA layer and a PAA/PAH multilayer in an acidic copper chloride solution.³⁵

To understand how our release method affected the properties of the released films, the surface topography of the (PMAA/PVPON)₆₀ film was analyzed with AFM at 10, 5, and 1 μm^2 areas for both the initial surface-anchored and the released films. The thickness of the released films was measured to be 142 ± 4 nm, which is similar to the initial surface-anchored film thickness of 149 ± 2 nm (Figure 6A,B). We did not observe any visible changes in the films transferred from the solution to a Si wafer after drying except for some occasional film folding due to film handling (Figure S3). The AFM topography images demonstrate smooth surfaces with the roughness slightly decreased from 0.52 ± 0.04 nm before release to 0.41 ± 0.05 nm after the release (Figure 7A). Also, the silica layer dissolution with HF did not affect the mechanical properties of the released film, with Young's modulus of 1.8 ± 0.1 GPa after release compared to 1.9 ± 0.2 GPa before release (Table 1, Figure 7B). These results indicate that the hydrogen-bonded film thickness and morphology and, consequently, composition and physical properties remain largely unaffected by the dissolution of sacrificial SiO₂, unlike other methods where holes, pores, and wrinkles were observed on the released film surfaces.^{30,32,35,37} Thus, the release method introduced here can be successfully used to obtain nanothin films without any changes in film properties.

To lift the PMAA hydrogel films, the release method was modified to account for the amphoteric behavior of EDA-crosslinked PMAA hydrogels. These multilayer hydrogels swell under both acidic and basic conditions with the minimum swelling observed in the $4.5 < \text{pH} < 5.5$ range where there is ionic pairing between the ionized carboxylic groups of PMAA and protonated amine groups from the one-end attached EDA crosslinker.⁹ The increase in thickness at pH values below and above this range is due to the electrostatic repulsion between

