Retention of poly(*N*-isopropylacrylamide) on 3-aminopropyltriethoxysilane

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(Received 9 February 2017; accepted 10 April 2017; published 27 April 2017)

Silane coupling agents are commonly employed to link an organic polymer to an inorganic substrate. One of the widely utilized coupling agents is 3-aminopropyltriethoxy silane (APTES). In this study, the authors investigated the ability of APTES to retain thermo-responsive poly(*N*-isopropylacrylamide) (pNIPAAm) on hydroxylated surfaces such as glass. For comparison purposes, the authors also evaluated the retention behaviors of (1) polystyrene, which likely has weaker van der Waals interactions and acid–base interactions (contributed by hydrogen-bonding) with APTES, on APTES as well as (2) pNIPAAm on two other silane coupling agents, which have similar structures to APTES, but exhibit less interaction with pNIPAAm. Under our processing conditions, the stronger interactions, particularly hydrogen bonding, between pNIPAAm and APTES were found to contribute substantially to the retention of pNIPAAm on the APTES modified surface, especially on the cured APTES layer when the interpenetration was minimal or nonexistent. On the noncured APTES layer, the formation of an APTES-pNIPAAm interpenetrating network resulted in the retention of thicker pNIPAAm films. As demonstrated by water contact angles [i.e., 7°–15° higher at 40 °C, the temperature above the lower critical solution temperature (LCST) of 32 °C for pNIPAAm, as compared to those at 25 °C] and cell attachment and detachment behaviors (i.e., attached/spread at 37 °C, above LCST; detached at 20 °C, below LCST), the retained pNIPAAm layer (6–15 nm), on both noncured and cured APTES, exhibited thermo-responsive behavior. The results in this study illustrate the simplicity of using the coupling/adhesion promoting ability of APTES to retain pNIPAAm films on hydroxylated substrates, which exhibit faster cell sheet detachment (≤30 min) as compared to pNIPAAm brushes (in hours) prepared using tedious and costly grafting approaches. The use of adhesion promoters to retain pNIPAAm provides an affordable alternative to current thermo-responsive supports for cell sheet engineering and stem cell therapy applications. © 2017 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4982248>]

I. INTRODUCTION

Retaining polymers on a surface is often necessary for their various applications. Polymer retention is often achieved by using different grafting methods^{1–5} or by utilizing coupling agents/adhesion promoters.^{6–9} When silanes are used as the adhesion promoters, they are normally deposited on the inorganic/metal surface and cured to form a network, which is expected to interdiffuse with the polymer once the polymer is placed on it.^{10–12} Thus, an effective adhesion promoter should consist of appropriate groups that would react/interact with the polymer as well as exhibit a suitable solubility with the polymer to form an interdiffused interphase. A common practice for generating thermo-responsive surfaces for cell sheet engineering and therapeutic treatment for damaged tissues and organs is grafting thermo-responsive polymers, such as poly(*N*-isopropylacrylamide) (pNIPAAm), as brushes to a surface.^{2–4,13} This approach could be more expensive and tedious

as compared to the coupling/adhesion promoting approach.^{2–5}

However, the thermo-responsive and cellular behaviors on these retained pNIPAAm films as well as the mechanistic study of this coupling/adhesion promoting approach to retain these thermo-responsive polymers, to the best of our knowledge, have not been investigated.

In this study, 3-aminopropyltriethoxysilane (APTES) a common coupling agent that exhibits many unique properties due to its molecular nature, especially when it is fully hydrolyzed (containing three –OH groups), is being used for retaining pNIPAAm on a silica based surface. Its functional –NH₂ group and the –OH groups not only react with the active groups on hydrolyzed substrates, but also allow both intermolecular and intramolecular hydrogen bonding.^{7,8,14–18} The short backbone alkyl chains (three –CH₂) of APTES provide insufficient intermolecular van der Waals forces to form an ordered monolayer, instead forming a disordered loose three-dimensional multilayered network that anchors to the substrate with fewer chemical bonds.^{7,17–20} This loose network allows the interdiffusion of

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polymer chains, especially for polymers that are compatible with APTES. The network can be tightened when the APTES molecules cross-link with each other under thermal annealing to firmly lock the diffused polymer chains in the network.⁶⁻⁸ In addition, to further enhance the coupling, hydrolyzed APTES, with its amino and hydroxyl groups, might allow acid-base (AB) (e.g., H-bonding) interactions with the amide group in pNIPAAm (Ref. 21) as well as van der Waals interactions. Therefore, by simply placing pNIPAAm on top of the APTES network that is bound to a surface and subjecting the sample to a thermal treatment, pNIPAAm can be retained on the surface.

Based on the above hypothesis, we investigated the retention of pNIPAAm on the APTES layers with different extents of cross-linking. The retention of pNIPAAm on APTES was determined by rinsing and soaking the sample in water. All the cured (i.e., a tight APTES network) and noncured (i.e., a loose APTES network) layers were able to retain pNIPAAm. As a comparison, polystyrene (PS), which has lesser interactions with APTES, was also studied. Only the noncured or slightly cured APTES layers could retain PS, and the retained PS layer was much thinner than that of pNIPAAm. To further verify the uniqueness of the APTES layer in retaining pNIPAAm, the silane coupling agents 3-mercaptopropyltrimethoxy silane (MPTMS) and triethoxysilylbutyraldehyde (TESBA), which have a similar structure to APTES but are less likely to form hydrogen-bonding, were briefly assessed on their ability to retain pNIPAAm. pNIPAAm retained on the noncured MPTMS and TESBA layers were 20%–30% of that retained on APTES; the cured MPTMS and TESBA basically retained no pNIPAAm. All the retained pNIPAAm films (6–15 nm) on APTES exhibited thermo-responsive behaviors (TRBs), verified by water

contact angle, film thickness, and cell attachment/detachment studies done at temperatures above and below the lower critical solution temperature (LCST) of pNIPAAm (i.e., 32 °C). The TRB was more pronounced for a thicker film. The pNIPAAm films generated using our simple approach are comparable, in terms of cell attachment/proliferation and cell sheet detachment, to those pNIPAAm brushes generated through laborious approaches. More importantly, our approach is more economical and environmentally friendly and it eliminates the use of agents that might be needed for promoting cell adhesion. The pNIPAAm films retained by APTES could be a cost-effective, attractive alternative to current thermo-responsive supports for cell sheet engineering applications and damage-free cell harvesting.

