Three-dimensional photonic crystal sensors

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ABSTRACT

Photonic crystal templating of optically active hydrogel sensors is a topic of growing interest in materials chemistry. When interactions between a mesostructured hydrogel and analyte molecules cause a reversible dimensional change of the hydrogel, the corresponding shift in optical diffraction can be detected either spectroscopically or visually. Using poly(styrene) photonic crystals as templates, we synthesized inverse opal hydrogels through photopolymerization of 2-hydroxyethylmethacrylate and various functional monomers, and demonstrated the ability to sense pH and glucose at different ionic strengths and other experimental conditions. The diffraction of the pH sensitive hydrogel shifted from 544 nm to 850 nm when the pH was increased from 4 to 7, while the diffraction of the glucose sensitive hydrogel changed from 599 nm to 719 nm when the glucose concentration was raised from 0 mM to 100 mM. Diffraction response kinetics on the order of ~30 minutes were observed, which may be attributed to diffusion of analyte molecules through the thin (12-24 μm) hydrogel samples. These mechanically robust inverse opal hydrogel sensors may form a starting point for chemical and biological sensing using diffractive three-dimensional mesostructures.

Keywords: colloidal crystal, photonic crystal templating, inverse opal hydrogel, optical diffraction, pH sensor, glucose sensor.

1. INTRODUCTION

Photonic crystal templating of hydrogels is an increasingly popular method to fabricate mesostructures that are both optically and chemically/biologically active. Hydrogels may be functionalized to reversibly swell and contract through changes in factors such as solvent 1,2, temperature 3,4, pH and ionic strength 5, and biomolecule binding 6-9. When polymerized in the presence of a photonic crystal, a hydrogel may adopt the 3-D mesosstructure of the photonic crystal with a periodicity on the order of the wavelength of visible light. The templated hydrogel on a planar substrate usually forms an inverse FCC structure with the (111) plane parallel to the substrate and diffracts light following Bragg’s law 10. The reversible swelling of a templated hydrogel due to analyte sensing may change its periodic spacing, resulting in a shift in diffraction wavelength that can be detected.

Several research groups have published results on photonic crystal templated hydrogel sensors. Asher and co-workers pioneered the method of synthesizing a dilute hydrogel inside a photonic crystal of highly charged colloids 11, leading to a composite structure called polymerized crystalline colloidal array (PCCA). The reversible diffraction shifts of the PCCAs have been coupled to stimuli such as force 12,13, complexion between crown ethers and metal ions 13-15, pH and ionic strength 16, and glucose binding 14,17,18. Our group and others have focused on the inverse opal approach, where a dried photonic crystal templates the polymerization of a dense hydrogel and is then etched to create a bicontinuous hydrogel/solvent structure. Among the advantages for this system are facile diffusion and thus fast response due to the interconnected pores and good mechanical stability of the concentrated hydrogel structure. Furthermore, a large variety of functional groups may be incorporated into the hydrogel to confer sensitivity to different analytes. Takeoka and Watanabe polymerized inverse opals consisting of temperature sensitive poly(N-isopropylacrylamide) (pNIPAM), which showed a reversible diffraction shift versus temperature due to lower critical solution temperature behavior 19. To demonstrate the applicability of inverse opal hydrogels to pH and biological sensing, we fabricated two different types of photonic crystal templated 2-hydroxyethyl methacrylate (HEMA) hydrogels functionalized with either acrylic acid (AA) or 3-acylamidophenylboronic acid (APBA). AA is a carboxylic acid with pKa ~4.5 20, so a AA functionalized hydrogel was expected to swell as pH was raised, with a maximum sensitivity at pH ~4.5. APBA binds to 1,2-cis diols such as glucose by forming a charged complex 21,22, and hydrogels functionalized with APBA have been
shown to swell when glucose was introduced. We found that the AA functionalized inverse opal hydrogels showed a reversible diffraction red shift when pH was changed from 4 to 7, and the APBA functionalized inverse opal hydrogels exhibited sensitivity to glucose at physiological concentrations and ionic strength. The kinetics of the diffraction shifts was also studied to determine the rate-limiting step of the optical response.

