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Preparation and characterization of a pH- and thermally responsive poly(N-isopropylacrylamide-co-acrylic acid)/porous SiO₂ hybrid

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Abstract

A multifunctional nanohybrid composed of a pH- and thermoresponsive hydrogel, poly(N-isopropylacrylamide-co-acrylic acid), poly(NIPAM-co-AAc) is synthesized in-situ within the mesopores of an oxidized porous Si template. The hybrid is characterized by electron microscopy and by thin film optical interference spectroscopy. The optical reflectivity spectrum of the hybrid displays Fabry-Pérot fringes characteristic of thin film optical interference, enabling direct, real-time observation of the pH- induced swelling and volume phase transitions associated with the confined poly(NIPAM-co-AAc) hydrogel. The optical response correlates to the percentage of AAc contained within the hydrogel, with a maximum change observed for samples containing 20% AAc. The swelling kinetics of the hydrogel are significantly altered due to the nanoscale confinement; displaying a more rapid response to pH or heating stimuli relative to bulk polymer films. The inclusion of AAc dramatically alters the thermoresponsiveness of the hybrid at pH 7, effectively eliminating the lower critical solution temperature (LCST). The observed changes in the optical reflectivity spectrum are interpreted in terms of changes in the dielectric composition and morphology of the hybrids.

Keywords

porous silicon; hydrogel; nanocomposite; volume phase transition; optical biosensor

1. Introduction

Porous Si is established as a unique nano-scale template for the incorporation of various polymers [1–5] and hydrogels [6, 7]. The potential of such hybrid materials in sensing [2], biosensing [6] and controlled drug delivery [2] has been demonstrated. Recently, we reported that a thermoresponsive hydrogel, poly(N-isopropylacrylamide) poly(NIPAM) synthesized in situ within an oxidized porous SiO₂ template, exhibits unique phase transition behavior [7]. In particular, the confined hydrogel undergoes a rapid volume phase transition

upon heating or cooling in comparison to bulk poly(NIPAM) hydrogels. We developed an optical approach to study the behavior of the hybrid, enabling direct, real-time observation of the confined hydrogel. Additionally, the porosity and pore size of the template, which are precisely controlled by the electrochemical etching conditions, strongly influence the extent and kinetics of the hydrogel phase transition.

Current research efforts are focusing on the study of responsive hydrogels. It is important for a hydrogel to display rapid response to stimuli, and miniaturization of domain size is one means to achieve this [8–12]. Poly(N-isopropylacrylamide-co-acrylic acid) (poly(NIPAM-co-AAc)) has emerged as a promising material for use in a variety of applications, including drug delivery [13, 14], sensing [15], microlenses [16, 17], and microfluidic devices [8, 18, 19].

In this work, we present the fabrication and characterization of poly(NIPAM-co-AAc)/porous SiO₂ hybrids. The resulting hybrid combines the optical properties of an electrochemically machined porous SiO₂ template with the multifunctionality of a pH- and thermosensitive hydrogel. Continuous, real-time monitoring of the state of the hydrogel in response to pH and thermal stimuli is achieved using the optical reflectivity properties of the porous SiO₂ template. The effect of the acrylic acid constituent on the pH-triggered and thermally induced volume phase transition is investigated. The results demonstrate the potential of the hybrid materials for applications in drug delivery and sensing, where both pH and temperature changes may be harnessed to induce a response.

2. Results

2.1 Preparation of Porous SiO₂ Templates

The synthetic scheme followed to produce poly(N-isopropylacrylamide-co-acrylic acid)/porous silica [poly(NIPAM-co-AAc)/porous SiO₂] hybrids is outlined in Figure 1. First, a porous Si template is prepared from a highly doped p-type single-crystal Si wafer using an anodic electrochemical etch. In this study, all porous Si templates are prepared by etching Si wafers at 385 mA/cm² for 30 s. The resulting freshly etched porous Si sample is then thermally oxidized at 800°C to create a hydrophilic porous SiO₂ matrix. The oxidation process also helps to protect the porous structure from degradation in aqueous media [6, 20].

2.2 Scanning Electron Microscopy of Templates

Cross-sectional SEM images of a typical layer after thermal oxidation are shown in Figure 2. The porous SiO₂ layer is approximately 7600 nm thick (Figure 2a). The pores display a nominal thickness of 60–100 nm (Figure 2b). It should be noted that the pores of this type of porous SiO₂ template are generally thought to be interconnected [21], which we have verified by ethanol infiltration of a portion of the porous film that was sealed on the top with a layer of impermeable polystyrene, fused to the surface by melt-casting.