II. EXPERIMENT

A. Materials and equipment

The main materials used for this study, along with their molecular formulas and some properties, are summarized in Table I. pNIPAAm with a number average molecular weight of 20–40 kg/mol and 99% APTES were obtained from Sigma-Aldrich. MPTMS and TESBA were obtained from Gelest, Inc. The ~29 kg/mol polystyrene standard (with a polydispersity index of 1.06) was obtained from Alfa Aesar. 200 hundred proof ethanol was obtained from EMD. High-performance liquid chromatography (HPLC)-grade toluene was obtained from BDH. Deionized (DI) water with a conductivity of $\sim 0.5 \mu\text{S}/\text{cm}$ was purified in house. Other chemicals used included 30% hydrogen peroxide, and 98% concentrated sulfuric acid from VWR. The cell culture medium used was Dulbecco's modified eagle's medium +

TABLE I. Basic information of the materials used in this study.

Substance	Molecular formula	Molecular weight M_w (g/mol)	Surface energy γ (mJ/m ²)	Boiling point (T _b) or glass transition (T _g) (°C)	Solubility parameter δ (MPa ^{1/2})	Radius of gyration R _g (nm)
APTES	H ₂ N(CH ₂) ₃ Si(OC ₂ H ₅) ₃ or $H_2N(CH_2)_3Si(OH)_3$	221.37	~38 ^a	217 at 760 mmHg	30.3 ^b	N/A
MPTMS	HS(CH ₂) ₃ Si(OCH ₃) ₃ or $HS(CH_2)_3Si(OH)_3$	196.34	41 ^c	93 at 40 mmHg	29.4 ^b	N/A
TESBA	O=CH(CH ₂) ₃ Si(OC ₂ H ₅) ₃ or $O=CH(CH_2)_3Si(OH)_3$	234.37	NA	85–87 at 1 mmHg	31.2 ^b	N/A
pNIPAAm		20 000–40 000	~36 ^a	~135	24.7 ^b	6.7–9.5
PS		~29 000	41.9 ^d	~100	18.9 ^b	8.5

^aData were from the study by Alghunaim *et al.* (Ref. 8).

^bValues were estimated using the group-contribution method (Ref. 23) for APTES, MPTMS and TESBA, and the hydrolyzed forms (structures shown in *italic* in the table) were used to estimate their solubility parameters since most of these silane molecules have been hydrolyzed during the process. R_g was estimated using $R_g = (NC_\infty)^{1/2} I$, where N is the degree of polymerization, I is the C–C bond length (0.154 nm), and C_∞ is the characteristic ratio of the polymer, which has a value of 10.6 for pNIPAAm and 10.8 for PS. NA: not available; N/A: not applicable.

^cData were obtained from Gelest's catalog.

^dData were taken from the study by Wang *et al.* (Ref. 22).

10% fetal bovine serum + 1% of antibiotic antimycotic solution (100×) and were obtained from Sigma-Aldrich. Unless otherwise mentioned, other reagents, such as 1× trypsin/ethylene diamine tetra-acetic acid and phosphate buffered saline, were obtained from Sigma-Aldrich. NIH3T3 cells (ATCC[®] CRL-1658, a type of mouse embryonic fibroblast) were obtained from ATCC. Other supplies included microscope glass slides obtained from VWR, silicon wafers (P type P<100) obtained from Silicon specialist, and treated 24-well plates and 35 mm culture dishes, both obtained from greiner bio-one.

Basic equipment used for this study included a spin coater (p-6000 Spin Coater, Specialty Coating System Inc., Indianapolis, IN), a UV/ozone cleaner (model 42, Jelight), analytical balances with an accuracy of 0.1 mg, a vacuum oven (VWR) and its pump (Welch), a humid CO₂ cell incubator (Model NU-5510, NuAire), a contact angle goniometer (Ramé-Hart Instrument Co., Netcong, NJ with a CCD camera attached), an ellipsometer (Rudolph Instruments, Inc., Fairfield, NJ equipped with a $\lambda = 632.8$ nm laser), a microscope heating stage (TP-110R, TOKAI HIT), a digital camera, an optical microscope with an eye-piece digital camera, a humidifier, and XPS (PHI VersaProbe II Scanning XPS microprobe) with a monochromatic Al K α excitation source (x-ray setting: 100 μ m, 25 W and 15 kV; Analyzer mode: FAT). A take-off angle of 45° was used.

B. Preparation of APTES, MPTMS or TESBA layers

Glass slides and Si-wafers were cut into $\sim 1 \times 1$ cm pieces and then cleaned using a freshly prepared piranha solution followed by copious DI water rinsing. The slides and wafers were dried with a stream of dry air and then oxidized for 8 min in the UV/Ozone chamber. These freshly cleaned slides and wafers were flooded with ~ 50 μ l of 1 wt. % APTES, MPTMS, or TESBA in 200 proof ethanol and spin-coated at a spinning speed of 2000 rpm for 30 s. At least two glass slides and two Si-wafer substrates were coated for each condition. To minimize the dewetting of the coating during spin-coating, the relative humidity inside the spin-coater was controlled to 20%–25% using a heat lamp. For APTES, the spin-coated slides and wafers were placed inside glass petri-dishes for at least 30 min under ambient conditions and then placed inside the vacuum oven to be cured for 1 h to 3 days at 160 °C. Some coated samples were also cured for 15 min at 100 °C inside the vacuum oven, a condition used by others to generate an APTES layer as an adhesion promoter.^{12,17} The cured samples, removed from the oven and cooled, were individually placed, along with the noncured APTES samples, into the wells of 24-well plates for storage.

