Nanoscale Characterization of the Equilibrium and Kinetic Response of Hydrogel Structures

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The use of hydrogel nanostructured patterns and films in biomedical micro- and nanodevices requires the ability to analyze and understand their response properties at the nanoscale. Herein, the thermoresponsive behavior of atom transfer radical polymerization (ATRP) grown poly(ethylene glycol) n dimethacrylate (PEGnDMA) cross-linked poly(3-isopropyl acrylamide) (PNIPAAm) hydrogel thin films over gold was studied. By controlling the mesh size of the hydrogel matrix through tuning the cross-linking density (i.e., using different molecular weight cross-linker and/or various amounts of cross-linker), the hydrogel volume swelling ratio was tailored for response applications. Thermoresponsive patterns exhibited a broad lower critical solution temperature (LCST) swelling transition, while rms roughness analysis of the hydrogel surface showed a sharp LCST transition. Mass and viscoelastic property changes were monitored using quartz crystal microbalance with dissipation (QCM-D), and the rapid response behavior of the thin hydrogel films was observed. The tunable response behavior along with the controlled growth of the hydrogel achieved via ATRP at the nanoscale make them applicable as functional components in diagnostic and therapeutic devices.

Introduction

Hydrogels have gained interest as functional components in biomedical devices such as microfluidic channels, drug delivery micro-actuators, biosensing microcantilevers, microrelays, and micropumps.1–5 The key element that influences the use of hydrogels for such applications is their unique ability to respond to environmental stimuli in a preprogrammed, intelligent manner.6 Poly(N-isopropyl acrylamide) (PNIPAAm), which has a lower critical solution temperature (LCST) of around 32 °C, is used as the paradigm polymer for various thermoresponsive studies.7 The unique property to swell in response to temperature changes has enabled PNIPAAm to be used widely for controlled drug delivery applications.8–11 PNIPAAm and other responsive hydrogels have been patterned at specific locations of device surfaces using conventional yet cumbersome UV photolithography. Recently, for applications related to devices, tethering of PNIPAAm over surfaces has been carried out using soft lithography of an initiator from which PNIPAAm is grown bottom-up via a surface initiated polymerization technique. Using microcontact printing (μCP) and atom transfer radical polymerization (ATRP), several responsive polymeric brushes including PNIPAAm have been successfully amplified from patterned monolayers of initiators.10–15

The majority of the prior PNIPAAm and other brush studies have focused on the synthesis and the response behavior by elucidating how the brush thickness is dictated by ATRP and the change in structural conformation by the surrounding environment. However, the synthesis of spatially controlled, cross-linked responsive hydrogels over surfaces has been relatively unexplored. Unlike polymeric brushes, the transport properties of molecular species into and out of the hydrogel mesh network can be tailored by the type, molecular weight, and amount of cross-linker used. Cells can be entrapped inside the hydrogel mesh network and manipulated for therapeutic reasons.16 In addition to the loading of hydrogels with drug molecules,17 hydrogel nanocomposites can be synthesized by in situ precipitation of nanoparticles inside their matrix. These advantages, along with the increased mechanical integrity and stability provided by the cross-linked matrix, make responsive hydrogels a better alternative to polymeric brushes for a variety of biomedical diagnostic and therapeutic applications.

We recently reported the synthesis of controlled thin patterns of poly(ethylene glycol) n dimethacrylate (PEGnDMA; n = 200/400/600; n denotes the average molecular weight of the PEG chain and corresponds approximately to 4.5, 9, and 13.5 repeating units) cross-linked PNIPAAm hydrogel over gold via μCP followed by ATRP.18 In various studies, researchers have shown that the swelling behavior of a hydrogel can be tuned by varying the cross-linking density used.19,20 Therefore, the swelling response of