2.3 Determination of Porous SiO₂ Template Thickness and Porosity

Sample porosity and thickness were quantified using gravimetry and the Spectroscopic Liquid Infiltration Method (SLIM). SLIM involves measurement of the reflectivity spectrum before and after infiltration of a liquid with a known refractive index [22]. The effective optical thickness (EOT) of the sample is determined from the interferometric reflectance spectrum for each filling liquid (e.g., ethanol, hexane). The data are then fit to a two-component Bruggeman effective medium approximation. The refractive index of the SiO₂ layer is assumed to be 1.455, and the values of the refractive indices of the different liquids are obtained from the literature or measured with a refractometer. The value of the open porosity (pore volume accessible to the probe molecule) and the sample thickness are

determined from the fit [23]. This model has been shown to predict the porosity and thickness of porous Si and oxidized porous Si in reasonable agreement with gravimetric and SEM determinations [7, 24, 25]. In this work, the SLIM-determined values of open porosity and thickness were $71 \pm 2\%$ and 7860 ± 150 nm, respectively. The thickness is somewhat larger than the value determined by SEM. Sample porosity was verified by gravimetry, involving determination of the sample mass before and after removal of the oxidized porous layer. The gravimetric method yielded porosity of $77 \pm 5\%$, in agreement with the value obtained by the SLIM method. The gravimetric method determines the total porosity of the template, including both the open and the closed porosity of the sample. Thus, the slightly larger value of porosity determined by gravimetry may be a reflection of the generation of inaccessible voids (closed porosity) within the porous network upon sample oxidation.

2.4 Synthesis of Composites: In-Situ Polymerization of Poly(NIPAM) in Porous SiO₂ Templates

The porous SiO₂ nanostructure was used as a template for the in-situ polymerization of poly(NIPAM-co-AAc) cross-linked with N,N'-methylenebis(acrylamide) (BIS). Details of the composition of the pre-gel solutions are summarized in Table 1. Pre-gel solutions, containing NIPAM, AAc, BIS and benzoyl peroxide as a free-radical initiator, all dissolved in 1,4 dioxane and purged with N₂ gas, were cast onto the porous SiO₂ film and allowed to react at 70°C for 24 h. The resulting porous SiO₂/polymer gel hybrid was thoroughly rinsed with pH 4 phthalate buffer over a period of 5 days and allowed to reach equilibrium swelling state at room temperature. Cross-sectional scanning electron micrographs (SEM) reveal the morphology of the porous poly(NIPAM-co-AAc)/porous SiO₂ hybrids (Fig. 3). The average thickness of the porous SiO₂ film is ~7600 nm, and the hydrogel layer is infused into the pores to a depth of ~5300 nm (Fig. 3a). Incomplete infiltration of the polymer in the polyNIPAM/porous SiO₂ hybrid was also observed in our earlier study [7], and it was attributed to the freeze drying/freeze fracturing process used to prepare the hybrids for SEM analysis. It is believed that the incomplete filling of the porous network observed in this work arises from the same phenomenon, although we cannot rule out the possibility of trapped air or high viscosity of the polymer precursor solution that inhibits complete pore filling. Figure 3b clearly reveals the nanostructure of the hybrid and the presence of fine polymer infiltrates throughout the porous scaffold.

2.5 Optical analysis of poly(NIPAM-co-AAc)/porous SiO₂ hybrids

Reflectivity spectroscopy provides a convenient means to monitor the phase transition behavior of the poly(NIPAM-co-AAc)/porous SiO₂ hybrids. Details of the experimental setup have been reported and they are included in the Experimental section. Briefly, Fabry-Pérot interference between the top and bottom interfaces of the hybrid thin film gives rise to a series of peaks in the reflectivity spectrum. The maxima of this interferogram are governed by the following relationship:

$$m\lambda = 2nL \quad (1)$$

where m is the spectral order of a fringe, λ is wavelength of incident light, and n and L are the total refractive index and the physical thickness of the hybrid film, respectively. The quantity “ $2nL$ ” is referred to as the effective optical thickness (EOT) of the hybrid in this work. Changes in either n or L lead to a shift in the interference maxima, resulting in a change in EOT [22, 26, 27]. As such, the spectrum of the sample allows real-time monitoring of the physical behavior of the nanoconfined poly(NIPAM-co-AAc) hydrogel under different pH and thermal conditions [7].

