Photoresponsive Nanometer-Scale Iron Alginate Hydrogels: A Study of Gel–Sol Transition Using a Quartz Crystal Microbalance

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Supporting Information

ABSTRACT: Alginate/Fe³⁺ hydrogels were fabricated on hyaluronic acid (HA) and poly(allylamine hydrochloride) (PAH) multilayers to yield photoresponsive nanometer-scale hydrogels. Light irradiation of the resulting hydrogels induced the photo-reduction of “hard” Fe³⁺ to “soft” Fe²⁺ cations, leading to changes in the mechanical properties of the hydrogels related to their cross-linking behavior. The buildup and the phototriggered response of the supported alginate hydrogels were followed *in situ* with a quartz crystal microbalance (QCM) using an open cell allowing light irradiation from an LED source on top of the hydrogel. The results were correlated to the release profiles of folic acid, employed herein as a drug model, obtained from light-irradiated supported iron alginate hydrogels.

INTRODUCTION

The design of novel functional biomaterials is nowadays focused on the employment of biobased polymers capable of responding to external stimulus, such as magnetic fields,^{1–3} temperature,^{4–6} pH,^{7,8} light,^{9,10} electrochemical potentials,¹¹ and mechanical signals^{12,13} in a well-controlled manner. Stimuli-responsive hydrogels are widely employed in different applications such as drug delivery,¹⁵ tissue engineering,¹⁶ sensors,¹⁷ actuators,¹⁸ and energy storage.¹⁹ Biopolymers are receiving increasing attention in engineering and biomedical fields due to their inherent properties, such as biocompatibility, biodegradability, and nontoxicity.²⁰ Alginate (Alg) is a polysaccharide isolated from brown seaweeds or certain bacteria, that consists of β -(1,4)-linked D-mannuronic acid (M) and α -L-guluronic acid (G) residues arranged in blocks of M fragments, G fragments, and alternating G and M fragments. Subject to ongoing research,²¹ alginate is able to form hydrogels by ionotropic gelation through interactions of G fragments with divalent and multivalent ions.²² One of the most employed cations to cross-link sodium alginate is Ca²⁺,²³ although transition metal ions, such as ferric ions (Fe³⁺), have also been reported as cross-linker ions for the formation of alginate hydrogels.²⁴ The coordination bonds between G fragments and Fe³⁺ ions create a covalent contribution not seen with Ca²⁺ ions which is attributed to the dynamic nature of the metal-coordination bond. This covalent link allows extra functionality by changing the metal-coordination environment, giving rise to new stabilities and mechanical properties for the

resulting alginate hydrogels.^{25,26} Fe³⁺ ions can be subjected to a photochemical reduction triggered by visible-light leading to ferrous ions (Fe²⁺), and this phenomenon has been employed to induce the gel–sol transition of bulk alginate/Fe³⁺ hydrogels.^{9,27} Fe²⁺ is a “soft” metal cation with lower ability than “hard” Fe³⁺ cations for cross-linking alginate affecting the alginate gel stability.²⁸ The reduction of Fe³⁺ to Fe²⁺ can also be achieved electrochemically on an electrode by application of reductive potentials.²⁹ As an example, iron-cross-linked poly(acrylic acid) hydrogels have been obtained through sol–gel transition using Fe²⁺/Fe³⁺ redox couple and application of an external stimuli such as light, air oxidation,³⁰ or an applied potential.³¹

In the past few decades, layer-by-layer (LbL) assembly, based on the sequential adsorption of polycations and polyanions over a substrate, has been studied as a prevalent method for the fabrication of nanoscale structures due to its simplicity and versatility.³² Multilayer thin films built up through LbL assembly have been widely employed in the biomedical field for controlled and sustained drug delivery applications because of its ability to safely load drugs and release them in a controlled manner, improving the drug efficacy.^{33–35} We report herein the synthesis and extensive characterization of a unique class of iron alginate hydrogels,