2. EXPERIMENTAL

2-hydroxyethyl methacrylate (HEMA), acrylic acid (AA), 3-aminophenylboronic acid (AmPBA), sodium dihydrogen phosphate, and 2-(cyclohexylamino)ethanesulfonic acid (CHES) were acquired from Acros Organics. Ethylene glycol dimethacrylate (EGDM, crosslinker) was obtained from Aldrich. Irgacure® 651 (photoinitiator) was donated by Ciba Specialty Chemicals. The polymerizable glucose sensing molecule, 3-acrylamidophenylboronic acid (APBA), was synthesized by coupling of AmPBA to AA using a carboximide (EDC, Pierce Biotechnology) following published procedure. The complete fabrication technique for the inverse opal hydrogels is detailed elsewhere. Briefly, photonic crystals were made from monodisperse poly(styrene) colloids (Interfacial Dynamics Corp.) by placing a flow cell on top of a sonicator (Fisher Scientific FS30), in a method modified from Xia and co-workers. The photonic crystals were allowed to dry overnight, and mixtures of HEMA, EGDM, Irgacure® 651, and the functional molecule were infiltrated into the photonic crystals. For pH sensing, AA was used as the functional molecule, and for glucose sensing, APBA was used. Several different hydrogel formulations were used to test their effects on analyte sensing (Table 1). Each templated hydrogel was formed by UV polymerization (B-100A UV lamp, UVF, Inc.) of the infiltrated photonic crystal for 50 minutes. The template was then etched overnight in chloroform, and the resulting inverse opal hydrogel was submerged in ethanol and then an appropriate buffer solution for spectroscopy.

Table 1. Hydrogel formulations used for photonic crystal templating

<table>
<thead>
<tr>
<th>Components</th>
<th>pH sensitive hydrogel</th>
<th>Glucose sensitive hydrogel</th>
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<tbody>
<tr>
<td>HEMA (mol %)</td>
<td>97.84</td>
<td>95.34</td>
</tr>
<tr>
<td>EGDM (mol %)</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Irgacure® 651 (mol %)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>AA (mol %)</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>APBA (mol %)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Additional H2O (% v/v)</td>
<td>25 (final H2O = 20% v/v)</td>
<td>None</td>
</tr>
</tbody>
</table>

Light reflected from the hydrogel in an analyte solution is collected by a microspectrometer consisting of a 10x objective (NA = 0.25) on an inverted optical microscope (Axiovert 135, Carl Zeiss Inc.) and fiberoptically coupled to a diffraction grating photodiode array detector (PDA-512, Control Development, Inc.) to collect the reflection spectra in the sample normal direction. A 100 μm diameter pinhole placed directly in front of the optical fiber limits the analysis area to ~10 μm² at the center of the image area. SEM was performed in a Hitachi S-4700 field emission microscope.

3. RESULTS AND DISCUSSION

The SEM of a typical, optically diffracting inverse opal hydrogel (2.5% AA) sample showed an ordered array of pores that were close packed, as shown by the triplet of contact areas between a pore and the pores on the next layer (Fig. 1). In addition, defects in the original photonic crystal such as drying cracks and point vacancies were duplicated in the mesostructured hydrogel. Finally, a shrinkage of ~5% to ~10% in the center to center distance between pores was observed when the hydrogel samples were placed in SEM, suggesting that while the hydrogel was not fully dense, it was mechanically stable to vacuum and resistant to pore collapse due to drying.
Figure 1. Structure of templated inverse opal hydrogel films. (a) SEM image of the 2.5% AA inverse opal hydrogel. Defect structures of the photonic crystal template, such as vacancies and point defects, were duplicated with high fidelity, yielding single domains ~10µm across. (b) High magnification SEM image of the same hydrogel, showing the expected interconnected triplet pore structure.

The optical responses of the functionalized mesostructured hydrogels are shown in Figure 2. As expected, the inverse opal hydrogels exhibited reversible diffraction response to the target analyte as a result of solvent influx due to ionization of the functional groups. At pH ≤ 4 in 0.1M phosphate buffer, the 5% AA hydrogel film diffracted 544 nm light, which was quite close to the value calculated by the Bragg equation (547 nm), assuming an inverse opal of hydrogel (n = 1.50) in water. As pH increased, the diffraction steadily red shifted until it reached 850 nm at pH 6.94 (Fig. 2a). The unitless diffraction shift (Δλ/λ₀) for the film is equal to 0.56. In comparison, diffraction of the 2.5% AA hydrogel red shifted from 517 nm at pH 3.9 to 652 nm at pH 7.0, a Δλ/λ₀ of 0.26. The diffraction blue shifted when pH was reduced until the original diffraction wavelength was recovered. Because microspectrometry probes dimensional changes in the sample normal direction, the fact that the ratio of acid concentration in the film is equal to the ratio of relative swelling expressed in terms of unitless diffraction shift strongly suggests that the swelling is limited to the sample normal direction, possibly due to pinning of the film to the substrate. The control sample containing no AA showed very little response to pH changes; its maximum Δλ/λ₀ of 0.03 may be caused by the small amount of AA in as received HEMA.

