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Removable interpenetrating network enables highly-responsive 2-D photonic crystal hydrogel sensors

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Responsive hydrogels functionalized with molecular recognition agents can undergo large volume changes upon interactions with specific chemical species. These responsive hydrogels can function as chemical sensing materials if the hydrogel volumes are monitored by using devices such as photonic crystals (PhC). An important criterion of merit is the responsiveness of these sensing hydrogels. Generally, hydrogel responsiveness is inversely proportional to the hydrogel crosslink density because the elastic constants scale with the crosslink density. The responsivities of these hydrogel sensors dramatically increase as their hydrogel crosslinker concentrations decrease. Unfortunately, the resulting highly responsive hydrogels become fragile at low crosslink densities, and are hard to fabricate and utilize. To temporarily increase the mechanical strengths of these highly responsive hydrogels we developed a method to incorporate a removable reinforcing interpenetrating hydrogel network. We demonstrate the utility of this approach by incorporating an interpenetrating PVA hydrogel within a weak, low crosslinked pH sensitive hydrogel through a freeze–thaw process. These interpenetrating PVA hydrogels are indefinitely stable at room temperature, but easily dissolved on transient heating to 70 °C. The pH sensing hydrogel response is unaffected by this incorporation and subsequent dissolution of the interpenetrating PVA hydrogel. These sacrificial hydrogels enable the fabrication and utilization of highly responsive hydrogel sensing materials.

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Introduction

Hydrogels are polymer networks that stabilize a water mobile phase. The unusual properties of hydrogels enable their applications as responsive smart materials. Responsive hydrogels have been used in applications such as sensors,^{1–6} mechanical actuators,^{7,8} and for drug delivery.^{7,9–11} Chemical sensors have been fabricated that utilize hydrogel volume phase transitions (VPT); the volume of the hydrogel changes in response to chemical or physical stimuli. We, as well as others, fabricated hydrogels to sense pH,^{5,12–15} heavy metals,^{1,4–6,15,16} humidity,¹⁷ and biological targets such as creatinine¹³ and glucose.^{1,18,19}

A wide range of methods have been developed to monitor the VPT of hydrogels. Originally measurements were conducted by measuring the size of the hydrogel.^{20–22} Later, groups developed the use of photonic crystals (PhC)^{6,13,15,17,23–27} and holograms^{12,18} to measure hydrogel VPT. Our group pioneered the use of 2D^{3,25,28–30} and 3D,^{1,4,6,15,16,19,23} PhC embedded within responsive hydrogels to monitor the VPT.³¹ PhC can be used to optically monitor the volume of the hydrogel by measuring the wavelength or the

angle of light diffracted. The diffraction depends on the spacing of the attached PhC periodicity which depends on the responsive hydrogel volume.^{1,32}

Chemically responsive hydrogels can be fabricated where their VPT are selectively induced by specific chemical species. These hydrogels change volume in response to chemically induced osmotic pressures. The three typical sources of hydrogel osmotic pressures are osmotic pressures due to the free energy of mixing (Π_m), due to the elastic free energy (Π_{el}), and due to the ionic free energy (Π_i).³³ Equilibrium occurs when the total osmotic equals zero ($\Pi_m + \Pi_{el} + \Pi_i = 0$).

The elastic free energy osmotic pressure is *roughly* proportional to the crosslink density times the extent to which the hydrogel volume departs from the synthesized hydrogel volume. The hydrogel VPT volume response due to changes in the free energy of mixing osmotic pressure, and/or due to changes in the immobilized charge-induced osmotic pressure, will increase as the crosslinker concentration decreases. The hydrogel becomes more volume responsive as the elastic restoring force is decreased. Unfortunately, decreasing the crosslinker concentrations also decreases the hydrogel mechanical strength.³⁴ Mechanically weak hydrogels are difficult to fabricate and handle.

The utilization of mechanically weak hydrogels requires hydrogel reinforcement. Unfortunately, structural reinforce-

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ment of mechanically weak hydrogels will reduce the magnitude of their VPT response. In order to utilize highly responsive but weak materials we developed a method to transiently reinforce the system to enable its fabrication and handling. We then remove the reinforcing PVA hydrogel restoring the high responsiveness. As shown below we incorporate an interpenetrating hydrogel of physically crosslinked poly(vinyl alcohol) (PVA) within a weak highly responsive hydrogel.