Slides and Si-wafers were also modified by submerging them in 0.2 wt. % APTES in HPLC-grade toluene for 2 h and then removed. They were sonicated in toluene for 2 min and then in ethanol for 2 min. The modified slides and wafers were either cured for 3 days at 160 °C in vacuum or stored inside a petri-dish under the ambient condition for 3 days.

C. pNIPAAm or PS on APTES, MPTMS or TESBA

pNIPAAm of 0.6 wt. % in 200 proof ethanol or PS of 0.6 wt. % in HPLC-grade toluene was prepared. Each of these solutions was spin-coated to the APTES, MPTMS, or TESBA coated/deposited slides or wafers prepared in the above step, after flooding each surface with ~ 50 μ l of pNIPAAm or PS solution, at a spinning speed of 2500 rpm for 60 s. The samples of pNIPAAm on silane were then cured in the vacuum oven for 3 days at 160 °C, while the PS on APTES samples was cured in the vacuum oven for 3 days at 125 °C.

D. Characterizations of silane layers and polymer films

The films on the Si-wafer were mainly used for thickness measurements. They were occasionally used to verify the water contact angles measured on the films prepared on glass slides. The film thickness for each sample was measured after the sample was removed from the oven, after it was thoroughly rinsed with DI water (for pNIPAAm) or toluene (for PS) and after it was soaked in DI water or toluene for 3 days in ambient conditions. The film thickness was measured using an ellipsometer with a $\lambda = 632.8$ nm laser; the values were used to assess the retention of the films on the surface. The refractive indices of 1.45 and 1.50 [estimated from the average refractive indices of the silane (1.42) and pNIPAAm (1.47) and those of silane and PS (1.57), respectively] were used to estimate the retained film thickness of pNIPAAm and PS, respectively, on silane in air. For the retained pNIPAAm films on silane in water, the laser intensity was closely followed during the heating and cooling cycles to ensure there was indeed a change in the thickness as the temperature change passed the transition point (~ 32 °C). For the case where the laser intensity change was observed, the thickness was estimated using the refractive indices of 1.38 at 25 °C (<LCST) and 1.40 at 40 °C (>LCST). These refractive indices were estimated based on the pNIPAAm refractive indices in water at these temperatures.²⁴ When no laser intensity change was observed, a refractive index of 1.42 was used for both 25 °C (<LCST) and 40 °C (>LCST).

The water contact angle on the film was measured using a contact angle goniometer equipped with a heating/cooling stage. For APTES layers with different extents of curing, water contact angles (advancing, receding, and static angles) were measured at room temperature. For the thoroughly rinsed pNIPAAm on APTES, advancing and static water contact angles were measured at 40 and 25 °C by placing the sample on the heating stage. After the heating stage reached the set temperature (40 or 25 °C), the sample was placed on the stage and allowed to equilibrate for ~ 5 min. Then, a water drop (~ 10 μ l) was placed on the sample, with the needle in the drop, and more water was slowly added until the drop was ready to advance, the point at which the image was captured. The time from adding the drop to taking the image was ~ 1 min. After three advancing measurements, the needle was withdrawn and the drop (~ 20 μ l) was allowed to sit on the sample for ~ 1 min before the image was taken for the static contact angle. A drop of DI water was added to the

sitting drop and again allowed to equilibrate for ~ 1 min before taking another image for the static contact angle. The contact angles were measured from the captured images using IMAGEJ software.

The samples used for XPS scans were freshly prepared (within 1 week) and were degassed in a vacuum oven overnight prior to XPS scanning. Survey scans for the binding energy (BE) of 0–700 eV were obtained for the samples using a pass energy of 117.40 eV, a resolution of 0.5 eV, and a time per step of 50 ms. Three sweeps were applied to obtain each data point for the survey scans. The high-resolution scans for C1s were acquired using five sweeps under a pass energy of 11.75 eV, a resolution of 0.1 eV, and a time per step of 50 ms. For the cases of pNIPAAm, the BE range of 278–290 eV was scanned, and the BE range of 278–294 eV was used for the cases of PS. The dual beam charge neutralization was applied as per the manufacture's standard for all the scans.

MULTIPAK software (Physical Electronics) (PHI) was used to process the data. All the binding energies were referenced to the main C1s (i.e., C–C/C–H) peak designated at 284.8 eV. The atomic concentration was determined from the survey scans using the peak areas under each peak component and after using a Shirley background subtraction. The high resolution C1s peaks were decoupled to show the peaks associated with C–H/C–C, C–N, and N–C=O. For the cases of pNIPAAm, the peak at ~ 285 eV could be further fitted into two bands, one at 284.9 eV for the two $-\text{CH}_3$ groups and the other at 285.3 eV for the $-\text{CH}_2-$ in the pNIPAAm backbone, but for a better comparison to the reported results,^{1,25,26} we decided to combine these two (C–H/C–C) peaks as normally adopted by others.^{1,25,26} The peak resolution was performed first using a Shirley background subtraction, followed by a seven point Savitzky-Golay smoothing operation, and then by applying a mixed Gaussian-Lorentzian peak. After roughly determining the location and the height of each band, the full-width at half maximum (FWHM) values were set to values that gave the best fits and made the most physical sense of the data. The FWHM values for the two or three bands for decoupling each C1s peak were normally within 5% of each other.

E. Cellular behaviors on retained pNIPAAm films

Approximately 150 000 cells/ml were seeded in a 35 mm dish containing a pNIPAAm retained glass slide after the slide was thoroughly rinsed with cold DI water and allowed to incubate at 37 °C ($>$ LCST of pNIPAAm) for 2–3 days until the cells reached confluence. The dish was removed from the 37 °C incubator and immediately placed on the microscope stage, and as the medium was cooled to and maintained at room temperature (~ 23 °C, $<$ LCST), the cell detachment process was followed using a microscope-video system and using a 10 $\times$ phase objective. A sequence of images was captured with a preset time interval (5, 10, 20, or 30 s) until the entire sheet was detached or the attached cells were detached.