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Scheme 1. Fabrication of Supported Nanometer-Scale Alginate Hydrogels by LbL Dipping

supported on a multilayer film, with light-responsive behavior. To that aim, alginate hydrogels cross-linked with iron ions were fabricated on top of multilayer films of hyaluronic acid (HA) and poly(allylamine hydrochloride) (PAH). To the best of our knowledge, this is the first time that the formation and photodissolution of nanometer-scale iron alginate hydrogels has been studied and followed *in situ* with a quartz crystal microbalance with dissipation (QCM-D) opening the route for the formation of new materials combining multilayer films and gels at the nanometer scale. Finally, folic acid was employed as a model drug to demonstrate the concept of phototriggered release from supported iron alginate hydrogels.

EXPERIMENTAL SECTION

Materials. Sodium alginate (Alg) was supplied by Sigma-Aldrich (A2158, lot 090M0092V). According to the fabricant, viscosity was 136 cps (2% w/v in water at 25 °C). Molecular weight (M_w) was 166 kDa, as determined by capillary viscosimetry at 25 °C using as solvent 0.1 M sodium chloride and applying the equation of Mark–Houwink ($k = 2 \times 10^{-5}$ dL/g, $a = 1.0$).³⁶ Hyaluronic acid (HA), M_w 301–450 kDa was purchased from Lifecore Biomedical. Poly(allylamine hydrochloride) (PAH) with a molecular weight of 58 kDa was supplied by Aldrich (283223, lot MKBJ4277 V). Iron(II) chloride tetrahydrate (Fe^{2+}) and poly(ethylene imine) (PEI) with a molecular weight (M_w) of 25 kDa were supplied by Aldrich. Sodium L-lactate, 98%, was supplied by Alfa Aesar. The compounds were used as received. The folic acid was obtained from a commercial formulation called ACFOL that contains 5 mg of folic acid per pill. The pill was dissolved in Milli-Q water and filtered first in a cotton-plugged Pasteur pipet and second in a syringe filter and diluted until reaching the desired concentration. The polyelectrolytes, PEI, HA, and PAH, were dissolved in 0.15 M NaCl. The other compounds were dissolved in Milli-Q water.

Preparation and Characterization of Bulk Iron Alginate Hydrogels (Alg-Fe). Bulk Alg-Fe hydrogels were prepared by mixing, in a vial, 0.5 mL of an aqueous solution of Alg (5 or 10 mg/mL) and 2.24 mg/mL of sodium lactate with 0.5 mL of an aqueous solution containing Fe^{2+} (4 or 6 mg/mL) and 1 mg/mL of folic acid. Gel formation was ascertained through the inverted vial test. Samples were denoted as $\text{Alg}_x\text{-Fe}_y$, where x stands for the concentration of alginate in mg/mL and y stands for the concentration of Fe^{2+} in mg/mL. Phototriggered gel dissolution was carried out by irradiation of the Alg-Fe gel with an LED source emitting 405 nm light at a laser power of 10.2 W/cm². Samples were located at a distance of approximately 3 cm from the LED source. The time to achieve gel–sol transition was determined for bulk alginate hydrogels with 5 and 10 mg/mL alginate concentrations and 4 and 6 mg/mL Fe^{2+} concentrations.

Preparation and Characterization of HA/PAH Multilayer Films. HA/PAH multilayers were built on silicon slides (20 × 40 mm, Siebert) previously cleaned with piranha solution (3:1 $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$) for 10 min and rinsed extensively with water. As the silicon substrate is negatively charged, a layer of PEI (1 mg/mL) was deposited as a first layer to get a homogeneous positively charged

substrate. For that, substrates were immersed in a solution of PEI for 5 min and then in a solution of Milli-Q water for 2 min. HA/PAH multilayer films were produced through sequential dipping of a silicon substrate into the HA (polyanion) and PAH (polycation) solutions for 5 min with a rinsing step in 0.15 M NaCl for 2 min after each polyelectrolyte deposition. Substrates were dried after each rinsing step. The cycle was repeated until the desired number of bilayers (n) was obtained. Samples were denoted as $(\text{HA}_z/\text{PAH}_w)_n$, where z and w stand for the concentration of HA and PAH in mg/mL, respectively.