2.6 Optical Response Recorded During pH Cycling of Hybrids

In order to study the pH responsiveness of the hybrids, samples were placed in a custom-made flow cell assembly attached to a mechanical liquid pump. The optical spectrum was monitored through a glass window ~2 mm above the chip surface, and the value of EOT was computed in real-time. The hybrid was conditioned by exposure to a flowing stream of pH 4 buffer for 1 h followed by pH 7 buffer for 1 hour. The process was repeated for a total of 3 complete cycles. Figure 4 demonstrates changes in the EOT for the third pH cycle for a 15% AAc hybrid. A small (– 4 nm), abrupt decrease in the value of EOT (blue shift) occurs upon changing pH from 4 to 7. Following this initial transient, the value of EOT increases (red shift) to a steady-state value (+ 35 nm). Changing the pH from 7 back to 4 leads to a recovery of EOT to its initial value. The negative EOT transient is also observed on the transition from pH 7 to 4. The behavior is the same on subsequent cycles.

During the sample conditioning procedure, the steady-state EOT values of the hybrids were observed to drift somewhat from cycle to cycle. A total of three 5-min cycles followed by a constant flow of pH 4 buffer for 2 h led to stable, reproducible values on subsequent cycles. The initial instability of the process is attributed to the dynamic flow environment within the flow cell. Previous work on pH-sensitive hydrogel actuators in microfluidic devices has determined that the forces of aqueous flow on constrained hydrogels can lead to variability in pH response [28].

2.7 Effect of Acrylic Acid Content on the Steady-State Optical Response to pH Cycling of Hybrids

The presence of the acrylic acid species within the hydrogel component of the hybrid has a strong effect on the overall optical response recorded in the pH cycling experiments. Figure 5 illustrates the effect of AAc content on the value of EOT in response to pH. All the samples in this Figure were prepared using identical porous SiO₂ templates. Overall, the optical responses of the hybrids are similar. Each undergoes an initial small transient decrease in the value of EOT followed by an increase to a constant value when the pH is switched from 4 to 7.

The steady-state optical response of the hybrids was quantified by measuring the difference in EOT values between pH 4 and 7. Figure 6 presents these responses as a function of acrylic acid content. The magnitude of the response is at a maximum (~50 nm) for hybrids containing 20% AAc. For lower (5, 10, 15%) and higher (25%) quantities of AAc, the magnitude of the steady-state response is smaller.

2.8 Effect of Acrylic Acid Content on the Temporal Response of the Hybrids to Changing pH

As with the magnitude of the steady-state response when the pH is switched from 7 to 4, the rate of change of EOT displays a nonlinear relationship to AAc content. For samples containing 5 and 25% AAc, EOT of the hybrid returns to the baseline (pH 4) value very soon after introduction of pH 4 buffer. For samples containing 10, 15, and 20% AAc, the transition back to the baseline value is more gradual (Figure 7). The sample containing 25% AAc can be repeatedly cycled between pH 4 and 7 within 5 min, resulting in approximately half of the total magnitude Δ EOT observed when the sample is allowed to equilibrate for 2 h between cycles (Figure 7).

2.9 Optical Response Recorded During Thermal Cycling of Hybrids

Poly(NIPAM) hydrogels are well-known to undergo a volume phase transition at the lower critical solution temperature of the material.[29–32] The inclusion of relatively small amounts of AAc leads to dramatic changes in the thermal response of the hybrid.[33–36]

Previous work on poly(NIPAM)/porous SiO₂ hybrids demonstrated the utility of using the optical properties to study the volume phase transition of a hydrogel hybrid nanostructure [7]. Figure 8 presents the change in EOT observed during thermal cycling of a 10% AAc hybrid in pH 7 buffer. Upon heating, the hybrid is observed to undergo a decrease in EOT, consistent with the previous findings. As the hybrid cools, EOT returns to the original value.

3. Discussion

Previous work on thermo-sensitive hydrogel/porous SiO₂ hybrids showed that the optical properties of the porous SiO₂ template can be used to monitor changes in optical thickness and to relate these changes to physical phenomena within the hydrogel phase of the hybrid as it undergoes heating and cooling cycles [7]. In the present work, we used the same methods to study a hydrogel phase that is multifunctional, containing both pH- and thermo-sensitive components: AAc and NIPAM, respectively.