III. RESULTS AND DISCUSSION

A. Thermal curing of APTES layers

Figure 1(a) shows the curing of the APTES layer spin-coated from 1 wt. % of APTES in ethanol at 160 °C in vacuum. The uncured (i.e., 0 h) spin-coated APTES layer, after drying inside a fume hood under ambient conditions for 2 h, had a thickness of ~ 5 nm, which indicates a multilayer of APTES, since the APTES monolayer thickness is ~ 0.7 nm. After rinsing the uncured layer with ethanol, a ~ 3.2 nm or ~ 4.5 -molecule thick layer of APTES remained. After thoroughly rinsing the non-cured APTES layer with water, a ~ 2 nm or ~ 3 -molecule thick layer of APTES resulted. Soaking this noncured APTES layer for 3 days in cold water did not result in an additional thickness reduction, suggesting that the layer is strongly grafted to the substrate. When the spin-coated APTES layer was thermally cured at 160 °C for

FIG. 1. Thickness of (a) APTES layers cured for different durations, (b) the retained pNIPAAm and (c) the retained PS, which had been thermally annealed for 3 days on these APTES layers. The rinsing and soaking were done using cold DI water for pNIPAAm and toluene for PS. The red and green dashed lines are used to indicate, respectively, the thickness of APTES alone after rinsing and after soaking. The silica layer thickness (1.5–2 nm) was subtracted and is not included in the thickness values.

1 h or longer (up to 72 h), the cured and rinsed APTES showed a similar thickness of ~ 3 nm, and soaking the cured APTES layer in water resulted in an increased layer thickness of ~ 4 nm. The reduction in the thickness of the cured film, as compared to 5 nm of the noncured APTES layer, likely resulted from the evaporation of nongrafted APTES in vacuum at 160°C , and the remaining APTES molecules were strongly adsorbed, physically and/or chemically, to the substrate. Additional thermal annealing (2–72 h) at 160°C did not result in noticeable changes in the APTES layer thickness [Fig. 1(a)].

The APTES layers without thermal curing had advancing and static water contact angles of 53° and 40° [Fig. 2(a)], respectively, which agrees with the values reported by others on the freshly prepared APTES layers,^{7,27} where some of the $-\text{NH}_2$ and $-\text{OH}$ groups are exposed to the surrounding. The water contact angles on the cured APTES layers continuously increased and plateaued in 4 h to the values of $\sim 88^\circ$ and 80° , respectively, for advancing and static angles. The increased water contact angle, implying hydrophobicity, could be due to the consumption of the $-\text{OH}$ groups in APTES to form siloxane bonds and the rearrangement of the APTES molecules to expose the more hydrophobic moieties (e.g., $-\text{CH}_2$ backbone) to minimize the total surface energy. The contact angle hysteresis (i.e., the difference between the advancing and receding angles) was slightly higher (35° – 40°) at a shorter curing time (≤ 2 h), suggesting a slightly greater heterogeneity of the APTES layer and/or the interactions between the APTES layer and water. The heterogeneity might be the result of the APTES layer being disordered,^{7,17,18,20,27,28} which would increase both surface roughness and chemical heterogeneity. These interactions could come from the hydrogen-bonding. When more $-\text{OH}$ and $-\text{NH}_2$ groups in APTES molecules are easily accessible, these interactions become greater.

Soaking the APTES layers in cold water for 3 days led to a reduction in all the water contact angles (reduced by $\sim 10^\circ$ and 20° for the advancing and static angles, respectively), especially the receding angles (reduced by 30°), for all the APTES layers [Fig. 2(b)]. The reduction could mainly result from the penetration of water molecules into the APTES (Ref. 27) to hydrogen bond with the $-\text{NH}_2$ and the unreacted

Fig. 2. Water contact angles of APTES layers (prepared by spin-coating 1 wt. % APTES in ethanol) cured with different curing times at 160°C inside a vacuum oven (a) before and (b) after rinsing + 3 days of soaking of the APTES layers in cold DI water.

$-\text{OH}$ groups in APTES molecules. These water molecules also swelled the network and even retained within the APTES layer after it was blown dry by air. The swelling is evidenced by the increase in the layer thickness, from ~ 3 to ~ 4 nm, as shown in Fig. 1(a). It is also possible that some rearrangement of the hydrophilic moieties of the APTES molecules rose to the surface when the sample was submerged in water, and these moieties remained on the surface after drying by air and played a role in the water contact angle reduction. The higher contact angle hysteresis ($\sim 50^\circ$) observed for all the water soaked APTES layers indicates a stronger interaction, e.g., hydrogen-bonding, between the soaked APTES layer and water molecules. The contact angles of soaked samples still increased with curing time and levelled off at ~ 6 h of curing at 160°C . These results illustrate that after the APTES layer is cured for 4–6 h at 160°C , it would result in little to no further change in the network structure with a longer curing time. Earlier studies^{7,18} have reported that by thermal curing an APTES layer at 80 and 120°C , the water contact angle values plateaued after ~ 15 and 10 h of curing, respectively. At a higher temperature (e.g., 200°C), a much shorter curing time was noticed for the water contact angles to level off, and therefore, the results of APTES curing in this study are in accord with earlier results.^{7,15,16,18,29,30}

B. Retention of the pNIPAAm film and PS on the APTES layer

When pNIPAAm films (~ 30 nm) were placed on top of the APTES layers and allowed to interdiffuse at 160°C ($\sim 25^\circ\text{C}$ above the T_g of pNIPAAm) for 3 days, pNIPAAm retention on the APTES layers was clearly evidenced as shown in Fig. 1(b). After rinsing, the layer thickness decreased slightly, ranging from ~ 17 nm for the noncured APTES layer to ~ 9 nm for the APTES layer cured for 2 h or longer. The slightly thicker pNIPAAm film retained on the noncured and 1 h cured APTES layers could be the result of easier penetration, i.e., forming the interpenetrating network (IPN), of the pNIPAAm chains into the less cross-linked APTES network. Three days soaking in cold water removed an additional ~ 2 nm of pNIPAAm. As shown in Fig. 1(a), soaking the APTES layer in cold water resulted in the swelling of the APTES layer, which would allow some of the entrapped pNIPAAm chains to be diffused out into the surrounding water.