Measurements of $(\text{HA}_z/\text{PAH}_w)_n$ multilayer film thickness were carried out with a PLASMOS SD 2300 ellipsometer operating at the single wavelength of 632.8 nm and a constant angle of 70°. Due to the inherent limit of ellipsometry to simultaneously determine the refractive index and the film thickness for very thin films, the refractive index of all films was assumed to be constant at $n = 1.465$.⁸ While this procedure will lead to slightly incorrect values with respect to the absolute film thicknesses, it allows for the quick and precise determination of relative film thicknesses. Thickness values obtained with the assumption of a fixed refractive index for all films are of better precision than required for the comparison of film growth data as in this report. For each substrate studied, 10 different thickness measurements were randomly taken on different regions over an area of a few cm² of the film surface.

The topography and surface roughness of $(\text{HA}_z/\text{PAH}_w)_n$ multilayer films were evaluated by atomic force microscopy (AFM) using a Veeco MultiMode scanning probe microscope (SPM) with a Nanoscope Iva controller employing a tip RTESP7 at a frequency of oscillation of 265–309 kHz. All measurements were performed in air, at room temperature, on films supported on silicon wafers. Samples were scanned over square regions of 50 μm . For thickness determination, a scratch was applied at the center of the film, leaving a part of the substrate exposed. The images were taken in an area of 50 × 50 μm that comprises the scratch. For the characterization of the thickness, three different regions of the image were evaluated. The image processing was performed with the software Nanoscope 6.14 R1.

Fabrication of Supported Alginate Hydrogels on HA/PAH Multilayer Films. As a first step, a HA/PAH multilayer film of HA (2.5 mg/mL) and PAH (2.0 mg/mL) with a total number of bilayers of 10 was built as described previously and immersed into an aqueous solution of alginate (5 mg/mL) and sodium lactate (2.24 mg/mL) for 15 min. Without rinsing, the sample was introduced in an aqueous Fe^{2+} solution (4 mg/mL) for 5 min and left to air-dry overnight. To encapsulate a drug (folic acid) inside the gel, Fe^{2+} was dissolved in an aqueous solution of folic acid (1 mg/mL). After the process, samples were left to dry at room temperature (20 °C) in a horizontal position for 12 h in a closed Petri dish of 60 mm diameter to ensure that the gel layer remains homogeneously distributed along the substrate surface. Three samples were measured for each condition under study to obtain the average and standard deviation. A schematic representation of the procedure is shown in Scheme 1.

Quartz Crystal Microbalance with Dissipation (QCM-D) Study. QCM-D experiments were carried out in an open QCM-D cell on a Q-Sense E1 apparatus (Q-Sense AB, Gothenburg, Sweden)

Figure 1. Gel formation (left) and photodissolution (right) of Alg_Fe hydrogels obtained at different concentrations of Alg and Fe^{2+} and fixed concentrations of sodium lactate and folic acid (a) Alg5_Fe4 after 24 h, (b) Alg5_Fe6 after 24 h, and (c) Alg10_Fe4 after 72 h. The gel dissolution was triggered by light irradiation.

at 22 °C by monitoring the resonance frequencies of the gold-coated crystal, as well as the dissipation factors at four frequencies, the fundamental frequency at 5 MHz ($\nu = 1$), and the third, fifth, and seventh harmonics ($\nu = 3, 5$, and 7) at 15, 25, and 35 MHz, respectively. The QCM-D experiments give information on the adsorption process, as well as on viscoelastic properties of the adsorbed film. The results corresponding to changes in the frequency shifts ($\Delta f_i/\nu$) and dissipation (ΔD_i), obtained from QCM-D experiments, were analyzed using the Voigt–Voinova model to determine the film thickness. The density was fixed at 1 g/cm³ and the viscosity at 0.91 mPa·s.³⁷