Bulk poly(NIPAM-co-AAc) hydrogels with varying compositions have been extensively studied.[33–36] The inclusion of acrylic acid imparts a pH-responsive characteristic to the hydrogel.[34] In contrast, neat polyNIPAM hydrogels display no sensitivity to pH over the range studied in the present work.[33] Furthermore, bulk hydrogels require significantly longer periods of time to achieve equilibrium swelling in response to changes in pH, typically on the order of hours [33] to days.[34] At pH values less than the pK_a of AAc, it has been suggested that the carboxylic acid groups engage in intra- and intermolecular hydrogen bonding with each other and with amine residues on NIPAM and BIS, effectively compartmentalizing water within the hydrogel and lowering the total water content of the gel.[33, 36] The swelling ratio of these hydrogels exhibits a strong pH dependence.

The pH dependant swelling ratio of an anionic hydrogel such as poly(NIPAM-co-AAc) is determined by two main forces: electrostatic repulsion between the carboxylic acid groups of AAc and osmotic pressure generated from the attraction of ions in the mobile phase to those covalently bonded to the hydrogel network. The abrupt change in osmotic pressure between the hydrogel and its surrounding environment is known as the Donnan equilibrium.[9, 34, 37] Varying the AAc content within the hydrogel leads to dramatically different equilibrium swelling ratios.[33, 34]

In poly(NIPAM-co-AAc)/porous SiO₂ hybrids, the feature size of the hydrogel component is dictated by the template morphology.[7] With a nominal pore size of 60–100 nm, the hydrogel is compartmentalized into domains that are significantly smaller than those typically studied in the bulk, resulting in a more rapid response to stimuli such as pH or temperature changes.[7, 37, 38] Furthermore, the optical properties of the template are sufficiently sensitive to allow detection of very small changes in refractive index of the solutions contained within the hydrogel phase of the hybrid, as well as the minute changes in thickness that can occur during pH cycling.

The initial decrease in EOT upon transition from pH 4 to 7, as observed in Figs 4, 5, and 7, is attributed to a change in refractive index of the hybrid film. The measured refractive indices of the pH 4 and the pH 7 buffers are 1.3355 and 1.3350, respectively. It should be noted that both buffers have the same ionic strength of 0.05 M—hydrogels can be especially sensitive to ionic strength. As the pH 7 buffer replaces the pH 4 buffer in the porous network, the dominant effect is to lower the overall refractive index of the hybrid. As a result of this decrease in refractive index, the magnitude of EOT decreases (see eq. 1). Shortly after the initial decrease, a large increase in the value of EOT is observed, attributed to physical changes occurring in the hydrogel phase of the hybrid.

Mixing of the new buffer (pH 7) with the remaining buffer (pH 4) causes the aggregate pH of the solution contained within the hydrogel to increase to values larger than the pK_a of the acrylic acid constituent, reported to be 4.25 at 25 °C [34, 36]. At this stage the charge distribution on the pendant acid groups changes, leading to a conformational change in the hydrogel. This behavior has been observed previously in both bulk [34] and thin films [9] of poly(NIPAM-co-AAc) hydrogels. Electrostatic repulsion of the anionic groups causes the hydrogel to swell. The conformational change of the hydrogel has been modeled as an increase in polymer chain-to-chain distance, or, alternatively, an unfolding of the crosslinking chains into an extended linear conformation [9, 35]. The observed increase in EOT is attributed (in part) to swelling of the hydrogel, resulting in an increase in the physical thickness, L , of the entire hybrid (see eq. 1). We suggested in our previous study of poly(NIPAM)/porous SiO_2 hybrids that some of the observed optical changes are due to changes in the thickness of the hybrid as a result of the volume phase transition in the hydrogel [7]. The other main contributor to the increase in EOT is attributed to a change in refractive index of the composite (n in eq. 1) as the carboxylic acid groups of the AAc component become deprotonated. A consequence of the change in ionized state within the hydrogel is an increase in hydrophilicity [9], and thus, wettability. The influx of water and the accompanying counterions needed to screen the negative charge of the ionized AAc groups [34, 36], leads to the observed increase in refractive index. Another possible parameter that may contribute to the increase in the EOT is further infiltration of the hydrogel, deeper into the porous scaffold. This could occur if the porous Si template is not completely filled with hydrogel initially.