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PNIPAAm hydrogel nanopatterns synthesized via ATRP can be tuned by changing the cross-linking density. In Figure 1, a schematic of the temperature dependent swelling response of a nanoscale hydrogel pattern is shown. This schematic also illustrates the potential for a different response at the surface of the hydrogel as compared to the bulk of the pattern. This phenomenon and the general response properties of hydrogel patterns are discussed in the following. Since the kinetics of hydrogel swelling responses are typically diffusion limited, the size of the hydrogel structure can dramatically impact its response time. It is expected that the response will become instantaneous as the size of the hydrogel structures is decreased to the nanoscale due to the direct proportionality between the characteristic diffusion time and the square of the length scale.21 There have been prior studies investigating the response time of micro- and macroscale hydrogel structures, but minimal prior experimental work has been done in studying the response properties of nanoscale hydrogel films or patterns.22 Also, the authors are not aware of any previous studies related to the differences in surface response to that of bulk response.

A detailed understanding of the tunable response properties of hydrogels is crucial for their applications in biomedical micro- and nanodevices. Our primary aim in the work reported here was to understand in detail the response properties of tethered, thermoresponsive PNIPAAm hydrogels on gold. Atomic force microscopy (AFM) measurements were completed for direct visualization of the hydrogel phase transitions. The difference in the surface and bulk response was also investigated using AFM analysis. In addition to AFM, quartz crystal microbalance with dissipation (QCM-D) analysis was performed to obtain a fundamental understanding of the effect of cross-linking amount and type on the response kinetics and hydrogel equilibrium stability.

**Experimental Section**

**Synthesis of Temperature Responsive Hydrogel Patterns Using μCP and ATRP.** The various cross-linked PNIPAAm hydrogel square patterns of controlled thickness were synthesized using μCP followed by ATRP as detailed in a previously published paper.18 Briefly, 1-octadecanethiol (ODT; Sigma) was patterned over the gold surface as a hydrophobic thiol outside the required square patterns (50–25–10 μm) via μCP. The hydrophilic initiator bromoisobutyrate undecyl disulfide (Br-Ini; ATRP Solutions) thiol was then backfilled in the non-ODT patterned gold squares from which ATRP of the respective hydrogels was carried out under nitrogen atmosphere for different reaction times (12 h in this work). Poly(ethyleneglycol) n dimethacrylate (n = 400/600; Polyscience, Inc.) was used along with PNIPAAm (Sigma) as the different cross-linkers and monomer, respectively. Mole ratios of 90:10 mol % and 70:30 mol % (PNIPAAm/PEGnDMA) were used to study the effects of the amount of cross-linker/cross-linking density employed for response studies. For studying the response behavior effects between the bulk versus surface, half-coated gold surfaces were first

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**References**

synthesized, then scratched, and finally characterized for thickness along the scratch.

**Synthesis of Thermally Responsive Hydrogel Films over Gold Coated Quartz Crystals.** The various hydrogel thin films were also grown over gold coated QCM-D crystals using ATRP. The crystals were chemically pretreated using ammonium peroxyde solution for 10 min followed by UV-ozone treatment for 20 min to remove impurities. The resulting clean gold crystals were then used in the QCM-D setup for obtaining its fundamental resonant frequencies. Ethanol was initially passed to obtain the stable frequency and dissipation baseline. A 3 mM solution of Br-Ini was then passed through the cells (except the blank cell) at a flow rate of 300 μL/min for about 1 h. The crystals were functionalized with Br-Ini for 24 h equivalent to the patterned samples. A post initiator attachment cleansing was done with ethanol to remove any unattached initiator thiol. A “no catalyst” ATRP monomer—cross-linker solution devoid of catalyst copper bromide and ligand 2,2'-dipyridyl was then passed through the cells to neutralize the frequency and dissipation baseline shift caused by differences in the ATRP solution density and viscosity. Once baseline correction was carried out, a nitrogen purged ATRP solution containing the catalyst and ligand was then passed through the respective cells for about 1 h. ATRP of PEGnDMA cross-linked PNIPAAm hydrogels was allowed to proceed in the respective cells for about 1 h. The reaction was stopped by a DI water purge step, and the hydrogel grown cells were used as such for thermoresponsive studies. A blank cell with water passed instead of initiator and ATRP solution was also used for comparative reasons. A blank cell with water passed instead of initiator and ATRP solution was also used for comparative reasons. (Initial dry thickness data: 10 mol % PEG600DMA, 293 nm; 30 mol % PEG600DMA, 268 nm; 10 mol % PEG400DMA, 260 nm; 30 mol % PEG400DMA, 221 nm).