Figure 2. Diffraction response of photonic crystal templated inverse opal hydrogel sensors. (a) Diffraction wavelength of AA functionalized hydrogels vs. pH for 5% AA (diamonds), 2.5% AA (triangles), and control film (squares). Inset: Raw reflection spectra of 5% AA hydrogel at various pH. (b) Diffraction response dependence of APBA functionalized hydrogels on glucose concentration: 6.25% APBA (squares), 1.25% APBA (circles); control film (triangles). Solid/open symbols indicate increasing/decreasing glucose concentration. Inset: Plot of diffraction wavelength vs. log of glucose concentration for 6.25% APBA hydrogel. The greatest response to glucose is at 1-10 mM, which is close to physiological concentrations (~5 mM).
The APBA functionalized hydrogels were found to be sensitive to glucose in 0.075 M CHES buffer with 0.13 M NaCl to simulate physiological ionic strength (Fig. 2b). At pH ~6, the 6.25% APBA hydrogel diffracted 539 nm light. When pH was increased to 9.0, the diffraction wavelength increased to 599 nm due to deprotonation of the acid moieties. The increase in diffraction wavelength corresponds to a diffraction shift due to acid deprotonation (Δλ/λo) of 0.11. Increasing the glucose concentration at pH 9.0 resulted in a gradual red shift of the diffraction wavelength until it reached 719 nm at 100 mM glucose, leading to a total shift (Δλ/λo) of 0.33. The diffraction shift due to phenylboronic acid-glucose binding (Δλ/λo) = 0.20. Thus, even at physiological ionic strength, the APBA functionalized inverse opal hydrogel film exhibited sensitivity to glucose that was easily detected. The diffraction response of the 1.25% APBA hydrogel at different glucose concentrations is also shown in Fig. 2b. A reversible red shift in diffraction wavelength was observed, going from 539 nm at pH ~6 (not shown), to 575 nm in 0 mM glucose at pH 9.0, to 592 nm in 100 mM glucose at pH 9.0, leading to a (Δλ/λo) of 0.03. In contrast, the control sample showed no sensitivity to glucose. Increasing glucose concentration to 100 mM at pH 9 caused the diffraction wavelength of the control film to shift from 566 nm to 565 nm, which corresponds to a slight shrinkage of the hydrogel when the increase in refractive index from a 0 mM to 100 mM glucose solution (~1%) was taken in account. Thus, the diffraction red shift observed in APBA functionalized hydrogels at pH 9 must be caused by complex formation between glucose and APBA.

Because the dimensional changes for both types of hydrogels were caused by ionization of the hydrogel structure (acid deprotonation and glucose-APBA complex formation), a change in the ionic strength of the solution, which controls the Debye screening length, should affect the degree of hydrogel swelling. Indeed, as ionic strength was decreased, the diffraction wavelength for both AA functionalized and APBA functionalized hydrogels increased. When the 5% AA hydrogel in unbuffered 1 mM HCl + 0.3 M KCl (aq.) solution was diluted with 1 mM HCl (aq.), its diffraction red shifted slightly from 549 nm to 565 nm (Fig. 3a). Because the hydrogel was in its neutral state at this acid concentration, the observed swelling (Δλ/λo = 0.03) is most likely due to increasing nonspecific hydrophilic interactions between the hydrogel network and water molecules as KCl concentration decreases, also known as the "salting out" effect 25. When KCl concentration was varied at 0.01 mM HCl (pH ~5), the 5% AA hydrogel, which was then negatively charged, exhibited reversible diffraction shift between 615 nm at 300 mM KCl and 825 nm at 0.1 mM KCl (Fig 3a). In the same fashion, the diffraction response of the 6.25% APBA hydrogel to 10 mM glucose became more pronounced as ionic strength was decreased. The diffraction wavelength red shifted from 634 nm at an ionic strength of 326 mM to 698 nm at an ionic strength of 38 mM, after which it remained constant as the ionic strength decreased further to 26 mM (Fig. 3b). When ionic strength was returned to 326 mM, the diffraction wavelength blue shifted to 628 nm. Thus, our inverse opal hydrogel exhibited diffractive optical response to glucose at physiological (~150 mM) and even greater ionic strengths, and the hydrogel became fully swollen at ionic strength below ~40 mM. We believe the ability of the inverse opal hydrogel to maintain sensitivity to glucose at high ionic strength may be attributed to the higher concentration of functional groups that can be attached to a dense hydrogel structure.