Uncrosslinked PVA has been used as a water soluble lift off layer.³⁵ PVA is easily physically crosslinked into indefinitely stable hydrogels (at room temperature) by freezing aqueous PVA solutions into cryogels.^{23,36,37} Upon freezing the PVA polymer chains form crystallites through hydrogen bonding. The crystals formed have the same structure as pure PVA crystals.³⁸ The crystallites are ~ 7 nm thick and spaced 15–20 nm apart.³⁸ The crystallites physically crosslink multiple PVA chains, forming a mechanically robust cryogel. PVA crystallites are stable at room temperature.^{36,37} At 70 °C, the PVA crystallites rapidly melt, dissolving the PVA cryogel.²³

Results and discussion

Fig. 1 shows the fabrication of the 2D PhC sensing hydrogels and the incorporation of interpenetrating PVA hydrogels. The 2D-PhC sensor is fabricated by depositing the polymerizable monomer solution on a slide containing a 2D PhC (Fig. 1A). A coverslip is placed on top of the solution and the solution is polymerized by UV light (Fig. 1B and C).^{17,24,31} After the sensor hydrogel is polymerized, the coverslip on the 2D array side is

removed (Fig. 1C and D). For mechanically robust hydrogels the sensor is ready for use after the other cover slip is removed (Fig. 1D–H).^{17,24,31} However, the mechanically weak hydrogels formed with low crosslinker densities are destroyed when the second cover slip is removed.

In this case we form a reinforcing interpenetrating physically crosslinked hydrogel within the sensing hydrogel which is attached to a pure PVA hydrogel substrate. This is accomplished by partially immersing the mechanically weak, highly-responsive hydrogel in a PVA solution (Fig. 1E). The PVA polymer is allowed to diffuse into the sensing hydrogel and then the PVA is physically crosslinked by a freeze–thaw process to form a PVA interpenetrating sensing hydrogel–pure PVA hydrogel bilayer system (Fig. 1F). This physically crosslinked PVA bilayer system is robust and is easily handled (Fig. 1G). The interpenetrating and pure PVA crosslinked hydrogel bilayers are easily dissolved away by heating the system briefly for 10 min to 70 °C (Fig. 1H).

We prepared highly pH responsive hydrogels by incorporating acrylic acid (AA) within acrylamide (Am) hydrogels cross-linked with low concentrations of bisacrylamide (BIS). The interpenetrating PVA cryogel was incorporated to mechanically strengthen the mechanically weak pH sensing hydrogel. Bulk polyacrylic acid has a $pK_a \sim 4.7$ (ref. 2) and increasingly deprotonates as the pH increases to form carboxylates that immobilize counterions, resulting in an osmotic pressure that swells the hydrogel.^{2,14} We sensitively monitor the hydrogel volume by monitoring the light diffracted from the embedded 2D PhC.

These poly-AA-Am-BIS hydrogels swell as the carboxyl groups deprotonate to form carboxylates at higher pH values.³⁹