The magnitude of the increase in EOT upon introduction of pH 7 buffer is a function of percent AAc content within the hydrogel (See Fig. 6). Previous work on bulk poly(NIPAM-co-AAc) hydrogels indicates that the % AAc content exerts a dramatic effect on the equilibrium swelling ratio of the hydrogels, particularly in the range of compositions studied in this work.[33, 34] As mentioned above, the carboxylic acid groups of the AAc constituent participate in hydrogen bonding interactions with each other at pH values less than the pK_a of the carboxylic acid. When $pH > pK_a$, the hydrogen bonds break and the hydrogel swells. The increase in magnitude of the pH-induced steady-state value of ΔEOT observed when the AAc content increases from 5 to 20% is attributed to this initial hydrogen bonding state within the gel. At lower AAc concentrations, there is a lesser extent of hydrogen bonding, and water can more effectively permeate the hydrogel at any pH. As the % AAc increases, there are more opportunities for the formation of hydrogen bonds, leading to lower water content when $pH < pK_a$. When $pH > pK_a$, the influx of water and counter ions is more extensive for gels containing a larger AAc content, resulting in the larger observed pH-induced increase in EOT.

The trend of larger ΔEOT values for higher AAc content reverses for the samples containing the highest percent content of AAc (25%, Fig. 6). In previous work involving poly(NIPAM-co-AAc) hydrogels, a substantial decrease in equilibrium swelling ratio has been observed for samples containing > 20% AAc [33]. The authors of this prior work did not suggest an explanation, as they did not observe a clear pattern in equilibrium swelling ratios in samples containing > 20% AAc. We suggest that the number and proximity of hydrogen bonding interactions in films containing > 20% AAc is so large that domains of multiply hydrogen bonded groups exist within the porous SiO_2 template. These more extensively interconnected domains are proposed to be insensitive to pH because they are too constricted to allow effective interchange of aqueous solution on the timescale of the experiments.

As the pH is changed from 7 back to 4, a reversal of the optical spectrum is observed. There is a rapid decrease in the value of EOT, likely stemming from expulsion of water and counterions from the hydrogel as the carboxylic acid groups become protonated and reform

hydrogen bonds. In the 5% AAc hybrids, the value of EOT actually decreases to a value smaller than in the original pH 4 baseline. For the 10, 15, 20% AAc hybrids, the recovered value of EOT is slightly larger than the initial baseline value. In all these cases, changing from one buffer to the other generates transient decreases in EOT. For the hybrids containing 5 and 25% AAc, the recovered value of EOT is close to the original baseline, and no further changes are observed after recovery. The response to pH changes is generally slower for the hybrids containing a larger amount of AAc (10, 15, 20, and 25%) compared with the hybrids containing the least AAc (5%). The slower response is attributed to the greater amount of AAc in these samples, as the diffusion of water out of the hydrogel phase (observed as a decrease in EOT) is expected to take more time in these samples.

Two negative controls (not shown) were studied to ensure that it was indeed the hydrogel leading to EOT changes and not a result of charge restructuring on the porous SiO₂ surface or some other pH dependent effect. In the first control, a neat template containing no hydrogel was tested. The optical response was observed to scale with the refractive index of the buffer used, with a ~20 nm decrease in EOT between pH 4 and pH 7. The system exhibited two distinct baselines (one at pH 4, one at pH 7) and no other optical response. The EOT immediately decreased upon changing from pH 4 to pH 7 solution. It should be noted that for samples containing the copolymer hydrogel, the decrease in EOT observed when the buffer solutions are changed from pH 4 to pH 7 never exceeded -12 nm. This may be caused by a decrease in overall free volume due to the presence of hydrogel in the pores, or by rapid swelling that counteracts the change in refractive index between the two buffers. In the second control, a poly(NIPAM)/porous SiO₂ hybrid lacking the AAc crosslinker was prepared and subjected to pH cycling. Since this hybrid contained no acid-sensitive moieties, the film is expected to behave similarly to the first control. This is indeed the case; the value of EOT decreases by 10–15 nm as the buffer is changed from pH 4 to pH 7 and recovers when the buffer is changed back to pH 4.

Previous work on poly(NIPAM)/porous SiO₂ hybrids allowed for accurate prediction of the lower critical solution temperature (LCST) of polyNIPAM by monitoring changes in EOT of the hybrid [7]. In this previous work, the optical transitions were observed to occur close to the LCST of polyNIPAM. In the present work, the transitions are observed to occur over a much larger temperature span.