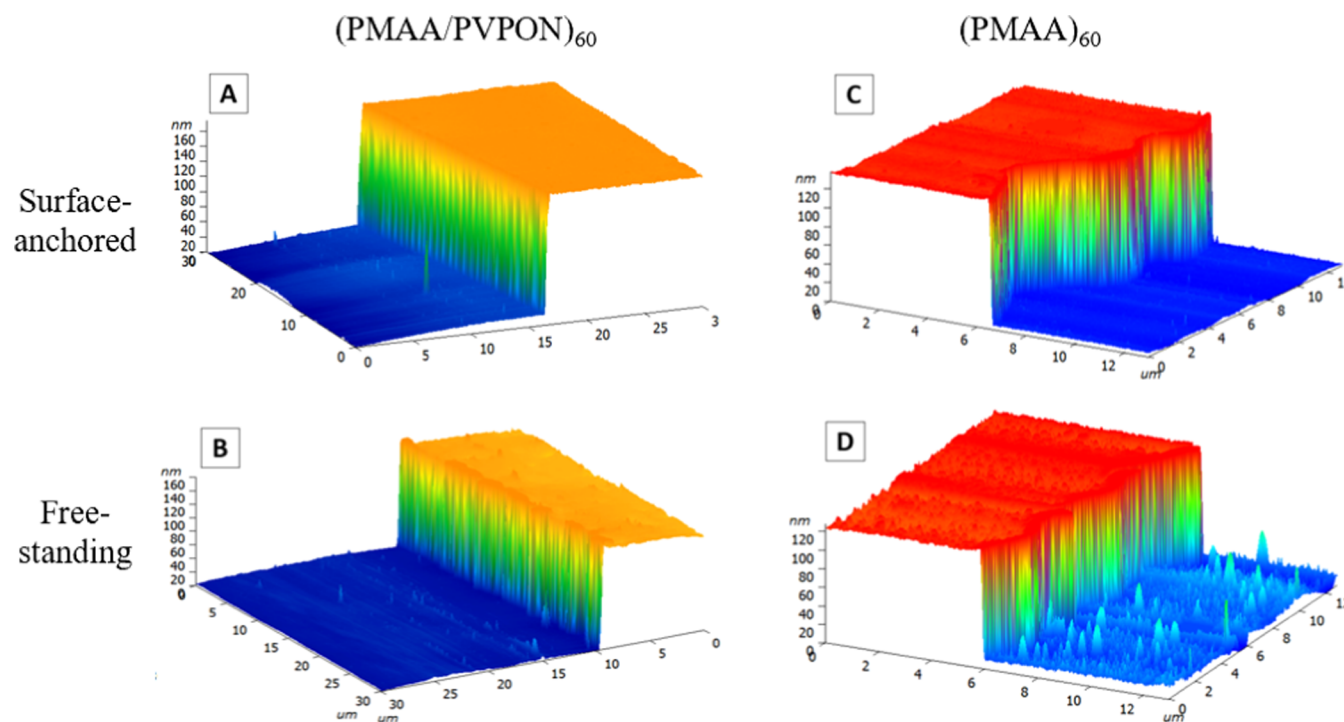


Figure 6. Three-dimensional AFM topography images of (PMAA/PVPON)₆₀ films (A, B) and (PMAA)₆₀ hydrogels (C, D) as surface-anchored (A, C) and free-standing coatings (B, D) alongside a scratched surface of Si wafer (shown in blue). The height (*z*)-scale is 160 nm (A, B) and 120 nm (C, D).

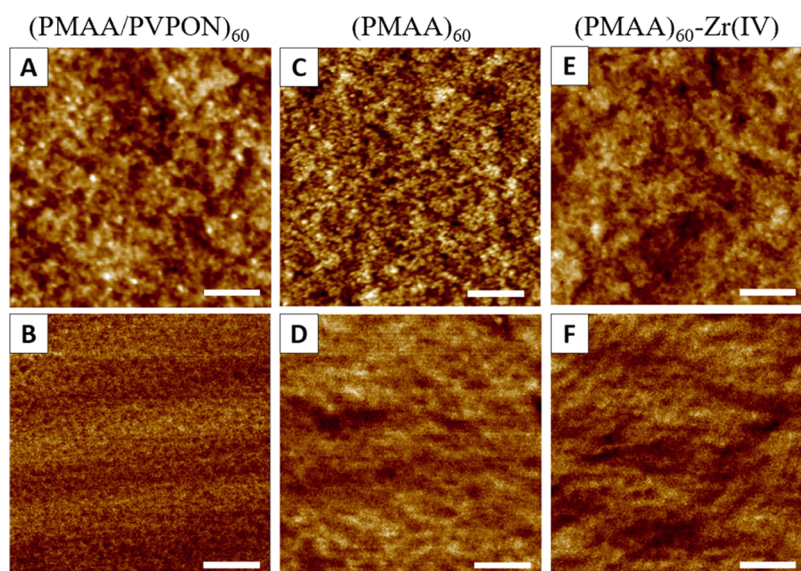


Figure 7. AFM dry-state topography (A, C, E) and Young's modulus images (B, D, F) of free-standing films: hydrogen-bonded (PMAA/PVPON)₆₀ films (A, B), 16 h crosslinked (PMAA)₆₀ (C, D), and (PMAA)₆₀-Zr(IV) hydrogels (E, F). The scan size is 1 μm^2 . The scale bar is 200 nm. The height (*z*)-scale is 3.6 (A), 3.6 (C), and 3.0 nm (E).

either excess positive (at $\text{pH} < 4.5$) or negative (at $\text{pH} > 5.5$) charges from protonated EDA amine groups or ionized carboxylic groups of PMAA at high pH, respectively.⁹ The amine group protonation at low pH causes the swelling of PMAA hydrogels with higher water uptake, and, consequently, a drastic decrease in the refractive index of the film rendering the hydrogel fragile and transparent to the naked eye.⁶⁸ Therefore, to ensure the visibility of the free-standing hydrogel at its minimum swelling, the SiO_2 layer etching was carried out in the mixture of $\text{HF}/\text{NH}_4\text{F}$ at $\text{pH} = 5$, followed by exchanging the etching solution with DI water at $\text{pH} = 5$ (Figure 1D). Figure 5B,C shows the (PMAA)₆₀ and (PMAA)₆₀-Zr(IV) hydrogel films of ~ 7 mm in size free floating on the DI water surface ($\text{pH} = 5$) after the sacrificial SiO_2 layer dissolution. The hydrogel films were transferred onto Si wafers, dried, and analyzed.