Phototriggered Drug Release. Folic acid release was determined from bulk and supported iron alginate hydrogels as follows: samples were introduced to a vial in 1 mL of Milli-Q water and irradiated with light from an LED source (50 mW at $\lambda = 405$ nm). Aliquots of 0.5 mL were extracted and replaced by pure water at different times. All the aliquots were measured using NanoDrop One^C (Thermo Scientific) at $\lambda = 280$ nm. For comparison, the same protocol was applied to hydrogels not exposed to light.

RESULTS AND DISCUSSION

Multilayer films based on PAH and HA are developed to act as a support and seed layer for the fabrication of iron alginate hydrogels at the nanometer scale. The interaction between the multilayer film HA/PAH and alginate is studied to analyze its effect in the phototriggered release of folic acid.

Bulk Iron Alginate Gel Formation and Phototriggered Gel–Sol Transition. We first optimized the experimental conditions (iron and alginate concentration) for alginate gel formation and its photodissolution in the bulk. The alginate gel formation in the presence of Fe^{2+} is a combination of ionotropic gelation and acid gelation. The ionotropic gelation is produced by the interaction of an ionic polymer with an oppositely charged cross-linker and the acid gelation is the regular way of gelation of alginic acid salts. In this case, the interactions will occur between free COO^- groups of alginate

and Fe^{2+} ions used as cross-linker.²² Fe^{2+} cations have a limited ability to cross-link alginate, giving rise to “soft” hydrogels. In our case, Alg_Fe²⁺ gels were put in contact with air to induce the oxidation into Fe^{3+} , leading to the formation of “hard” hydrogels. To choose the best concentration of alginate and iron to form a gel, different concentrations of Alg (5 and 10 mg/mL) and Fe^{2+} (4 and 6 mg/mL) were tested with a fixed concentration of sodium lactate (2.24 mg/mL). Figure 1 (left) shows the images corresponding to the inverted vial test of gels Alg5_Fe4 (Figure 1a) and Alg5_Fe6 (Figure 1b) obtained after 24 h at room temperature. The sample Alg10_Fe4 (Figure 1c) gelled after 72 h at room temperature according to the inverted vial test. In the absence of Fe^{2+} ions, the gelation did not take place (Figure S1).

Figure 1 (right) shows the photodissolution of Alg_Fe hydrogels in the presence of an LED source ($\lambda = 405$ nm). Fe^{3+} cations are reduced to Fe^{2+} through photochemical reaction, inducing the dissolution of the gel. Sodium lactate was chosen as photoreducing agent to enhance the dissolution of the gel thanks to its oxidative decarboxylation concomitant with Fe^{3+} reduction.^{38,39} Alg5_Fe4 gel was completely dissolved after light irradiation for 65 min in contrast to Alg5_Fe6 gel, obtained with a higher quantity in Fe^{2+} , for which some lumps of gel remained in suspension after light irradiation for 90 min as shown in Figure 1b. Likewise, the sample Alg10_Fe4, in which the alginate concentration was increased up to 10 mg/mL, did not dissolve after being subjected to light irradiation for 2 h (Figure 1c). Therefore, the increase of iron concentration with respect to the alginate concentration resulted in an increase of the time for gel–sol transition for light irradiated iron alginate hydrogels. For the following experiments, we chose to work with the hydrogel that presented the shortest time for sol–gel transition, Alg5_Fe4 (alginate concentration 5 mg/mL and iron concentration 4 mg/mL).