Broad temperature transitions have been observed in bulk thermoresponsive AAc-containing hydrogels as well [33, 35]. In some cases, the inclusion of acid moieties leads to loss of a sharp LCST transition at pH > pK_a [35]. There is a significant temperature dependence of the heat capacity of these samples at pH 7 in the temperature range 23–50 °C [33]. A combination of electrostatic repulsion forces and increased hydrophilicity of the sample is proposed to account for this [33]. At sufficiently large pH values, electrostatic forces between charged polymer sidechains further broadens the transition. Furthermore, the volume phase transition in bulk polyNIPAM hydrogels is generally thought to result from a hydrophilic-to-hydrophobic transition within the material [29–32]. This transition is damped in the poly(NIPAM-co-AAc) copolymer because the ionized AAc species impart significant hydrophilic character to the hydrogel.

3. Conclusions

A poly(NIPAM-co-AAc) hydrogel was synthesized in situ within a mesoporous SiO₂ template. The interconnected pore structure of the template combined with the unique properties of poly(NIPAM-co-AAc) hydrogels results in a novel hybrid material with a combination of thermo- and pH-responsive properties. The optical properties of the porous SiO₂ template enable real-time observation of morphological changes in the hydrogel phase

of the hybrid during both pH and thermally induced volume phase changes, suggesting the utility of the optical method in studies of the behavior of hydrogels confined in a nanometer-scale porous network. The extent of AAc in the hydrogel phase of the hybrid exerts a dramatic effect on the magnitude of the optical change. The inclusion of AAc in the hydrogel produces a different thermal response, as predicted by theory and previous experimental work. In particular, a more rapid response is observed [7]. This acceleration of the swelling transition is important for various applications, including microfluidics [8, 28], drug delivery, and sensor devices [37, 39].

4. Experimental

Materials

Aqueous HF (48%) and ethanol (99.9%) were obtained from Fisher Scientific and AAPER, respectively. Porous Si samples were prepared from single crystalline, highly doped p-type Si ($9.0 \times 10^{-4} \Omega\text{-cm}$ resistivity, <100> oriented, B-doped, from Siltronic Corp.). Aqueous buffers of pH 4.0 and pH 7.0 were obtained from Fisher Scientific. *N*-isopropylacrylamide (NIPAM), Acrylic Acid (AAc) and 1,4-dioxane were obtained from Sigma-Aldrich Chemicals. *N,N'*-methylenebis(acrylamide) (BIS) was obtained from Fluka Chemicals. Reagent-grade benzoyl peroxide (97%, Sigma-Aldrich Chemicals) was purified by recrystallization from ethanol. All other reagents were analytical grade and used as received.

Etching of Samples

Porous Si samples were prepared by anodic etch of Si in a solution of 3:1 v/v 49% aqueous HF:EtOH employing a two-electrode configuration with a platinum counter electrode. CAUTION: Hydrofluoric acid is highly toxic and should be handled with care. Medical attention should be sought in case of contact with skin or inhalation. Si wafers were placed on an aluminum back contact and mounted in a Teflon etching cell. An area of 1.33 cm^2 of Si was exposed to the etching solution. Samples were etched at a constant current density of 377 mA/cm^2 for 30 s in the absence of light. After etching, samples were rinsed extensively with ethanol and dried under a stream of purified nitrogen.

Oxidation of PSi films

Freshly etched porous Si films were thermally oxidized in a ceramic tube furnace (Lindberg Blue M). Samples were heated at 800°C for 1 h in air, and then cooled to room temperature.

Preparation of poly(NIPAM-co-AAc) and poly(NIPAM-co-AAc)/porous SiO₂ Hybrids

Poly(NIPAM-co-AAc) hydrogels were synthesized by free-radical polymerization of NIPAM and AAc monomers using BIS as the cross-linking agent. NIPAM, AAc and BIS were dissolved in 1,4 dioxane at a total concentration of 0.9 mol/L, and the NIPAM:BIS concentration ratio for all preparations was 110. The mole ratio of AAc monomer was varied relative to NIPAM monomer. The pre-gel solutions were deoxygenated by bubbling with purified nitrogen gas for 30 min. The polymerization reaction was initiated with benzoyl peroxide and carried out at 70°C for 24 hr. The resulting poly(NIPAM-co-AAc)/porous SiO₂ hybrids were soaked in pH 4 buffer, rinsed thoroughly over a period of five days to reach the equilibrium swelling state.