Figure 3: Ionic strength dependence of diffraction response. (a) Diffraction wavelength of 5% AA hydrogel in unbuffered 1 mM and 0.01 mM HCl (aq) solutions. Inset: Reflection spectra of inverse opal hydrogel with 5% AA in solution of 0.01 mM HCl and different concentrations of KCl (in mM). (b) Diffraction wavelength of 6.25% APBA hydrogel in 10 mM glucose, pH 9, versus ionic strength. Decreasing the ion concentration caused the diffraction to red shift (solid squares), and the effect was reversed when increasing the ion concentration (open squares). Inset: Reflection spectra of 6.25% APBA hydrogel at several ionic strengths.
By recording the time evolution of diffraction wavelength when a new pH or glucose concentration solution was introduced, the kinetics of diffraction response for both types of functionalized hydrogels was studied. Figure 4a presents the diffraction shift kinetics of the 5% AA inverse opal hydrogel between pH 4 and 5.4. Increasing pH from 4.03 to 5.40 (diamonds) caused diffraction to slowly red shift from 557 nm at t = 0 s to 599 nm at t = 480 s, followed by a fast red shift to equilibration at 693 nm by t = 1200 s. The kinetics of the fast diffraction shift points to a diffusion limited process (Δλ/Δt 0.5). Decreasing pH from 5.39 to 4.08 resulted in a similar behavior in reverse, where diffraction slowly blue shifted from 696 nm at t = 0 s to 682 nm at t = 320 s followed by a fast blue shift to equilibration at 562 nm by t = 1120 s. Figure 4b shows the diffraction shift kinetics of the same hydrogel as pH increased from 5.42 to 6.81. Again, a delayed diffusion-limited diffraction shift was observed: the diffraction increased from 703 nm at t = 0 s to 705 nm at t = 60s, followed by a large red shift to 782 nm by t = 1120 s. Several conclusions can be drawn from the diffraction shift kinetics. First, the equilibration times are quite close to one another, suggesting that the overall diffusions kinetics in the three cases is similar. In addition, the actual equilibration times are on the same order as, but larger than, the equilibration time (~400s) calculated from a delayed diffusion model of pH sensitive hydrogels proposed by Tanaka and co-workers 20. Because the ions are diffusing in a dense hydrogel (20% v/v water) rather than a dilute aqueous solution, their diffusivity is probably lower than the bulk value (~8x10^-5 cm^2/s) 20, leading to an increase in equilibration time. Second, the diffraction shift kinetics in all three cases consisted of a slow initial shift followed by a fast shift that was approximately diffusion-limited. As we explain later, this phenomenon was probably caused by the time required for the solution front with the new pH to reach the hydrogel. Finally, while an apparent hysteresis in diffraction response exists for pH sensitive hydrogel between increasing and decreasing pH (Fig. 2a), Figure 4b inset shows that when the hydrogel swelling is allowed to equilibrate, the diffraction wavelengths of the hydrogel during the increasing pH sweep (diamonds) overlap those during the decreasing pH sweep (triangles), suggesting that the hysteresis in diffraction observed in Figure 2a is due to the short time allowed between pH steps (~400 s).
Figure 4: Diffraction shift kinetics of inverse opal hydrogel sensors. (a) Diffraction shift kinetics during pH increase from 4.03 to 5.40 (diamonds) and during pH decrease from 5.39 to 4.08 (squares). Both exhibit diffusion-limited response with an initial time delay. (b) Kinetics of pH-induced diffraction shift as pH increased from 5.42 to 6.81. The initial time delay is smaller, while the overall equilibration time remains unchanged. Inset: Final diffraction wavelengths of 5% AA hydrogel at various pH, showing that the hysteresis in diffraction observed in Figure 2a is due to diffusion-limited kinetics. (c) Normalized diffraction response of the 6.25% APBA hydrogel for increasing glucose concentration. Squares, 0.1 mM to 1 mM; triangles, 1 mM to 10 mM; circles, 10 mM to 100 mM. (d) Normalized diffraction response for decreasing glucose concentration. Circles, 100 mM to 10 mM; triangles, 10 mM to 1 mM. Insets of (c) and (d): Diffraction wavelength as a function of elapsed time. The vertical black line marks the time when the pump was turned off and flow stopped.