Figure 2. (a) Evolution of the thickness, measured by ellipsometry, vs the number of bilayers of (HA1.2/PAH1.0)_n (red ●), (HA1.2/PAH2.0)_n (blue ▲), (HA2.5/PAH1.0)_n (■), and (HA2.5/PAH2.0)_n (pink ▼) films. (b) Evolution of (HA2.5/PAH2.0)_n film thickness, measured by AFM, vs the number of bilayers. Dashed lines are a guide to the eye.

Figure 3. (a) Fundamental frequency shift, measured at 5 MHz by QCM-D, as a function of time during the alternated depositions of HA (pink ●) and PAH (blue ▲) using HA (2.5 mg/mL) and PAH (2.0 mg/mL). (b) Fundamental dissipation versus the fundamental frequency shift at each deposited layer of HA (●) and PAH (▲). (c) Evolution of the HA/PAH film thickness, determined by fitting the experimental data with the Voigt–Voinova model, as a function of the deposited layers.

Figure 4. (a) Normalized frequency shift ($-\Delta f_1/1$), measured at 5 MHz by QCM-D, as a function of time during the buildup of the hydrogel Alg_Fe with (red line) and without lactate (green line) on top of the (HA/PAH)₁₀ multilayer film. (b) Fundamental dissipation versus the fundamental frequency shift with (red line) and without (green line) lactate.

Study of the Buildup of (HA/PAH)_n Multilayer Films.

The buildup of HA/PAH multilayers was optimized using different polyelectrolyte concentrations. Figure 2a shows the thickness evolution with the number of bilayers, measured by ellipsometry for films prepared with HA and PAH concentrations ranging from 1 to 2.5 mg/mL. The thickness of (HA/PAH)_n films increases exponentially with the number of bilayers up to five bilayers in all cases. The film thickness increase is more pronounced for films fabricated with the highest concentration of HA (2.5 mg/mL). At constant HA concentration, the growth of films is higher for the highest PAH concentration tested (2.0 mg/mL). The exponential growth was attributed to the presence of NaCl in the assembly process because films assembled without NaCl showed a linear growth, as previously reported.^{40,41} Exponentially growing

films are dynamic entities where diffusion and exchange processes of at least one of the polyelectrolytes through the entire film thickness take place at each deposition step.⁴² In our case, the exponential growth could be related to the diffusion of PAH in and out of the multilayer film at each deposition step of PAH and HA, respectively, as previously reported.⁴³ A higher concentration of PAH provides more amine groups to diffuse into the structure, increasing the thickness. Taking into account the restrictions of ellipsometry to determine thicknesses of films with more than five bilayers, AFM performed in a dry state was used to image scratched (HA2.5/PAH2.0)_n films (Figure S2 in SI). The film thickness increases from 160 nm for films with five bilayers to 650 nm for films with 15 bilayers, thus confirming the exponential growth of (HA2.5/PAH2.0)_n. For the following experiments,

Scheme 2. Formation of Alg_Fe-Supported Hydrogel Built on Top of HA/PAH Multilayer Film by Deposition of Alg and Contact with Fe^{2+} Solution, Further Oxidized in Air^a

^aThe supported alginate hydrogel is formed by an alginate pregel obtained by a partial complexation of the alginate chains with PAH chains from the multilayer HA/PAH film and a Alg_Fe nanogel formed by the free alginate chains, which do not interact with PAH, and the iron(II) ions. Only the part of the supported hydrogel corresponding to the Alg_Fe nanogel is dissolved by UV irradiation while the alginate pregel formed by complexation between Alg and PAH remains stable after UV irradiation.

(HA2.5/PAH2.0)₁₀ multilayer films with a thickness of 280 nm were chosen as substrates for the deposition of supported iron alginate hydrogels and designated as (HA/PAH)₁₀.