Porous SiO₂/poly(NIPAM) hybrids were prepared by casting the pre-gel solution described above (without AAc) onto the porous SiO₂ sample. The sample was covered with a glass slide to minimize the amount of free monomer above the porous template layer, and the polymerization reaction was performed at 70°C for 24 hr. The resulting hybrids were carefully soaked in Milli-Q water, rinsed thoroughly over a period of five days and allowed to reach an equilibrium swelling state.

Scanning Electron Microscopy

Scanning electron microscope (SEM) images were obtained using a FEI Quanta 600 environmental scanning electron microscope, operating at an accelerating voltage of 20 keV. High resolution-SEM images of the PSiO₂/hydrogel hybrid scaffolds were obtained using a Carl Zeiss Ultra Plus HR-SEM, at an accelerating voltage of 1 keV. Porous SiO₂/hydrogel hybrids were prepared for SEM analysis by first placing the samples directly into liquid nitrogen for 15 min, followed by freeze drying. The samples were then freeze-fractured for SEM analysis.

Gravimetric Determination of Porosity

Three porous SiO₂ samples were weighed on a laboratory microbalance to obtain their initial mass (m_1). Samples were then placed in a solution of 3:1 49% aqueous HF:EtOH in order to dissolve the SiO₂, and the sample was reweighed (m_2). Porosity was determined using the following equation:

$$P = V_{\text{total}} - V_{\text{SiO}_2} / V_{\text{total}} \quad (2)$$

Where V_{total} is the total volume of material removed from the bulk silicon substrate, determined by:

$$V_{\text{total}} = At \quad (3)$$

Where A is the projected area of the etched film (determined by the diameter of the oring used in the etching cell), and t is the total thickness of the porous film, measured by cross-sectional SEM. The total volume of SiO₂ removed from the porous layer, V_{SiO_2} , was calculated from eq. 4:

$$V_{\text{SiO}_2} = (m_1 - m_2) / d \quad (4)$$

Where d is the density of bulk SiO₂, taken as 2.6 g cm⁻³.

Measurement of pre-gel solution and buffer refractive indices

The refractive index of the pre gel solutions and buffers used in this study were measured using a Milton-Roy refractometer.

Measurement of Interferometric Reflectance Spectra

Interferometric reflectance spectra of the porous SiO₂/hydrogel hybrids was measured using an Ocean Optics CCD S-2000 spectrometer. A bifurcated fiber optic cable was used to attach microscope optics to the spectrometer. The other end of the fiber optic cable was attached to a tungsten light source that was focused through the optics to a spot size of *ca.* 1–2 mm². Spectra were collected in the wavelength range of 400–1000 nm, with a 20 ms spectral acquisition time. 100 individual spectra were averaged together for each spectral acquisition. Illumination of the sample and detection of reflected light were both performed at 0 ° relative to the surface normal. The pH cycling experiments were carried out in a custom flow cell apparatus attached to a VICI m50 pump system. The solution pH was held constant for one hour increments, and buffers were introduced to the sample compartment containing the hybrid at a fixed flow rate of 1 mL/min. Flow was temporarily suspended when changing buffer solutions in order to clean the outside of the tubing and change reservoirs. A total of 3 pH cycles were conducted on each sample. Thermal cycling experiments on the hybrid were conducted on top of a standard laboratory hot plate. Samples were immersed in 3 mL of buffer and a k-type thermocouple was attached to the Si wafer surface to measure its temperature. Samples were monitored at room temperature for one

hour, after which the temperature was raised at a rate of ~ 1 °C/min until a final temperature of 47 °C was achieved. Samples were held at this temperature for one hour after initiation of the heating cycle. Samples were then allowed to cool back to room temperature by convection. A total of 2–5 thermal cycles were performed on each sample.

Measurement of Template Porosity and Thickness by the Spectroscopic Liquid Infiltration Method (SLIM)

The thickness and porosity of the templates used in this study were measured spectroscopically using the previously-described SLIM method [20, 40]. Briefly, SLIM is an indirect method to determine porosity and thickness by comparison of reflectivity spectra obtained with the sample immersed in various liquids of known refractive index or in air. The values of nL , or the optical thickness (OT) are determined from the thin film interference spectrum as described below. In this study we used ethanol, hexane, acetone, and the 15% pre gel solution, with refractive indices of 1.359, 1.372, 1.357, and 1.428, respectively. The data were fit to a two-component Bruggeman effective medium approximation.

Determination of Optical Thickness

The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm. The data spacing of the spectrometer is approximately 0.4 nm. The x-axis was inverted and a linear interpolation was applied such that the data were spaced evenly in units of nm^{-1} . A Hanning window was applied to the spectrum, it was redimensioned to 4096 data points and zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was applied. The Fourier transform of the spectrum yields a peak whose position on the x-axis corresponds to the value of $2nL$ in eq. 1.