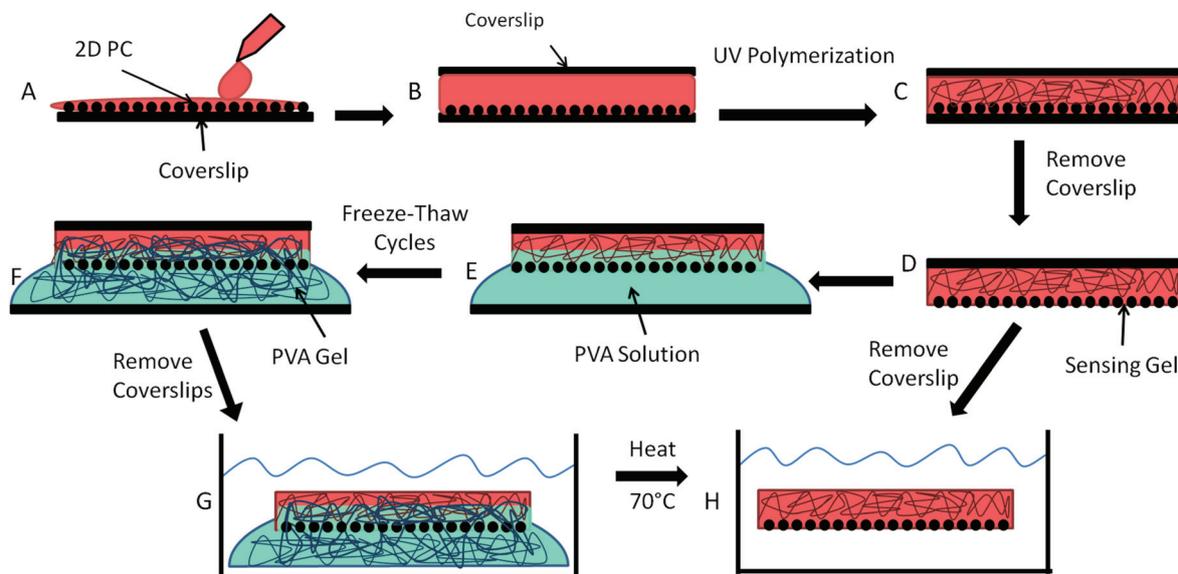


Fig. 1 Fabrication and dissolution of thermo-reversible PVA physically crosslinked hydrogel-2D PhC sensing hydrogel bilayer system. A solution of polymerizable monomers containing a UV initiator is deposited upon a 2D PhC attached to a coverslip (A). A coverslip is placed onto the solution (B) and the system photopolymerized (C).³¹ The 2D PhC sensing hydrogel is peeled off the glass slide (D) and partially immersed in a PVA solution (E). After two freeze–thaw cycles an interpenetrating PVA hydrogel forms within the sensing hydrogel and a pure PVA hydrogel forms below (F). The coverslips are removed (G). Upon heating, the PVA hydrogel dissolves leaving the original sensing hydrogel.

The extent of hydrogel swelling increases as the crosslinker concentration decreases. Unfortunately, the decreasing crosslinker concentration decreases the hydrogel mechanical strength.³⁴ These hydrogels become difficult to fabricate and handle. To enable the fabrication and utilization of minimally crosslinked, more highly responsive hydrogels, we incorporated the poly(vinyl alcohol) cryogels to temporarily mechanically reinforce the hydrogel.

Fig. 2 shows photographs of pH sensing hydrogels that were polymerized with decreasing BIS crosslinker concentrations of 1%, 0.1%, and 0.05%. The top row of pictures show hydrogels fabricated without the PVA hydrogels as depicted in Fig. 1 going from D to H without steps E–G. The low crosslink density 2D PhC pH sensing hydrogels are fragile and easily tear during fabrication. For example, the top left 1% BIS hydrogel is easily removed from the coverslip, while the 0.1% BIS hydrogel tore easily during handling, as shown in the top center of Fig. 2. The very fragile 0.05% BIS hydrogels tore into multiple small pieces because of its mechanical weakness (Fig. 2 top right).

Incorporation of the interpenetrating PVA hydrogel results in a more mechanically robust hydrogel bilayer system as shown in the middle row of photographs. The resulting bilayer hydrogel systems show significant wrinkling due to the differential swelling of the sensing interpenetrating PVA hydrogel composite compared to the pure PVA hydrogel. Wrinkling relieves strain caused by the differential swelling of the hydrogel bilayers.⁴⁰

These interpenetrating PVA-sensing hydrogel-PVA hydrogel bilayers are stable for over three months in pH 3.18 buffer at

room temperature. The bottom row of Fig. 2 shows the resulting sensing hydrogels after PVA hydrogel dissolution at 70 °C (Fig. 1G and H) most notable is that the 0.05% BIS hydrogel remains intact.

Fig. 3 shows the pH dependence of the inter-particle spacing of a series of 2D PhC AA-Am-BIS hydrogel sensors polymerized at decreasing crosslinker concentrations of 1%, 0.1% and 0.05%. As expected, the sensing hydrogel pH responsiveness increase as the crosslinker concentration decreases. At pH 3.76 the particles spacing is 646 ± 2 nm for the 1% BIS hydrogel, 970 ± 4 nm for the 0.1% BIS hydrogel, and spacing increases to 746 ± 4 nm for the 1% BIS hydrogel, 1148 ± 8 nm for the 0.05% BIS hydrogel. At pH 5.48 the particle spacing is 1220 ± 7 nm for the 0.1% BIS hydrogel, and 1562 ± 18 nm for the 0.05% BIS hydrogel. Thus, between pH 3.76 to 5.48 the particle spacing increases by 100 nm for a 1% BIS hydrogel, 251 nm for a 0.1% BIS hydrogel, 414 nm for a 0.05% BIS hydrogel. The pH sensing hydrogels increase their responsivity by four-fold as the crosslinker concentration decreases 20-fold from 1% BIS to 0.05%.

The linear response observed over this pH range was previously observed.^{2,5,14} This volume response is of complex origin. The hydrogel changes volume in response to the sum of changes in the ionic free energy, the free energy of mixing, and the restoring elastic force.³³ The equilibrium between these factors gives rise to a linear response to the titration of the carboxylate groups.