When PS (~ 30 nm) was spin-coated on top of the APTES layers and allowed to interdiffuse at 125°C ($\sim 25^\circ\text{C}$ above the T_g of this PS) for 3 days, the amounts of PS retained on the APTES layers were less [Fig. 1(c)]. Only the noncured and slightly cured (at 100°C for 15 min in Fig. S2,⁴⁵ the condition utilized for applying APTES as the coupling agent by researchers) APTES layers appeared to retain PS, with a thickness of ~ 4 nm, and this thickness remained after soaking the sample in toluene for 3 days. Once the APTES layer had been cured for 1 h or longer, a minimal amount of PS (≤ 1 nm) was retained.

The ability of the polymer chains to diffuse into the APTES network as it is being formed resulted in more

pNIPAAm retained on the noncured APTES layer than PS. The styrene unit ($\sim 4.5 \times 7 \text{ \AA}$) is bulkier than the NIPAAm unit ($\sim 2.7 \times 8 \text{ \AA}$ —estimated from bond lengths and its molecular structure), and the PS chains are likely stiffer due to the presence of benzene ring stacking. As a result, it would be harder for PS chains, as compared to pNIPAAm chains, to diffuse into the APTES network during the thermal curing process. In addition, the miscibility of pNIPAAm with APTES is greater than that of PS with APTES, as shown by the larger difference in solubility parameters between styrene and APTES ($\delta_{\text{fully hydrolyzed APTES}} - \delta_{\text{PS}} = 30.3 \text{ MPa}^{0.5} - 18.7 \text{ MPa}^{0.5}$ or $11.6 \text{ MPa}^{0.5}$) versus that between NIPAAm and APTES ($\delta_{\text{fully hydrolyzed APTES}} - \delta_{\text{pNIPAAm}} = 30.3 \text{ MPa}^{0.5} - 24.7 \text{ MPa}^{0.5}$ or $5.6 \text{ MPa}^{0.5}$). The higher miscibility of NIPAAm with APTES is further verified by the energy of mixing (ΔG_{mix}) estimated using the modified UNIQUAC Functional-group Activity Coefficients (UNIFAC) (Dortmund) model,^{31,32} and ΔG_{mix} of NIPAAm/APTES is found to be $\sim 30\%$ more negative than that of Styrene/APTES (see the supplementary material file).

More pNIPAAm retained on the APTES layer than PS could also result from stronger interactions, e.g., hydrogen-bonding, between pNIPAAm chains and the APTES molecules even when the APTES layer is fully cured. The more negative ΔG_{mix} of NIPAAm/APTES is an indication of such interactions. The hydrogen bonding might be unlikely for APTES and PS, but the aromatic rings in PS could have a possibility for accepting H from $-\text{NH}_2$ or $-\text{OH}$ of APTES.^{33–35} The proton donating and proton accepting ability of the two interacting compounds is defined as the AB interactions by van Oss and coworkers.^{36–39} They indicated that hydrogen bonding compounds might be treated as the Bronsted (proton donor–proton acceptor) theory of acids and bases, and hence, hydrogen bonding is a special case of acid–base interactions. van Oss *et al.*³⁹ also pointed out that a π electron system acted as a proton acceptor in hydrogen bonds. The bulky nature of the PS chain could potentially hinder the close contact of aromatic rings with the $-\text{NH}_2$ and $-\text{OH}$ groups, lowering the possibility of H-bonding as compared to pNIPAAm with APTES.

The pNIPAAm chains could also interact with the substrate (i.e., silicon oxide with hydroxyl groups on the surface); as a result, the 3 day cured pNIPAAm on the silicon wafer had a layer of $\sim 4 \text{ nm}$ retained after thorough rinsing. Even though pNIPAAm is highly soluble in cold water, there was still a $\sim 2 \text{ nm}$ layer remaining after three days of soaking in cold water (Fig. 3). On the other hand, in the case of PS, there was a very thin layer ($\sim 0.4 \text{ nm}$) detected on the silicon wafer after rinsing and 3 days of soaking by toluene (Fig. S2), indicating that the interactions were weaker and the retention was less than that of pNIPAAm.

C. Potential mechanism of retaining pNIPAAm on APTES

Based on the results, the potential mechanism of pNIPAAm retained on the APTES layer is proposed and illustrated in Fig. 4. When the pNIPAAm is placed on top of

FIG. 3. (a) Retained pNIPAAm layer (after rinsing with and soaking in cold DI water, measured in air) after pNIPAAm films had been thermally annealed on SiO_x and various noncured silanes for 3 days at 160°C . The variation of the retained film thickness on MPTMS and TESBA was greater as compared to that of APTES, indicating that MPTMS and TESBA could be more sensitive to the preparation conditions. (b) The thickness of one set of pNIPAAm on the above three silanes measured in water at a temperature above (40°C) and below (25°C) the LCST ($\sim 32^\circ\text{C}$) of pNIPAAm along with their values in air. The films in water were slightly thicker (1.5–2x) than those in air. No thickness difference in water at 25 and 40°C was observed for pNIPAAm retained on MPTMS and TESBA.

the noncured APTES layer [Fig. 4(a)], an interphase, i.e., an IPN, forms. During the early stage ($\leq 1 \text{ h}$) of thermal curing, the network is less cross-linked and the interpenetration is easier. In the meantime, both the van der Waals and acid–base interactions between the pNIPAAm and the APTES also contribute to the retention of pNIPAAm on the APTES layer. For the cured APTES layer, the retention of pNIPAAm on the APTES is mainly contributed by the van der Waals and acid–base interactions [Fig. 4(b)]. Such interactions would play a pivotal role in firmly retaining an organic polymer to an inorganic substrate when the IPN could not be easily formed.