AFM analysis of the released (PMAA)₆₀ (Figure 7C) and (PMAA)₆₀-Zr(IV) (Figure 7E) hydrogels revealed smooth, homogeneous surfaces with the rms roughness values of 0.45 ± 0.11 and 0.46 ± 0.03 nm, respectively (Table 1). There was no significant change in the thicknesses of the released (PMAA)₆₀ and (PMAA)₆₀-Zr(IV) hydrogels compared to the corresponding surface-anchored films. The surface-anchored (PMAA)₆₀ film of 127 ± 5 nm (Table 1, Figure 6C) was measured to be 119 ± 3 nm in thickness after the release and drying (Table 1, Figure 6D). Similarly, after the surface-attached (PMAA)₆₀-Zr(IV) hydrogel with the thickness of 132 ± 2 nm was released, its dry thickness was found to be 126 ± 3 nm.

Young's modulus collected over 1 μm^2 area of free-standing (PMAA)₆₀ (Figure 7D) and (PMAA)₆₀-Zr(IV) (Figure 7F) hydrogels showed no significant difference in the mechanical properties between the dry hydrogels with the corresponding elasticity modulus values of 1.9 ± 0.1 GPa for (PMAA)₆₀ and 1.9 ± 0.2 GPa for (PMAA)₆₀-Zr(IV) hydrogels (Table 1). Previously, film crosslinking was shown to prevent morphology changes in the released free-standing film otherwise observed in the noncrosslinked film upon lifting.^{32,35} Voegel and colleagues found that thermal crosslinking reduces the diameter of the holes from 40 μm for the released (PLL/

HA)₄₀/PLL ionically paired multilayer to 10 μm for its thermally crosslinked (PLL/HA)₈₁/PLL counterpart.³² Furthermore, Shen and colleagues demonstrated that ionically paired (PAA/PAH)₁₅ films immediately dissociated at $\text{pH} = 2$, while thermally crosslinked PAA/PAH films can be obtained as free standing after exposure to an aqueous solution at $\text{pH} = 2$.³⁵

Finally, we explored the effect of the crosslinking degree on the release of free-standing PMAA hydrogels. The surface-anchored (PMAA)₆₀ hydrogels with a lower crosslink density were produced by decreasing the crosslinking time from 16 to 8 h. The dry thicknesses of the 8 and 16 h crosslinked (PMAA)₆₀ hydrogels were similar and in the range of 124 ± 7 nm, as measured by AFM (Figure 8A). The decreased crosslinking time resulted in considerably more swelling for the 8 h crosslinked (PMAA)₆₀ surface-anchored film compared to that of its 16 h crosslinked counterpart as measured by *in situ* AFM (Figure 8A). For example, the 8 h crosslinked hydrogel increased in thickness by 4.1-fold ($124\text{--}514$ nm) when hydrated at $\text{pH} = 5$, while the 16 h crosslinked hydrogel swelled only by 3.5-fold under this condition ($127\text{--}434$ nm). Similarly, the further exposure of the 8 h crosslinked hydrogel to $\text{pH} = 6.5$ resulted in additional swelling to 700 ± 23 nm, again outpacing the swelling performance of the 16 h crosslinked hydrogel (541 ± 10 nm). These results correlate well with the previously reported decreased swelling of PMAA multilayer capsules with increasing time for the EDA exposure due to increased crosslink density.⁶⁹

We found that despite its larger degree of hydration at $\text{pH} = 5$ (76% hydration), which is expected to lead to a more fragile film with less mechanical rigidity, the 8 h crosslinked hydrogels can also be released from the substrate via the dissolution of the sacrificial thick silica layer using the approach developed herein (Figure 5D). The AFM analysis of the free-standing (PMAA)₆₀ hydrogels transferred to Si wafers revealed that they had a similar dry thickness (125 ± 5 nm) and the same swelling degree at $\text{pH} = 5$ (504 ± 17 nm) and at $\text{pH} = 6.5$ (694 ± 24 nm) as the surface-attached 8 h crosslinked (PMAA)₆₀ hydrogels (Figure 8A). The exposure of the 8 h