**AFM Imaging.** In situ imaging of the tethered hydrogel patterns in aqueous solution was performed using the liquid cell setup, noncontact mode AFM imaging (Agilent Technologies Inc.) with a BS-TAP300 tapping mode cantilever (Budget Sensors; force constant of 40 N/m). The AFM scans delineating the response behavior of the temperature sensitive PEGnDMA cross-linked PNIPAAm hydrogels was allowed to proceed in the respective cells for 12 h. The reaction was stopped by a DI water purge step, and the hydrogel grown cells were used as such for thermoresponsive studies. A blank cell with water passed instead of initiator and ATRP solution was also used for comparative reasons.

**QCM-D Measurements.** A commercial Q-Sense E4 system was used for measuring the shifts in frequency (∆f) and dissipation (∆D). An optically polished, AT-cut gold coated quartz crystal of diameter 1.4 cm and fundamental resonance frequency of 5 MHz was used for all QCM-D measurements (Q-Sense). The Saurbrey constant (C) of the crystal used is 17.8 ng/cm²·Hz. The solution temperature was controlled within ±0.02 °C. All results obtained in this current work were from the measurements of frequency changes in the third overtone (n = 3), although higher overtones were also measured. This is because the third overtone shear wave senses one layer of liquid over the crystal corresponding to the grown hydrogel thickness, unlike the fundamental frequency which senses two layers of liquid. Moreover, the third overtone values are reproducible and stable as compared to higher overtones. All data were measured dynamically with changes in temperature to analyze the response kinetics. The crystal as such had very low resonance and dissipation changes with respect to applied temperature changes, but necessary corrections were made for this effect.

**Results and Discussion**

**Effect of the Molecular Weight and Amount of Cross-Linker on Response Behavior.** The response behavior of temperature sensitive PNIPAAm hydrogels of varying cross-linker molecular weight and amount was studied at different temperatures using AFM and QCM-D. Figure 1a shows the three-dimensional response AFM topography images of a 25 × 25 μm², 12 h ATRP reacted, 10 mol % PEG400DMA cross-linked PNIPAAm hydrogel pattern at 25 and 40 °C. For the system shown here, we observe that the hydrogel pattern has a greater thickness at 25 °C than at 40 °C, which is the expected transition behavior of PNIPAAm based polymers responding to the varying temperatures below and above its LCST. A similar analysis was performed over the 10/30 mol % PEG400DMA/PEG600DMA cross-linked PNIPAAm hydrogel pattern combinations at increasing temperatures of 25, 30, 35, and 40 °C, and their thicknesses are plotted as a function of temperature (Figure 2). The initial dry

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The effect of cross-linking density modified by the amount of cross-linker and the molecular weight of cross-linker used on swelling response is observed in Figure 2. An increase in molecular weight of the PEGDMA cross-linker from \( n = 400 \) to 600 increased the change in swelling greatly (ethylene glycol chain length increased from 9 to 13.5 units). We observe from Figure 2 that, for both the PEG400DMA and PEG600DMA systems, the 10 mol % cross-linked hydrogels swell more than the 30 mol % cross-linked hydrogels at 25 °C (room temperature). This is due to the fact that the 10 mol % cross-linked hydrogel has a larger mesh size than the 30 mol % cross-linked hydrogel.24 As the temperature increased, the polymer–polymer interactions between the hydrogel PNIPAAm backbone increased over the polymer–water interaction, thereby collapsing to a lower thickness state. Although PNIPAAm in solution has a sharp LCST, we observed a broad transition of >10 °C from Figure 2. This broadened transition has been reported by Frimpong et al. in the case of PNIPAAm patterns were 293, 268, 260, and 221 nm, respectively.