In Situ Study of the Buildup and Photodissolution of Supported Iron Alginate Hydrogels through QCM-D. The entire buildup of (HA/PAH)₁₀ multilayer films followed by Alg_Fe hydrogel formation was monitored *in situ* by QCM-D using an open cell. During the buildup of the (HA/PAH)₁₀ multilayer film, the evolution of the normalized frequency shift ($-\Delta f_i/\nu$) and the dissipation (ΔD_i) show an exponential growth in the wet state which is consistent with the results obtained in the dry state (Figure S3 in SI). Figure 3a shows the normalized frequency shift $-\Delta f_i/1$ after each deposited layer, HA in pink and PAH in blue. To evaluate the rigidity properties of the film, Figure 3b shows the evolution of ΔD_i versus $-\Delta f_i/1$. The higher slope of the blue curve indicates a more rigid behavior of PAH-ending films in comparison with HA-ending films. This could be explained by the diffusion of PAH inside the film, when PAH is the last layer deposited, creating a PAH reservoir and rendering the film more dense. When HA is the last deposited layer, PAH is diffusing out of the film to complex with HA chains, leading to less dense film. The thickness of HA/PAH film, obtained by fitting the experimental data with the Voigt–Voinova model, reaches a thickness of $1.1 \pm 0.1 \mu\text{m}$ for 10 bilayers (Figure 3c).

After (HA/PAH)₁₀ film buildup, a layer of Alg (5 mg/mL) was deposited onto the multilayer film (with and without sodium lactate) followed by contact with an aqueous solution of Fe^{2+} cations (Figure 4a). In both cases, a high increase of $-\Delta f_i/1$, $\sim 1360 \text{ Hz}$, is obtained after the deposition of alginate and a smaller one, $\sim 100 \text{ Hz}$, after the injection of the aqueous solution containing Fe^{2+} cations. The contact with alginate solution leads to a high frequency shift due to the complexation of alginate chains with PAH chains. Indeed, as in the case of HA deposition, PAH is diffusing out of the film to complex with alginate chains, leading to the formation of PAH/Alg complexes. Further contact with Fe^{2+} cations gives

rise to a smaller increase in frequency shift which can indicate their complexation with alginate chains. In the presence of sodium lactate, the frequency shift signal slightly increases with the time due to the oxidation of “soft” Fe^{2+} to “hard” Fe^{3+} in contact with air giving rise to a hydrogel (zoom-in Figure S4 in SI). The influence of sodium lactate in the rigidity of the hydrogel was studied by QCM-D. Figure 4b exhibits the variation of ΔD_i versus $-\Delta f_i/1$ for hydrogels prepared with and without sodium lactate. The slope of the curve is lower in the presence of lactate indicating a lower rigidity of Alg_Fe gel (Figure 4b). This result is consistent with the elastic modulus, determined through oscillatory shear measurements, measured for bulk Alg_Fe hydrogels prepared at the same Alg and Fe^{2+} concentrations (5 mg/mL alginate concentration and 4 mg/mL iron concentration) (Figure S5 in SI). The value of the elastic moduli measured at 1 Hz frequency for Alg_Fe is $100 \pm 8 \text{ Pa}$. Lactate addition decreases the elastic moduli of the resulting gels to $65 \pm 5 \text{ Pa}$ yet preserving the gel formation as demonstrated through oscillatory frequency tests. This could be due to competitive complexation of Fe^{2+} with sodium lactate carboxylic groups thus preventing the interaction with alginate carboxylic groups. To determine the thickness of the supported iron alginate gel with incorporated sodium lactate fabricated on top of the multilayer (HA/PAH)₁₀ film, experimental data from QCM-D were fitted by using the Voigt–Voinova model. The thickness obtained for the supported alginate gel is $437 \pm 231 \text{ nm}$ before the addition of the aqueous solution of Fe^{2+} cations and increases slightly to $490 \pm 215 \text{ nm}$ with the addition of Fe^{2+} . These results highlight the fact that the main composition of the supported gel is PAH/Alg complexes.