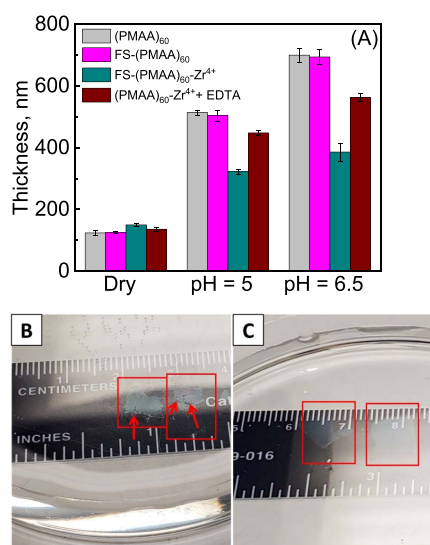


Figure 8. Thickness of 8 h crosslinked (PMAA)₆₀, FS-(PMAA)₆₀, FS-(PMAA)₆₀-Zr(IV), and FS-(PMAA)₆₀-Zr(IV) treated by EDTA hydrogels in a dry state and at pH = 5 and 6.5, as analyzed by AFM (A). Optical images of the free-floating 8 h crosslinked (PMAA)₃₀ (B) and (PMAA)₃₀-Zr(IV) hydrogels (C) in aqueous solutions at pH = 5. The red squares in (B, C) enclose the images of the hydrogel films. The red arrows point to breaking points in the film. The scale in (B, C) is in centimeters.

crosslinked free-standing (PMAA)₆₀ hydrogels with Zr(IV) suppressed the hydrogel swelling to 322 ± 9 nm at pH = 5 and to 386 ± 29 nm at pH = 6.5 (Figure 8A). Nonetheless, the exposure of the 8 h crosslinked (PMAA)₆₀-Zr(IV) hydrogels to 0.5 M EDTA solution for 2 days restored the swelling of the hydrogels to larger thicknesses of 415 ± 10 nm at pH = 5 and 505 ± 15 nm at pH = 6.5 due to chelating of Zr(IV) ions from the hydrogel network. The EDTA chelation of Zr(VI) from the PMAA hydrogel network was found to be slow, however, and required frequent changes of the EDTA solution. This behavior can be rationalized through competitive interactions of Zr(VI) species with the PMAA hydrogel's carboxylic groups on the one hand and with the carboxylate and tertiary amine groups of the EDTA chelator on the other hand. Strong complexation of Zr(VI) by carboxylates and EDTA with the corresponding constants of the complexes ($\log K$) in the range of 11.5–29.7⁶⁷ and 31–98,⁷⁰ respectively, has been previously reported to be very slow in the pH range 7–9.⁷⁰ Zr(VI) was also found to form mixed-ligand complexes in the presence of EDTA.⁷⁰ In our system, this could lead to one or more Zr ions simultaneously binding with EDTA and carboxylates of PMAA that could result in a very slow removal of Zr(VI) from the PMAA network under those conditions. Despite the slow Zr(VI) chelating by EDTA, these coordination crosslinks can be added to and EDTA-removed from the PMAA hydrogel multiple times without degrading the network as it is held by covalent links from the EDA crosslinker.

Our data show that the introduction of multivalent temporary coordination crosslinks can be used to improve the hydrogel mechanical rigidity and strength when ultrathin free-standing hydrogels need to be obtained. Indeed, the twice as thin 8 h crosslinked (PMAA)₃₀ and (PMAA)₃₀-Zr(IV) hydrogels with an average thickness of 66 ± 2 nm demonstrated a different degree of mechanical rigidity upon release from the template surface.