We examined the impact of the incorporation and dissolution of the PVA hydrogel on the response of a pH responsive 0.1% BIS hydrogel. A single 2D PhC AA-Am-BIS hydrogel was polymerized and cut in half. One half was stored in pH 3.18 buffer while the other had a PVA cryogel incorporated. Both hydrogels were similarly heated for 15 min at 70 °C. We independently demonstrated that this 15 min heating does not affect the pH response of the sensing hydrogels. We observe

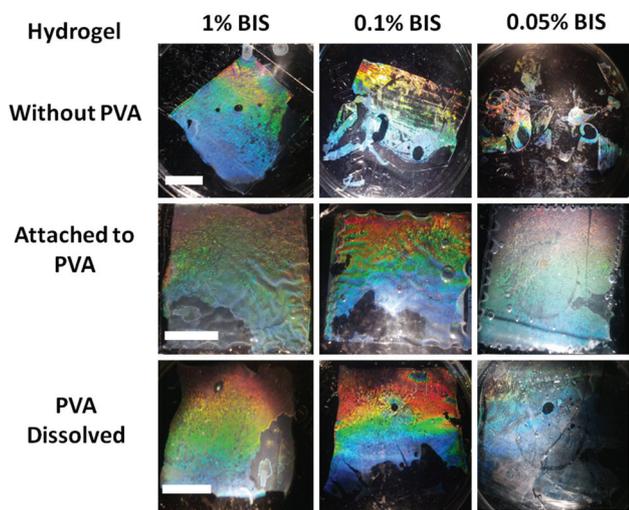


Fig. 2 Photographs of AA-Am-BIS sensing hydrogels. The top row show hydrogels fabricated without the use of PVA at 1%, 0.1%, and 0.05% (w/w) BIS crosslinker concentrations (Fig. 1D–H). The second row shows a second set of sensing hydrogels fabricated using PVA hydrogels (Fig. 1D–G). The third row shows this second set of sensing hydrogels after the PVA hydrogel is removed (Fig. 1G and H). White scale bar lengths are 1 cm.

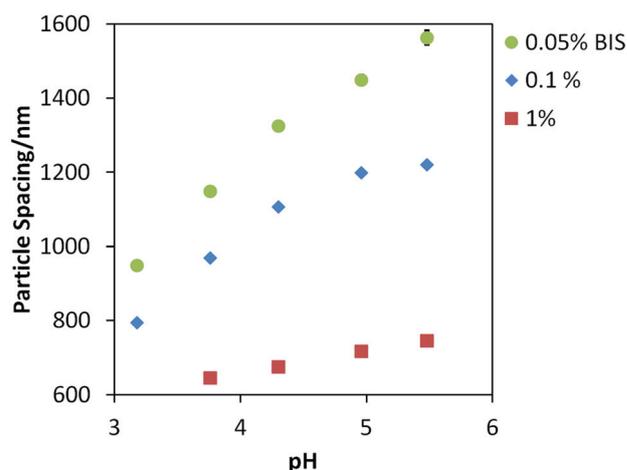


Fig. 3 Comparison of pH dependence of 2D array spacing of 2.5% AA-10% Am hydrogels prepared with 1%, 0.1%, and 0.05% concentrations of BIS crosslinker. Error bars indicate one standard deviation.

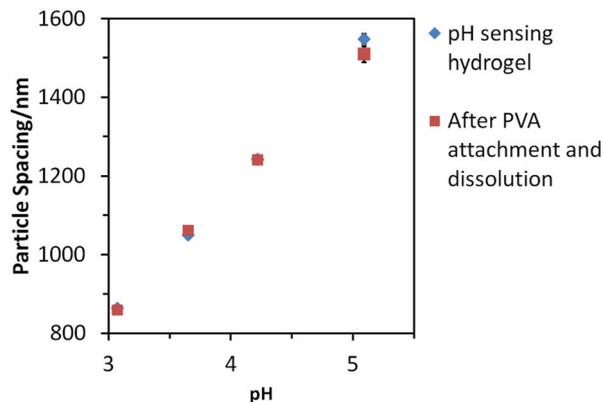


Fig. 4 Comparison of pH response of pH sensing 0.1% BIS hydrogel fabricated without a PVA hydrogel to that of a pH sensing hydrogel fabricated with an interpenetrating PVA hydrogel bilayer system that was subsequently dissolved. Error bars indicate one standard deviation.

essentially identical pH responses of these two pH responsive hydrogels (Fig. 4). The observed bright visible diffraction in Fig. 2 and a sharp well defined Debye ring after the PVA is dissolved indicates that the attached 2D PhC is well ordered. Neither the hydrogel sensor response nor the attached 2D PhC is irreversibly adversely affected by the incorporation and dissolution of the PVA hydrogel.