To determine the film compositions and confirm the film thickness retained on APTES, XPS survey scans were obtained [Figs. 4(c) and 4(d)]. The scan of pNIPAAm (14–15 nm) retained on the noncured APTES (c-2) was similar to that of an un-rinsed pNIPAAm film ($\sim 30 \text{ nm}$, c-1) with the atomic % ratios [Table II] of C:O:N close to theoretical values (75:12.5:12.5) and observed by others for pNIPAAm brushes and films.^{1,25,26} The scan of pNIPAAm retained on the 2 h (or longer) cured APTES layer (i.e., c-3) had a small Si2p peak (atomic % of 5.3) and a higher O content (14.3 atomic %) than c-1, but the spectrum still had the general shape as that of c-1, suggesting that the film was basically pNIPAAm. The high-resolution C1s peaks of these three samples were also similar in shape (Fig. 5). The decoupled peaks showed roughly 66.7%, 16.7%, and 16.7%

FIG. 4. Potential mechanisms of retaining polymers on APTES: (a) IPN formed during thermal annealing + van der Waals (vdW) + AB interactions for pNIPAAm on the noncured APTES layer and (b) vdW + AB interactions for the polymer on the cured APTES layer. (c) and (d) represent the XPS spectra that illustrate the retention of pNIPAAm and PS, respectively. **c-1** and **c-2** are for the pNIPAAm film cured on noncured APTES for 3 days at 160 °C before and after soaking (3 days) in cold water, respectively; **c-3** and **c-4** are for the pNIPAAm film cured on 2 h-cured APTES and on SiO_x, respectively, for 3 days at 160 °C and then rinsed with cold water; **d-1** and **d-2** are the PS film cured on noncured APTES for 3 days at 125 °C before and after rinsing with toluene, respectively; **d-3** and **d-4** are for the PS film cured on 2 h-cured APTES and SiO_x, respectively, for 3 days at 125 °C and then rinsed with toluene.

of areas under the C–C/C–H, C–N, and N–C=O peaks [Table II], which were in accord with the four C–C bonds, one C–N bond, and one N–C=O bond in the monomer unit of pNIPAAm and those reported by others.^{1,25,26} Since XPS typically probes the top 10 nm of the film,^{40,41} it is possible to detect a small amount of Si and O from the APTES beneath the 5–6 nm retained pNIPAAm. However, this small amount of detected APTES, whose molecule contains two C–C bonds and one C–N bond, appeared to have little contribution to the break-down of C–C, C–N, and N–C=O in the C1s peak of these samples. This result supports the thickness measured by ellipsometry and confirms the pNIPAAm films retained on both noncured APTES and 2 h cured APTES consisting primarily of pNIPAAm. For the pNIPAAm film on the SiO_x after rinsing with cold water, a thickness of ~4 nm was obtained, and the greater presence of Si (with an

TABLE II. (Top) Atomic % of C, O, N, and Si, obtained from the XPS survey scans of the layer on APTES or SiO_x presented in Fig. 4. (Bottom) Area % of C–H/C–C, C–N, and N–C=O of the decoupled high-resolution C1s peaks for the samples presented in Fig. 5. The details of the spectra are described in the caption of Fig. 4, briefly, **c-1** to **c-4** are the pNIPAAm film cured for 3 days at 160 °C; **c-1**—on noncured APTES, **c-2**—on noncured APTES, rinsed and soaked for 3 days in cold water; **c-3**—on 2 h-cured APTES, rinsed with cold water; **c-4**—on SiO_x, rinsed with cold water; **d-1** to **d-4** are the PS film cured for 3 days at 125 °C; **d-1**—on noncured APTES, **d-2**—on noncured APTES, rinsed with toluene; **d-3**—on 2 h-cured APTES, rinsed with toluene; **d-4**—on SiO_x, rinsed with toluene.

Elemental atomic %	c-1	c-2	c-3	c-4	d-1	d-2	d-3	d-4
C	76.6	75.7	69.6	59.7	100.0	61.7	52.1	20.4
O	11.5	12.0	14.4	19.3	0.0	19.1	25.7	37.9
N	11.9	11.9	12.0	8.8	0.0	4.0	2.9	0
Si	0	0.4	4.0	12.2	0.0	15.2	19.4	41.7
Area %	c-1	c-2	c-3	c-4	d-1	d-2	d-3	d-4
C–H/C–C	66.7	66.5	65.6	65.6	~100	94.1	65.6	69.6
C–N	17.8	17.2	16.8	16.8		5.9	16.8	—
N–C=O	15.5	16.4	17.6	17.6				

atomic % of 12.2) and O (with an atomic % of 19.3) was noticed. The decoupled C1s for this sample also showed that the area % of C–C, C–N, and N–C=O [Table II] was basically the same as that was expected for pNIPAAm.

For a thick PS film (~30 nm, **d-1**), a majority of C (98.1 at. %) were detected [Table II], and the tiny amounts of O and Si were also noticed, which could be caused by contamination. Its high resolution C1s peak clearly showed π - π^* shake up satellites at ~291.5 eV [Fig. 5(d-1)], associated with the aromatic ring,^{42–44} confirming that the film was PS. The dramatic reduction of C for PS films on the noncured APTES (**d-2**) and 2 h cured APTES (**d-3**) layers after rinsing with toluene suggested that most of the PS was removed, and the smaller C1s peak in **d-3**, as compared to **d-2**, suggested less PS retained on the cured APTES layer. The increase of O1s and Si2p peaks in **d-2** and **d-3** indicated the detection of the underneath APTES/SiO_x. Since the PS film retained on the noncured APTES (**d-2**) and 2 h cured APTES (**d-3**) was ~4 and ~1 nm, respectively, while the average probing depth was ~6 nm for the XPS used in this study, this result was expected.