Figure 8B shows that although (PMAA)₃₀ hydrogels could be liberated into solution, they exhibited a high degree of fragility with visible eroded areas at the film edges and in the middle of the film due to the hydrogel's small thickness and high hydration. These coatings could not be transferred onto the Si wafer as a whole film due to poor mechanical strength. Conversely, free-floating (PMAA)₃₀-Zr(IV) hydrogels did not show any visible erosion and kept their initial shape with defined edges and overall undamaged morphology (Figure 8C). Apparently, Zr(IV) ions increased the rigidity of ultrathin (PMAA)₃₀ hydrogels due to physical links between carboxylic groups and the ions rendering the hydrogel more tolerant to mechanical handling. These findings indicate that regardless of the small thickness and high hydration, nanostructured multilayer hydrogels can be successfully liberated from their templating surface and transferred to other substrates, e.g., Si wafers, without significant changes in the hydrogel morphology, thickness, and elasticity. Given the simplicity and versatility of the developed approach for the release of pH-sensitive, highly hydrated hydrogels that are ~10–100 times thinner than any previously reported hydrogels, this method can be extended to other ultrasoft nanostructured coatings that can be used as transferrable platforms for stimuli-responsive sensing.

CONCLUSIONS

We report on the synthesis of free-floating ultrathin (~100 nm dry thickness) nanostructured coatings made from PMAA/PVPON hydrogen-bonded multilayers and PMAA multilayer hydrogels. The coatings assembled through SA multilayer assembly were transferred into an aqueous solution via etching the ~300 nm thick sacrificial SiO₂ layer from a Si wafer template. We studied the effects of the release method on the surface morphology, thickness, and elasticity of the released (PMAA/PVPON)₆₀ and (PMAA)₆₀ films and demonstrated that these properties were not affected either by the film release or following transfer onto a Si wafer surface. We found that the 8 h crosslinked (PMAA)₆₀ hydrogels with 60% larger swelling at pH = 5 than that for the 16 h crosslinked (PMAA)₆₀ hydrogels could also be released into the solution and produce free-standing films with good mechanical integrity and strength. Conversely, a 2-fold decrease in thickness of the (PMAA)₆₀ hydrogel (~120 nm dry thickness) to (PMAA)₃₀ (~66 nm) resulted in the decreased mechanical rigidity of the hydrogel film, and the film could not be completely transferred onto a Si wafer surface due to poor mechanical integrity. We explored the incorporation of Zr(IV) metal ions into PMAA hydrogels with low crosslink density and small thickness to improve mechanical rigidity of thin hydrated PMAA hydrogels through temporary coordination links. The improved mechanical strength in these Zr(IV)-containing films allowed us to obtain very thin (~60 nm) free-floating (PMAA)₃₀-Zr(IV) hydrogels that could be further transferred onto Si wafers. This finding can be potentially relevant to other multivalent ions that could be used to obtain free-standing highly hydrated hydrogel films that are ~10 to 100 times thinner than any previously reported hydrogels. Accounting for the simplicity and versatility, the developed approach of releasing nanoscale PMAA hydrogel films can be useful for obtaining other thin polymeric hydrogel materials that can be used as transferrable platforms for the development of lab-on-a-chip systems, nanocomposite pressure sensing platforms, rapid optical

biosensors, and responsive platforms for regulating cell adhesion and more effective cell sheet recoveries.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsapm.1c00511>.

Information on AFM topographical images of a film-free silicon wafer with the SiO₂ layer, with a PGMA/PMAA layer, and with surface-anchored and released hydrogen-bonded (PMAA/PVPON)₆₀ films (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Eugenia Kharlampieva – Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294, United States; Center for Nanoscale Materials and Biointegration, University of Alabama at Birmingham, Birmingham, Alabama 35205, United States; orcid.org/0000-0003-0227-0920; Phone: 205-934-0974; Email: ekharlam@uab.edu

Authors

Maksim Dolmat – Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294, United States; orcid.org/0000-0002-4918-7342

Veronika Kozlovskaya – Department of Chemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294, United States; orcid.org/0000-0001-9089-4842

Donald Cropek – U.S. Army Engineer Research and Development Center, Construction Engineering Research Laboratory, Champaign, Illinois 61822, United States

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsapm.1c00511>

Author Contributions

^{||}M.D. and V.K. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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