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Figure 1.

Schematic representation of the preparation of poly(NIPAM-co-AAc)/SiO₂ hybrids. A porous Si layer is prepared from a single-crystal Si wafer by anodic electrochemical etch. The freshly etched sample is then thermally oxidized at 800 °C. A pre-gel solution is then cast onto the porous SiO₂ sample. The sample is covered with a glass slide, purged with nitrogen and allowed to polymerize at 70 °C for 24 h. The resulting hybrids are soaked in pH 4 phthalate buffer, rinsed thoroughly over a period of five days and allowed to reach equilibrium swelling state at room temperature. It should be noted that this schematic does not depict the detailed morphology of the hybrid nanostructure.

Figure 2. Cross-sectional scanning electron micrographs (secondary electron image) of a thermally oxidized porous Si layer. (a) Interface between the porous SiO₂ layer (etched for 30 s at 385 mA/cm²) and the single crystal Si substrate. (b) Higher magnification image of the porous SiO₂ layer, revealing the pore morphology.

Figure 3. Cross-sectional scanning electron micrograph of poly(NIPAM-co-AAc)/porous SiO₂ hybrid. The porous SiO₂ layer was prepared by etching of Si for 30 s at 385 mA/cm², followed by thermal oxidation. The poly(NIPAM-co-AAc) hydrogel precursor solution containing 15% AAc was then infused into the porous SiO₂ template. (a) The image shows the lower portion of the hydrogel/porous SiO₂ hybrid and the interface between porous SiO₂ and the bulk Si substrate. (b) Higher magnification high-resolution SEM micrograph of the hydrogel/porous SiO₂ hybrid, revealing its detailed nanostructure.

Figure 4.

EOT changes of a 15% AAc hybrid during a single pH cycle between 4 and 7. Samples are contained in a liquid flow cell assembly and exposed to a constant flow of 1 mL/min of solution. Optical changes in the hybrid are monitored while the pH is cycled between 4 and 7. Positive values of ΔEOT indicate an increase in the quantity 2nL.

Figure 5.

Optical response of hybrids containing different compositional percentages of AAc upon cycling of pH between 4–7. The samples were measured during a constant buffer flow of 1 mL/min at 25 °C. EOT changes are triggered by cycling the pH within the flow cell. Templates were all prepared under the same conditions (anodic etch at 385 mA/cm² for 30 s, followed by thermal oxidation at 800 °C).

Figure 6. Change in effective optical thickness (Δ EOT) observed upon cycling between pH 4 and 7 as a function of AAc content in poly(NIPAM-co-AAc)/porous SiO₂ hybrids. A total of three sample runs are averaged for each data point. The value of Δ EOT is determined as the difference between the minimum EOT value recorded during the transition from pH 4 to 7 and the steady-state EOT value obtained at pH 7. The solid line is included as a guide to the eye. Error bars indicate standard deviation.

Figure 7. Rapid pH cycling of a 25% poly(NIPAM-co-AAc)/Porous SiO₂ hybrid. EOT changes are triggered by changing the buffer solution feeding the flow cell every 5 min. Buffer flow rate is 1 mL/min at 25 °C. Dead volume in the flow cell introduces ~3 min. time lag.

Figure 8.

Thermal cycling of a 10% AAc poly(NIPAM-co-AAc)/porous SiO₂ hybrid immersed in pH 7 buffer. A pronounced decrease in the EOT is observed upon heating. As the hybrid cools to room temperature, the EOT returns to its initial value, indicating thermal reversibility of the hybrid.

Table 1

Composition of pre-gel solutions

Pre-gel Component	Structure	Solution and Composition [*]				
		A	B	C	D	E
N-isopropylacrylamide (NIPAM)		1.47	1.47	1.47	1.47	1.47
Acrylic Acid (AAc)		0.085	0.170	0.255	0.340	0.425
N-N'-methylene bisacrylamide (BIS)		0.027	0.027	0.027	0.027	0.027
Benzoyl peroxide (BP)		0.028	0.028	0.028	0.028	0.028
Total Solids (%)		17.58	18.21	18.76	19.34	19.90

^{*} All concentrations presented as molarity (M). Solutions A–E represent increasing concentration of acrylic acid monomer. All other components are held at the fixed concentrations indicated.