Scheme 2 represents the proposed mechanism for the formation of Alg_Fe hydrogel supported on a HA/PAH multilayer film. (HA/PAH)₁₀ film constitutes a reservoir of PAH chains, due to the exponential growing behavior of the film (Scheme 2a). The contact of the alginate solution with this film leads to a partial complexation of the alginate chains

Figure 5. (a) Percent remaining supported gel and normalized frequency shift ($-\Delta f_1/1$), measured at 5 MHz by QCM-D, as a function of time for the supported iron alginate hydrogels in contact with water non-light-irradiated (black line) and light-irradiated (red line). (b) Evolution of the UV-vis absorbance, measured at 236 nm, as a function of time of the supported iron alginate hydrogel exposed to light from LED source. The dashed line is a guide to the eye. The inset shows a photo of the supported hydrogel after being immersed for 6 h in water. The red rectangle marks the area subjected to light irradiation.

Figure 6. Release of folic acid in water from (a) supported iron alginate hydrogels non-irradiated (black points) and light-irradiated (red points) and (b) schematic representation of the mechanism proposed for the folic acid release for nonirradiated supported iron alginate hydrogels (left) and for light irradiated hydrogels (right).

with PAH chains forming a “pregel” and the presence of free alginate chains on top of the “pregel” (Scheme 2b). When the architecture is put into contact with Fe^{2+} ions solution, free alginate chains are complexed by “soft” Fe^{2+} ions and subsequently oxidized to “hard” Fe^{3+} ions in the presence of air, giving rise to the supported iron alginate hydrogel (Scheme 2c) with a slight increase of the obtained thickness. The proposed formation mechanism for Alg_Fe hydrogels is analogous to that described for Alg_ Ca^{2+} gels built on top of PLL/HA exponentially growing multilayers.⁴⁴ Light exposure ($\lambda = 405$ nm) leads to gel-sol transition of the supported hydrogel (Scheme 2d). Bruchet et al.⁴⁵ have reported a partial photochemical dissolution of iron alginate hydrogels in bulk by creating a diffusion gradient of lactate through the hydrogel. In that case, a solution of lactate was put in contact with the top of the iron alginate hydrogel to be then irradiated with UV light, allowing the etching of the thin layer of hydrogel, which was in contact with lactate, while the rest of the gel without contact with lactate remained stable. In our case, we follow a different strategy by creating a supported hydrogel composed of an alginate pregel with sodium lactate incorporated, which is not dissolved by UV irradiation due to its complexation with free PAH chains from the multilayer film HA/PAH acting as a support, and a Alg_Fe nanogel over it, which is phototriggered by UV irradiation.

The gel-sol transition of light-irradiated iron alginate hydrogels was followed by QCM-D by placing the LED source on the top of the open cell (Figure 5a). For non-

irradiated hydrogels, $-\Delta f_1/1$ is constant during the entire experimental time, showing stability of the hydrogel in contact with water for at least 2 h (100% of remaining gel). In contrast, under irradiation, the supported hydrogel loses 7% in frequency shift in 2 h. The fundamental frequency shift of the iron alginate hydrogel decreases $-\Delta f_1/1 \sim -100$ Hz over an initial value of -1460 Hz. This dissolved part of the supported gel is the one formed after the addition of Fe^{2+} (Figure 4a). Most of the supported hydrogel (93%) is stable in contrast to the bulk gel which is completely destroyed after 65 min (Figure 1a).

The photodissolution of the supported hydrogel was also ascertained through UV-vis spectroscopy (Figures 5b and S5a in SI). For that purpose, the whole sample was immersed in water and only the area marked by a red rectangle was placed under LED source for 6 h. Outside of the red rectangle, the supported hydrogel keeps its light orange color characteristic of the presence of Fe^{3+} inside the gel (Figure 5b). There is no change detected in the UV-visible spectra over time (Figure S6b in SI). The supported hydrogel area subjected to light irradiation loses its light orange color (red rectangle in Figure 5b). A decrease of the absorbance at 236 nm is observed over time until reaching a steady state after 2 h. This is due to the photoreduction of Fe^{3+} into Fe^{2+} triggered by light.