The diffraction shift kinetics of the APBA functionalized hydrogel to glucose followed the same general diffusion limited trend, but with several fundamental differences. The first major difference is the geometry of the experimental setup, where a microfluidic flow cell with dimensions of 1.8 cm x 1.8 cm x 170 μm was used to introduce the glucose solution to the inverse opal hydrogel. At a flow rate of 2.5 μL/s, the flow is equivalent to a Reynolds number Re ~15,330, so the flow of glucose solution above the inverse opal hydrogel was approximately laminar. In addition, after a fixed lag time for the glucose solution to reach the entrance of the flow cell, the introduction of glucose to the hydrogel was essentially instantaneous, as seen from the time evolution of diffraction wavelength for three stepwise changes in glucose concentration at pH 9: 0.1 mM to 1 mM, 1 mM to 10 mM, and 10 mM to 100 mM (Fig. 4c inset). For all three concentration increases, the diffraction wavelength red shifted gradually for ~10 s and then increased rapidly until it approached an equilibrium value. In all cases, when the flow was stopped, the diffraction wavelengths decreased ~2 nm to their final equilibrium value. To clarify the nature of the diffraction response kinetics, the diffraction wavelength values were normalized to their steady state values before the flow was stopped and plotted versus the square root of elapsed time t (Fig. 4c). In all three cases, a single straight line could be fit to the data for the majority of each experimental run, suggesting that the kinetics was proportional to t^1/2, which is characteristic of a diffusion limited process. For the two stepwise decreases in glucose concentration (100 mM to 10 mM and 10 mM to 1 mM), the diffraction shift kinetics followed the same t^1/2 relationship (Fig. 4d).

For the change from 0.1 mM to 1 mM glucose (Fig. 4c, squares), the diffraction shift began to slow down at t ~1000 s but did not flatten completely during the time of the experiment, with an equilibration time of ~1500 s. When glucose concentration was increased from 1 mM to 10 mM (Fig. 4c, triangles), the diffraction shift began to slow at t ~500 s, and equilibrium was reached at t ~1100 s. For 10 mM to 100 mM, even faster kinetics was observed (Fig. 4c, circles); the slow swelling regime was reached at t ~100 s, and by t ~250 s the diffraction wavelength had reached its equilibrium value. Decreasing glucose concentration resulted in diffraction blue shifts with slower rates than when glucose concentration was increased (Fig. 4d). For example, an equilibration time of ~1000 s was found for 100 mM to 10 mM, and the equilibration time was ~2100 s when glucose concentration dropped from 10 mM to 1 mM. The apparent diffusivity of glucose in the dense hydrogel, D_app, can be estimated from pseudo 1-D diffusion, using the diffusion equation x^2 = 2 D_app t, where x is the film thickness and t is the equilibration time. From the equilibration time values, it was found that D_app ranged from 2.5 x 10^-6 cm^2/s to 2 x 10^-7 cm^2/s, which is much smaller than the diffusivity of glucose in water, 6.7 x 10^-6 cm^2/s. Since the HEMA monomer mixture used in this case contained no water, the value of D_app is expected to be low initially and then increase as the volume fraction of water in the hydrogel phase increases due to glucose binding. Because APBA-glucose binding removes free glucose from solution, the effective diffusivity of glucose through the hydrogel should decrease. Finally, the diffraction response hysteresis of the APBA functionalized hydrogels (Fig. 2b) persisted for the time frame of the kinetic experiment (~30 minutes, Fig. 4d), perhaps due to kinetically trapped regions in the interior of the hydrogel that became accessible to glucose only in the swollen state.

4. CONCLUSION

Utilizing dried photonic crystals as templates, we synthesized 3-D mesostructured hydrogel pH and glucose sensors. SEM of the inverse opal structure showed an interconnected array of FCC pores with a ~5% to ~10% shrinkage in characteristic dimensions compared with the photonic crystal template. Acrylic acid functionalized hydrogels exhibited ionization induced diffraction shift between pH 3 to 7, and the magnitude can be adjusted by varying the AA concentration. A maximum unitless diffraction shift of 0.56 was reported for the 5% AA hydrogel. Similarly, APBA
functionalized hydrogels exhibited sensitivity to glucose at physiological concentrations and ionic strength, with a maximum diffraction shift due to glucose binding of 0.20 for 6.25% APBA hydrogel at 100 mM glucose. 1-D hydrogel swelling was observed in both systems, possibly due to substrate pinning. A strong dependence of hydrogel swelling on ionic strength was observed for the ionized form of the pH and glucose sensitive hydrogels, while the uncharged hydrogel showed minimal response to varying ionic strength. Diffraction response kinetics followed a t^1/2 relationship corresponding to diffusion-limited kinetics. For the AA functionalized hydrogel, a constant equilibration of ~1200 s was observed, while the APBA functionalized hydrogels exhibited equilibrium times that are dependent on the degree of hydrogel swelling, with an equilibration time of ~1100 s for physiological ionic strength and glucose concentrations. In both cases, the diffusivity of the analyte molecules through the hydrogel structure is much lower than their dilute solution values, consistent with the dense, mechanically robust hydrogel structures.

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6. REFERENCES