The swelling dependence on the type and amount of cross-linker used can be characterized using the volume swelling ratio measured from the change in thickness with respect to dry state thickness \( (Q) \). The two assumptions used in this calculation are the following: (1) the swelling and collapse of the surface-tethered hydrogel pattern are spatially confined, and therefore, the swelling is restricted primarily to the direction perpendicular to the gold surface; (2) the hydrogels are formed evenly over the entire patterned 25 × 25 μm² area. With these assumptions, \( Q \) is defined as the ratio of the average thickness of the swollen hydrogel pattern at a particular temperature to its average thickness in the dry state. Table 1 shows the \( Q \) values for the different hydrogel patterns of varying cross-linker molecular weight and density at different temperatures. The volume swelling ratio decreases as the amount of cross-linker increases. The \( Q \) values for the collapsed state at 40 °C were observed to be independent of the cross-linking density. At 40 °C, all patterns collapse to similar \( Q \) values (i.e., 1.13–1.18), implying that at higher temperatures all PEGnDMA based PNIPAAm hydrogels behave similarly. Here, tuning the cross-linking parameters/mesh size of the hydrogel was shown to control the extent of swelling change from the swollen to the collapsed state.

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<th>( T ) (°C)</th>
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Table 1. Volume Swelling Ratio \( (Q) \) of the Different Hydrogel Patterns at Different Temperatures, Measured from the Change in Thickness with Respect to Dry State

The dry thickness values for 10 mol % cross-linked PEG600DMA, 30 mol % PEG600DMA, 10 mol % PEG400DMA, and 30 mol % PEG400DMA cross-linked PNIPAAm patterns were 293, 268, 260, and 221 nm, respectively.

Surface versus Bulk Temperature Dependent Swelling Response. The surface response behavior analyzed by the rms roughness (Figure 3) is clearly quite different from the response behavior of the entirety of the hydrogel pattern (Figure 2). The surface or the skin of the hydrogel pattern shows a sharper response transition over about 2.5–3 °C. The entire pattern behaves similarly to a macroscale cross-linked polymer in solution exhibiting a swelling transition of >10 °C. This difference between the presence of a transition temperature over the hydrogel surface and a much broader transition in the case of the entirety of the pattern is hypothesized to be a result of PNIPAAm chains at the surface being only partially cross-linked into the network and, thus, able to respond closer to free PNIPAAm chains or PNIPAAm grafts. This phenomenon poses interesting questions.

References:

about the dependence of hydrogel response behavior on the thickness of the patterns. For this reason, we synthesized 10 mol % PEG400DMA cross-linked hydrogels over gold surfaces with shorter ATRP reaction periods and studied their response characteristics. The response behavior by thickness change is represented as $Q_t$ in Figure 4. The lines in the plot are only to assist in guiding the eye.