Phototriggered Drug Release. Folic acid was employed as a model drug to demonstrate the concept of phototriggered drug release. The results corresponding to cumulative drug

release of folic acid from supported alginate hydrogels with and without light are depicted in Figure 6.

For non-irradiated supported hydrogels, ~60% folic acid was released within 60 min and ~100% of folic acid was released within 24 h. Light irradiation of iron alginate hydrogels greatly accelerates folic acid release, and ~80% folic acid was released within 30 min (Figure 6a). Folic acid release profiles from bulk hydrogels (Figure S7 in SI) present a similar trend, that is, light irradiation accelerates folic acid release; ~15% folic acid was released within 15 min for non-irradiated alginate hydrogels and increases to ~100% folic acid release for light-irradiated alginate hydrogels.

The results obtained for supported alginate hydrogels can be correlated to those obtained by QCM-D (Figure 5a) which shows that supported iron alginate hydrogels remain stable in contact with water with no notable dissolution in the absence of light exposure. In contrast, light irradiation leads to the dissolution of 80% of the supported hydrogel in 30 min which leads to an increased rate of folic acid release. The results can be further explained, taking into account the two phases proposed for iron alginate hydrogels depicted in Figure 6b: Alg/Fe³⁺ gel and the alginate pregel. In the absence of light, Alg/Fe complexes have high interaction with Fe³⁺, giving rise to low release of folic acid throughout this gel layer (bottom). With light, the absence of the Alg/Fe³⁺ gel due to its phototriggered gel–sol transition leads to a faster folic acid release.

A comparison of the release profiles obtained from supported and bulk hydrogels allows us to conclude that for non-irradiated hydrogels, the initial drug release (within the first 10 min) was accelerated for supported alginate hydrogels with respect to bulk alginate hydrogels. This might be attributed to the fact that quick diffusion of folic acid from the surface layer of the nanometer-scale iron alginate hydrogels might play a dominant role during the initial drug release period.⁴⁶ Conversely, light-irradiated supported hydrogels show a more sustained folic acid release over time with respect to bulk hydrogels due to the presence of an alginate pregel consisting of PAH/Alg complexes that hinders the burst release of folic acid seen in bulk alginate hydrogels.

CONCLUSIONS

Nanometer-scale alginate hydrogels have been formed on top of a multilayer HA/PAH film employing the dipping-assisted LbL technique. The buildup process has been followed *in situ* by QCM-D, allowing us to determine the gel thickness, ~490 nm, and the influence of sodium lactate on the elastic properties of the gel. The iron and alginate concentrations needed for the formation of bulk iron alginate hydrogels and the time for phototriggered gel–sol transition were previously determined by inverted tube tests. In addition, the optimal experimental parameters for the buildup of HA/PAH multilayer films, in terms of the number of bilayers and concentration of HA and PAH, were determined by ellipsometry and AFM. The phototriggered dissolution of the supported hydrogels through Fe³⁺ reduction into Fe²⁺ was followed by QCM-D and UV–vis spectroscopy, showing a complete dissolution of the supported alginate hydrogel after light exposure for 2 h. The potential application of the supported nanometer-scale hydrogels for phototriggered drug release was proven by incorporation of folic acid during gel formation and subsequent release triggered by light from an LED source. With light irradiation, the design of supported

nanometer-scale photoresponsive alginate hydrogels constitutes a strategy for a more sustained drug delivery over time with respect to bulk alginate hydrogels.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.9b01224.

AFM topographic images corresponding to (HA/PAH)_n films; QCM-D data for (HA2.5/PAH2.0)₁₀ and for the nanogel Alg_Fe on top; oscillatory shear measurements for bulk iron alginate hydrogels; UV–vis spectra of (HA/PAH)₁₀-Alg_Fe in contact with water and/or LED irradiation; release of folic acid from bulk iron alginate hydrogels (PDF)

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Notes

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