Experimental

Materials

Irgacure 2959, DMSO, acrylamide (Am), 1-propanol and *N,N'*-methylenebisacrylamide (BIS) were acquired from Sigma Aldrich ($\geq 99\%$ purity) and used as received. Acrylic acid (AA) was acquired from Sigma Aldrich and purified through distillation. Glacial acetic acid was supplied by Fisher and used as received. Sodium acetate was acquired from EM Science and used as received. 98% mole hydrolyzed 78 000 MW poly(vinyl alcohol) (PVA) was acquired from Polysciences Inc. and used as received.

Fabrication of pH sensitive hydrogels

2D photonic crystal array (2D PhC) pH sensing hydrogels were fabricated as previously described.³ 490 nm diameter polystyrene particles were synthesized by emulsifier-free polymerization.⁴¹ A 2D PhC was fabricated by carefully layering a 3 : 1 volume dispersion of these 490 nm polystyrene particles (15 wt%) in 1-propanol onto a water surface.²⁸ As shown previously²⁸ these particles self assemble on the water surface into a 2D PhC. The 2D PhC on the water surface was transferred to a glass slide by inserting the slide under the 2D PhC and carefully lifting it.

The sensing hydrogel monomer solution was prepared by adding 20 μL of a photoinitiator solution of 33% (w/w) Irgacure 2959 in DMSO to a 1 mL aqueous solution containing 10% (w/w) Am, 2.5% AA (w/w), and between 1% to 0.05% (w/w)

BIS crosslinker in a pH 3.18, 0.1 M acetate buffer. 130 μL of this monomer solution was deposited onto the glass slide attached to the 2D PhC (Fig. 1A). A coverslip was placed on top and the solution was UV polymerized for 15 min by illuminating the solution with a UV lamp (UVGL-55, UVP) to form a 100 μm thick 2D PhC BIS-AA-Am hydrogel (Fig. 1B).

One of the glass coverslips was removed, Fig. 1C and D. The adhesion of the hydrogel to the opposite cover slip enabled this removal. The adhesion to the opposite coverslip distributes the mechanical stress from the coverslip removal evenly over the hydrogel. An interpenetrating PVA hydrogel-bilayer system was fabricated within and around the 2D PhC sensing hydrogel by partially immersing the 2D PhC sensing hydrogel in a 7% (w/w) aqueous PVA solution (Fig. 1E). The system was temperature cycled twice by cooling to -20°C for 2 h and thawing for 2 h at room temperature. At the lower temperature the PVA forms small crystallites that physically crosslink the PVA into an interpenetrating network within the 2D PhC sensing hydrogel and an adjacent pure PVA hydrogel.

The PVA hydrogel was dissolved by heating the system in a pH 3.18 acetate buffer to 70°C , Fig. 1G and H. The dissolved PVA solution was removed and the 2D PhC pH sensor was washed with and stored in a room temperature pH 3.18 acetate buffer.

pH sensing

The 2D PhC pH sensing response was studied in acetate buffers at concentrations between 0.01 M to 0.1 M fabricated using sodium acetate and glacial acetic acid and adjusted to maintain a constant ionic strength of 0.01 M. Changes in hydrogel volume due to pH changes were monitored by measuring the 2D PhC particle spacing as previously described.³ A 532 nm laser pointer illuminated the 2D PhC sensing hydrogel at normal incidence. The light is diffracted by the 2D PhC at an angle determined by the particle spacing. This would give rise to six diffraction spots for a perfectly ordered 2D array. However, the randomly oriented 2D PhC micro-domains diffract the light to form a Debye ring.

The angle of diffraction, α was determined from the Debye ring diameter from $\alpha = \tan^{-1}(D/2H)$ where D is the diameter of the Debye ring and H is the distance between the measured ring and the illuminated hydrogel. The particle spacing was determined by the Bragg diffraction condition for 2D diffraction: $M\lambda = (\sqrt{3}/2)d(\sin \alpha)$. M is the order of the diffraction, λ is the wavelength of illumination or 532 nm, and d is the nearest neighbor particle distance.

Conclusions

Incorporation of interpenetrating PVA hydrogels enables the utilization of highly responsive, but mechanically weak sensing hydrogels. These interpenetrating PVA hydrogels can easily be removed by incubation at 70°C . This process neither alters the responsivity of the hydrogels nor significantly affects the attached 2D PhC. This approach of temporarily reinforcing

weak hydrogels will enable utilization of highly responsive hydrogel sensors that were previously inaccessible due to their fragility.

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