He et al. reported a broad transition similar to that of bulk hydrogels in solution for tethered PNIPAAm chains (Figure 1b, transition profile of tethered PNIPAAm). In Figure 4, the swelling appears to become sharper around 32.5 °C, as the thickness of the tethered hydrogel decreases. The error in the data and the limited data points do not currently enable a firm conclusion, but a general trend appears to be present. We hypothesize that those portions of the PNIPAAm backbone present at the surface or the “skin” of the pattern are not fully cross-linked with the adjacent chains and behave similarly to that of tethered polymeric brushes (Figure 1b, zoomed in scheme). The bulk or “body” of the cross-linked gel matrix lies between the tethered chains at the top of the surface (“skin”) and the gold substrate at the bottom, and this matrix “body” is filled with the surrounding fluid. It appears that the swollen matrix behaves closer to that of free solution than to that of the rigid gold surface, and this results in a different transition behavior than the broad transitional behavior of the non-cross-linked surface grafted chains observed by He et al. This surface response is similar to that of pure PNIPAAm in solution (Figure 1b, transition profile of free PNIPAAm in solution).
Rapid Response Behavior of Thin Film Hydrogels. Complementary QCM-D data were also collected to study the thermo-responsive behavior of PEGnDMA cross-linked PNIPAAm hydrogel thin films. With the gel to be evenly grown over the QCM-D crystal, the change in mass of the hydrogel tethered crystal ($\Delta m$) is directly proportional to the negative change in frequency shift of the crystal ($-\Delta f$) as per the Sauerbrey equation.\(^2\)

Figure 5 shows the temperature dependence of frequency and dissipation shift of 10 mol % PEG400DMA and PEG600DMA cross-linked PNIPAAm hydrogel systems, showing thermo-responsive behavior for three temperature change cycles.

Additional references:

adsorption in liquid-phase research applications.\(^{30-32}\) The dissipation factor (\(D\)) is defined as the measure of the ratio of dissipated energy to stored energy in a material during one oscillation. Rodahl et al. showed that the structure of the viscoelastic polymer layer on a piezoelectric resonator influences the change in dissipation.\(^{33}\) A collapsed rigid or glassy polymer layer has a small dissipation of energy, whereas a loosely swollen or rubbery polymer layer exhibits a larger dissipation of energy. \(\Delta D\) in Figure 5b decreased with temperature increase, and vice versa. This indicates that, as heating was introduced, the films collapsed into a more rigid state, whereas under cooling they became more flexible due to water intake. It is also worth remarking here that while AFM provided insight on the material property of the hydrogel via surface roughness measurements, QCM-D gives information of the hydrogel viscoelastic properties by measuring \(\Delta f\).

Figure 6 shows the AFM measured \(Q_t\) data of a 12 h reacted 10 mol \% PEG400DMA cross-linked PNIPAAm pattern at different temperatures and the \(\Delta f\) for the same system on a QCM crystal (\(\Delta f\) of 25–40 °C from cycle 2 is normalized and inverted for the sake of comparison). \(\Delta f\) is monitored continuously as the temperature is ramped over this temperature range. Since the normalized \(\Delta f\) values exhibit the same temperature dependence as the equilibrium \(Q_t\) values, we can conclude that the \(\Delta f\) values are also at equilibrium. Thus, the rate at which a responding thin hydrogel pattern achieves equilibrium must be significantly higher than the rate at which the temperature is being ramped in the QCM-D setup (1.2 °C/min). This equilibrium response with temperature change observed in the kinetic QCM-D analysis demonstrates the rapid response behavior of ATRP grown thin hydrogel patterns. These nanoscale patterns with rapid response behavior are attractive for point of care, rapid/instantaneous diagnosis, and therapeutic applications.