As compared to **d-2**, the O1s and Si2p peaks were more pronounced for **d-3** due to the detection of SiO_x beneath the APTES layer, which could overshadow the presence of N, and the N1s peak became slightly smaller (2.9 at. % vs 4.0 at. % in **d-2**). The high resolution C1s peak in **d-2** was relatively symmetric as that observed for a thicker PS film (**d-1**), with ~94% of the peak contributed by C–C/C–H. The small shoulder (~6%) at ~286 eV was most likely coming from the underneath APTES layer that consisted of C–N. For **d-3**, the contribution of the C–N peak increased to ~17%, suggesting that the relative amount of APTES in the case of PS retained on the cured APTES layer was greater than PS retained on the noncured APTES layer. There was also a peak (contributing ~17.6%) at ~288 eV observed in **d-3**. The exact reason why this peak was observed for a very thin layer (≤ 1 nm) of PS adsorbed on cured APTES was unclear.

FIG. 5. Decoupling of C1s peaks of samples **c-1**, **c-2**, **c-3**, **c-4**, **d-1**, **d-2**, and **d-3** presented in Fig. 4. The C1s peaks for **c-1**, **c-2**, **c-3**, and **c-4** are similar, and they can be decoupled into three main peaks at binding energies of ~ 285 eV (C–C/C–H), 286 eV (C–N), and 288.5 eV (N–C=O). For **d-1** (~ 30 nm PS film on APTES), clear π – π^* shake-up satellites were observed at ~ 291.5 eV, and this peak was barely noticed for **d-2** and **d-3**. Also, **d-1** has a symmetric peak, it could be forcedly decoupled into two peaks (aromatic C–C and aliphatic C–C), but one peak (C–C/C–H) showed a better fit. For **d-2** and **d-3**, a small peak associated with the C–N bond was observed, and for **d-3**, another small peak at ~ 288 eV was noticed. For **d-4**, broadening of the peak at high BE was noticed. The relevant numeric values of peak area % are summarized in Table II.

One potential reason could be the contamination of other hydrocarbons on the sample. Due to the extremely thin layer of the polymer (≤ 1 nm), the contribution of contamination became relatively greater as compared to those cases with thicker polymer layers (**c-1**, **c-2**, **c-3**, **c-4**, **d-1**, and **d-2**).

For the thoroughly rinsed sample of PS cured on SiO_x for 3 days at 125°C , the C1s high resolution peak intensity was about half of that of the PS cured on the 2 h cured APTES layer, and the high BE side of the peak was broadened, which could be due to small amounts of hydrocarbon contaminations during the sample processing and handling steps. The survey scan of PS cured on SiO_x , after rinsing with toluene, showed ~ 20 at. % of C and 38 and 42 at. % for O and Si. The close to 1:1 ratio of O:Si was similar to that observed for cleaned bare

SiO_x , which also contained ~ 7 at. % of C (spectrum not shown) without coming into contact with any polymer or organic solvent. It was not surprising that SiO_x with PS cured on top and then rinsed with toluene contained ~ 20 at. % of C, which could be from a very small amount of PS left on the sample as well as some hydrocarbon contaminations. These XPS results verified that, without interactions between PS and SiO_x , likely very little PS could be retained on SiO_x as evidenced from the thickness measurements (~ 0.4 nm).

D. Retention of pNIPAAm on MPTMS and TESBA

In order to further confirm that the above proposed mechanism is owing to the unique properties of APTES, i.e.,

TABLE III. Advancing and static water contact angles (θ_A and θ_S) above and below the LCST of pNIPAAm retained on (Top) spin-coated APTES cured for different periods of time at 160°C and (Bottom) various noncured silane layers. The pNIPAAm film was cured on the silane layer for 3 days in vacuum at 160°C and then rinsed with cold DI water prior to the contact angle measurements. The average and standard deviation were obtained from four to six measurements.

Curing time (h)	θ_A (deg) at $40^\circ\text{C} > \text{LCST}$	θ_A (deg) at $25^\circ\text{C} < \text{LCST}$	θ_S (deg) at $40^\circ\text{C} > \text{LCST}$	θ_S (deg) at $25^\circ\text{C} < \text{LCST}$
0	87.1 ± 2.0	80.6 ± 2.7	72.6 ± 1.4	63.5 ± 1.9
1	91.1 ± 2.2	77.1 ± 4.5	73.0 ± 0.8	64.2 ± 1.1
2	86.0 ± 1.3	70.3 ± 2.0	72.2 ± 2.6	59.8 ± 1.7
4	86.3 ± 1.7	70.1 ± 1.9	70.3 ± 3.0	57.2 ± 1.2
6	83.9 ± 1.6	72.1 ± 1.5	67.5 ± 3.0	60.3 ± 3.2
14	83.3 ± 2.1	74.3 ± 2.9	73.0 ± 0.8	63.4 ± 2.0
APTES ^a	88.1 ± 3.3	88.3 ± 2.5	82.6 ± 0.6	82.7 ± 2.4
SiO _x or silane	θ_A (deg) at $40^\circ\text{C} > \text{LCST}$	θ_A (deg) at $25^\circ\text{C} < \text{LCST}$	θ_S (deg) at $40^\circ\text{C} > \text{LCST}$	θ_S (deg) at $25^\circ\text{C} < \text{LCST}$
SiO _x	58.5 ± 2.1	47.6 ± 3.6	53.6 ± 2.2	45.8 ± 2.5
APTES	87.1 ± 2.0	79.4 ± 3.4	70.0 ± 3.1	61.8 ± 2.2
MPTMS	92.9 ± 2.8	83.3 ± 2.4	79.0 ± 3.2	63.0 ± 2.4
TESBA	80.8 ± 1.8	73.8 ± 2.5	69.2 ± 3.3	49.9 ± 1.7

^aThis is the cured APTES layer without pNIPAAm on top.

strong inter and intramolecular hydrogen bonding to allow the formation of the APTES network and stronger interactions with pNIPAAm, the retention of pNIPAAm on two other silanes having a similar size and structure to those of APTES (see Table I) was briefly assessed. TESBA was chosen because its molecules could form a similar network to that of APTES to retain pNIPAAm, but it has a lower capability of forming H-bonds with pNIPAAm, i.e., between its aldehyde, $\text{H}-\text{C}=\text{O}$, and the $-\text{NH}-$ in NIPAAm. No hydrogen bonding was expected for MPTMS, but it could still form a different type (the S–S bond formation) of network that could retain pNIPAAm.