Figure 7 shows the relation between \(\Delta D\) and \(\Delta f\) for the three heating–cooling cycles for the 10 mol \% PEG400DMA and PEG600DMA cross-linked PNIPAAm hydrogels. We observed from the \(\Delta D\) versus \(\Delta f\) plot that, during the first temperature cycle (heating and cooling), both the hydrogels traced a loop characteristic of water loss and rigidity during the heating stage, followed by water intake and flexibility during the cooling stage. At the end of temperature cycle 1, both of the hydrogels had a different \(\Delta D\) and \(\Delta f\) value as compared to the start of the cycle (origin, in the case of the start of cycle 1), indicating an irreversible response transition similar to the hysteresis loop of PNIPAAm brushes.\(^{33}\) With subsequent temperature cycles (2 and 3), we observed that the loop of the curve extended in area on both directions (area 1–1’ < area 2–2’ < area 3–3’ for PEG400DMA; area a–a’ < area b–b’ < area c–c’ for PEG600DMA). Further, with each temperature cycle, the rate of area increase decreased (noticed by the distances 1–2, 2–3, 1’–2’, 2’–3’ for PEG400DMA; a–b, b–c, a’–b’, b’–c’ for PEG600DMA). The increase in area with each cycle is due to the increased response behavior of the hydrogels. Initially, the hydrogels are in a thermodynamically stable complex state, where the knotting and entangling of the networked polymer matrix occurs due to the various intrachain and interchain polymeric interactions. With each temperature cycle, the disentanglement of the various polymeric chains continues, thereby contributing to the increasing area along the \(\Delta D–\Delta f\) curve. However, with subsequent disentanglement over each temperature cycle, the need
for further disentanglement/rate of disentanglement decreases, thereby decreasing the rate of area increase. This phenomenon of the hydrogel increased response with each temperature cycle continues until the hydrogel reaches the final thermodynamic response equilibrium state, after which there is no significant change in the $\Delta D - \Delta f$ profile. Since the PEG600DMA cross-linked hydrogel has higher molecular weight polymeric chains, they should exhibit increased mobility and disentangle faster than the PEG400DMA hydrogel for the same temperature cycle number. The difference between distances $b - c$, $b' - c'$ of PEG600DMA and $2 - 3$, $2' - 3'$ of PEG400DMA confirms this effect. For cycle 3, PEG600DMA cross-linked hydrogel disentangles much closer to its final equilibrium response state than the PEG400DMA cross-linked hydrogel. Once the final response equilibrium state is reached, the hydrogel thin films can be effectively used for precise sensing applications for multiple cycles.

This study confirmed the potential of hydrogel patterns at the nanoscale for rapid sensing applications. Functionalized hydrogel thin films at the nanoscale can become the key feature of future point-of-care biomedical devices because their mesh networks have significant advantages over linear polymeric brushes such as entrapping biomolecules, controlled drug release, tunable response behavior, and better mechanical integrity.

**Conclusion**

Herein, we used AFM to visually observe the structural and thickness changes that originated from the thermoresponsive property of the PNIPAAm hydrogels. As the cross-linking density of the hydrogel increased, the mesh size of the hydrogel matrix decreased, thereby decreasing the volume swelling ratio of the hydrogel. The “bulk” nanoscale patterns were demonstrated to exhibit a broad swelling transition of $>10^\circ C$. The surfaces of the nanoscale patterns were observed through rms roughness analysis to exhibit a sharper swelling transition over about $2-3^\circ C$. QCM-D monitoring of the response kinetics of the different hydrogels complemented the results obtained from AFM. PEG600DMA

![Figure 7. $\Delta D$ versus $\Delta f$ plot showing the hysteresis loop ends of the 10 mol % PEGnDMA cross-linked PNIPAAm hydrogel systems at (a) 25 $^\circ C$ and (b) 40 $^\circ C$. The hydrogels try to reach an equilibrium response state at the particular temperatures in subsequent cycles. The PEG600DMA cross-linked hydrogel tries to reach equilibrium faster than that of the PEG400DMA system.](image-url)
cross-linked hydrogels had more water content than the PEG400DMA cross-linked hydrogels. For the same response cycle, PEG600DMA cross-linked hydrogels were closer to their equilibrium response state than the PEG400DMA cross-linked hydrogels. The fundamental understanding of the response properties of surface tethered hydrogel thin films is significant and holds promise in augmenting various biomedical applications that require “instantaneous” sensing and controlled drug delivery.

Supporting Information Available: Chemical notation of the hydrogel formed, details of the pattern present in the PDMS used, scratch sample analysis, topographical $2 \times 2 \mu m^2$ scan images for rms roughness analysis at different temperatures, and details of determining start and end transition temperatures of rms profile curves. This material is available free of charge via the Internet at http://pubs.acs.org.