As shown in Fig. 3, pNIPAAm retained on MPTMS and TESBA was significantly less than that retained on APTES, and there was no difference between the pNIPAAm films (~ 5 nm after rinsing, and 2–3 nm after soaking) retained on MPTMS and on TESBA. In this case, the MPTMS and TESBA layers were not cured or rinsed before pNIPAAm was spin-coated on them. When pNIPAAm was placed on the MPTMS and TESBA layers that had been cured for 3 days, the retained pNIPAAm was <0.5 nm after 3 days of soaking in cold water. The retention of pNIPAAm on the noncured MPTMS and TESBA could primarily be the result of forming an IPN during the formation of the silane network. The thinner pNIPAAm retained on these two silanes, as compared to APTES, would be the result of weaker interactions between MPTMS or TESBA with pNIPAAm.

E. Thermo-responsiveness of the retained pNIPAAm

We illustrate that the retained pNIPAAm via the simple coupling approach exhibited thermo-responsive properties as pNIPAAm brushes. The water contact angles on these surfaces were measured at 40°C (above LCST, or 32°C , of pNIPAAm) and 25°C (below LCST of pNIPAAm). Table III summarizes the advancing and static water contact angles on the cold water rinsed pNIPAAm films on various substrates. All the retained pNIPAAm films clearly exhibited hydrophobic-hydrophilic transition, with the difference in the contact angle of 7° – 16° . In contrast, cured APTES without pNIPAAm on top had a constant advancing angle of $\sim 88^\circ$ and a constant static angle of $\sim 83^\circ$ at both 40 and 25°C . The thermo-responsive behaviors of the retained pNIPAAm were also evaluated by measuring the film thickness in water at 40 and 25°C , and the pNIPAAm film (~ 15 nm) retained on noncured APTES showed a noticeable difference in the film thickness. For all other pNIPAAm films retained on the three silanes, the thickness difference in water at 40 and 25°C was small [Fig. 3(b)], which could be the result of the film being too thin to clearly show the extended-to-collapsed transition.

To further illustrate the thermo-responsiveness of the retained pNIPAAm and its potential use for tissue engineering, mouse embryonic fibroblast (NIH3T3) cells were seeded on these surfaces placed inside a culture dish and incubated at 37°C ($>\text{LCST}$). The cells attached and grew into a confluent cell sheet at 37°C within 2–3 days. When the dish was

removed from the 37°C incubator and left at room temperature ($\sim 23^\circ\text{C}$), the detachment of the cell sheet was observed. As shown in Fig. 6, the cell sheet detached from the pNIPAAm film retained on the noncured APTES for ~ 30 min, and the cell sheet detached from the pNIPAAm film retained on the 3-day cured APTES for 35–40 min. The detachment time is comparable to or even faster than some of the pNIPAAm brushes reported by others.^{2–4}

IV. SUMMARY AND CONCLUSIONS

In this study, the insights of utilizing silane coupling agents/adhesion promoters for retaining a thin layer of an organic polymer to a hydroxylated substrate were detailed. The focus was on a common coupling agent: 3-aminopropyltriethoxysilane

Fig. 6. Optical microscopy images of a sheet of mouse embryonic fibroblast (NIH3T3) cells detached from the pNIPAAm film retained on (a) a noncured and (b) a 3 day cured APTES layer. The time indicated in each image was the elapsed time of the culture dish being removed from the 37°C incubator and placed on the microscope stage in the ambient condition ($\sim 23^\circ\text{C}$, 1 atm). The scale bar is $500\ \mu\text{m}$.

(APTES), which has the ability to form inter and intramolecular H-bonds and a cross-linked network for entrapping polymer chains during a thermal curing process. It was hypothesized that the IPN formation would be the chief foundation for the enhanced coupling of the polymer to the substrate by APTES, and the van der Waals and acid–base interactions further enhance the coupling. Thermo-responsive pNIPAAm and PS were used as two model polymers. The results showed that while IPNs played a role in retaining these polymers, especially when the polymers were placed on the noncured APTES layer prior to thermally curing the samples, the interactions, especially the hydrogen-bonding, between the APTES layer and the polymer were found to contribute substantially to the retention of pNIPAAm on the hydroxylated substrates, especially on the cured APTES layer when IPN was minimal or nonexistent. The energy of mixing (ΔG_{mix}), estimated based on the modified UNIFAC model, and additional experiments, including the use of two networks forming coupling agents that have a similar structure to APTES, were carried out. The results supported the importance of the interactions for retaining polymer films on substrates. Also, a thin layer of pNIPAAm retained on APTES using this simple coupling/adhesion promoting approach exhibited thermo-responsive behavior compared to those observed on pNIPAAm brushes.

ACKNOWLEDGMENTS

The research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award No. 1R15GM097626-01A1 and by the National Science Foundation under Grant No. 1322061. Alghunaim acknowledges the King Abdullah Scholarships Program support for his graduate study from the Ministry of Education in Saudi Arabia. The authors are also grateful to Zhorro Nikolov for very helpful assistance with XPS measurements and analysis.

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